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Comparative Studies on Modelling and Optimization of Soil Parameters on Crude Oil Contaminated Mangrove and Clay Soils Remediation Using Box-Behnken RSM

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ABSTRACT

The combined effect of three selected soil parameters (moisture, nutrient, and pH) on the degradation rate of hydrocarbons in mangrove and clayey soils was successfully modeled using Box–Behnken design matrix of Response Surface Methodology (RSM). The method of study using the biostimulation technology aims to investigate, optimize and compare the relative *effects of the selected soil variables and textures on the degradation rate of Bonny light crude oil in the soil samples. Two sets of sixteen experimental buckets containing 1 kg of contaminated soil were set up for each soil sample. The soil factors studied at three levels was the independent variables. The residual (TPH) in the experimental buckets, monitored using gas chromatography/mass spectrometry was the dependent parameter. Results from each experimental unit showed a measurable reduction in TPH with time. The statistical analyses of the experimental results and model predictions reveal that nutrient has a pronounced major influence on crude oil degradation and that mangrove soil in comparison to clay soil favors faster crude oil degradation under atmospheric conditions. The predicted optimum parameters for nutrient, pH, and moisture were 0.143kg/kg soil, 6.7 and 0.23kg/kg soil, for the clay soil 0.160kg/kg soil, 8.00 and 0.140kg/kg soil for the mangrove soil. The predicted value of 81.48% for the mangrove soil and 63.5% for the clay soil at the optimum with a regression coefficient of 99.35% and 99.09 is suggesting that the developed quadratic regression models are accurate and reliable.*

Key Words: Box-Behnken, Crude Oil, Remediation, Optimization, Clay soil, Mangrove soil.

1 INTRODUCTION

The goal of bioremediation treatment that may a times involves the enhancement of the activities of primary microorganism in soil by adding nutrients or by augmenting with external organisms is to degrade contaminants in the environment by mineralizing the pollutants to an innocuous level. Enhanced bioremediation is a cleanup strategy that is effective in degrading crude oil pollutants and involves different technologies as electrokinetics separation [1], phytoremediation [2, 3, 4] Bioventing [5], Land farming [6, 7], biostimulation [8] etc.

The prospect of the bioremediation techniques in decontaminating polluted environments is well documented in the literature [9, 10, 11]. And their effectiveness in the treatment of hydrocarbon contaminated soils have also been proved, including treatments for moderate clay and mangrove soils contaminated with crude oil [12], sludge, lubricating oil and diesel [13]. Numerous remediation studies have acknowledged that the addition of nutrients (biostimulation) will speed up the rate of removal of the soil contaminants. The nutrient is normally added in the form of inorganic nitrogen and phosphorus salts [14, 15, 16] and the control of other environmental parameters have been proved adequate and acceptable in crude oil contaminated soil remediation [17, 18]. Soil parameters are acknowledged to limit crude oil removal from contaminated soil and the decontamination prospects of the soil is greatly affected by them. Therefore the soil factors must be optimal to achieve the maximum efficiency obtainable in such decontamination treatment. By adjusting the nutrient, pH and moisture contents of the soil, %TPH removal efficiency in the contaminated environment can have been fixed adequately.

In the quest by researchers to establish the optimum degradation conditions for hydrocarbon affected soils, most studies previously focused on sandy and agricultural course-textured soils. However, mangrove and clayey soils which are the common soils found in several crude oil producing areas especially the Niger Delta Region of Nigeria were rarely considered [19]. Knowledge of crude oil degradation rates and the optimal factor settings for such environments is thereby limited. The need exists to establish the correlation between the %TPH removal in soil and the selected soil factors through optimization.

Although the operating range for the factors in soils have well been established [9, 20, 21]. There is need to optimize the variables for crude oil degradation in such soils. Another critical parameter that must be considered while evaluating %TPH removal in the soil is the texture of the soil. Texture is a fundamental property of soil with significant influence on its physical and chemical properties. The soil texture controls the mineralization of contaminants in it and must be considered when assessing the progress of degradation in it. Namkong, et al, Ladioslao Et al, showed that the texture of the soil limits the rate and degree of contaminant degradation in it by accelerating or decelerating the movement of water, air, and nutrient through the profile [23, 22]. And there is increasing evidence to prove that many of the contaminants that persist in the environment are influenced majorly by the soil textural properties [24, 25, 26].

Clay soils have high water-holding capacity, low optimum moisture content, low air-filled porosity which often leads to anoxic conditions thereby affecting degradation rate. Clay soils are also electro-chemically active, possess many physical and chemical properties inimical to bioremediation [19]. They are maximally affected when impacted by hydrocarbon contaminants [27]. Mangrove soil is rich in features that aid biodegradation such as organic matters, moderate clay contents and water holding capacity, minerals, and exchangeable bases than clay soil making it more amenable to biodegradation treatment [28, 29].
Soil is also heterogeneous with variable composition, and the condition at any particular place governed by soil texture determines the extent of contaminant removal in that area [30]. Soil heterogeneity has also been proven as a major limiting factor in the application of laboratory-derived rate equation to the field [31]. Several reports have it that the degree of biodegradation of petroleum hydrocarbons contaminants is faster in sandy soils and generally slower in silty and clayey soils [32, 33].

1.1 Range of Soil Parameters

Studies show that large limitations are imposed on the natural attenuation process in the degradation of petroleum hydrocarbons by the unbalanced C: N: P ratio due to high level of carbon in hydrocarbon pollutant (Henrique, et al., 2011) the variation in pH of the soil [34] and the soil's moisture content [35] of the soil media. An optimal C: N: P ratio of 100:15:13 is desirable for hydrocarbon impacted soils [36]. Dibble and Bartha established a C: N and C: P ratios of 60: 1 and 800: 1. Crude oil pollutants in soil cause the carbon level for the soil microorganisms to rise to result in the raising of C: N: P ratio. Both the mineral nitrogen and phosphorus will then be mobilized into mineral biomass leading to a slowdown in the degradation process [26]. PHC biodegradation in most soils is optimal at between 50% and 70% of soil water holding capacity [35] although the desirable moisture range in the soil for biodegradation purposes is between 70 to 80% of field water holding capacity [34]. Also, the soil microorganisms require a specific range of pH for effective biodegradation [35]. pH is site specific and has a tremendous effect on the biodegradability of pollutants. The optimal pH for biodegradation is in the range of 5 - 7.8 [20] and is influenced by the relationship between the microorganism and the chemical constituents of the soil. The pH adjustment was made by adding calcium hydroxide $Ca(OH)_2$ using a neutralization curve, prepared according to the method Min Liu et al by direct titration [37].

1.2 Experimental design and optimization by RSM

Environmental researchers including Golam and Kalali, [36, 37] have utilized the Box-Behnken RSM optimization tool and Design Expert statistical software for crude oil degradation optimization studies because it permits the use of relatively few combinations of variables to determine a complex response function and its suitability for fitting quadratic surfaces. The primary feature of the data set in most optimization studies is described using the Box Behnken Response Surface Methodology utilizing Design Expert11 as the optimization software. Statistical analysis of variance (ANOVA) is usually employed to examine the quadratic relationship between the dependent variable, e.g. %TPH reduction and the independent parameters by fitting a quadratic equation to the observed data samples to define the degree to which the variables affects the dependent variable. The resultant values of the coefficients will be a measure of their contribution to the rate of change in the conditional parameter. Regression analysis can be performed to estimate the response function as a second order polynomial in the form of:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1, i<j}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j + \varepsilon \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1.1$$

A generic form of the second order multiple regression model as given in equation 1.2 where Y is the predicted response variable (%TPH) and the terms β_0; β_1, β_2, β_3; β_{11}, β_{22}, β_{33}; and β_{12}, β_{13}, β_{23} are the corresponding center point value of the fixed response, linear, quadratic and interaction coefficients regression terms while A, B and C are amendment soil variables [38].

$$Y= \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC \ldots\ldots\ldots 1.2$$

A resultant surface and contour plot is generated for the model using Design Expert 11 as a software solution to enable us to visualize and evaluate the relationship between the response variable and the independent parameters. The influence of interactions of the independent variables on the response is visualized from the surface plot, and the adequacy of the model

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justified through the analysis of variance (ANOVA). The quadratic regression equation and coefficient of determination (R^2) are evaluated to test the model fit.

This work aims to investigate and evaluate the optimal response of crude oil degradation in two soil textural classes (mangrove and clay) subjected to the same level of petroleum contamination and biostimulation treatment. We aim to develop and compare the model equations obtained for the soils and applied them to determine optimum processing conditions necessary for effective decontamination of crude oil contaminated mangrove and clay soils.

2 Materials and Methods

2.1 Sample Collection, Preparation, and Characterization

About 20kg of un-impacted soils samples were collected from two locations Akenfa in Yenagoa Local Government Area and Ekowe, Southern Ijaw Local Government Area, Bayelsa state - Nigeria. The samples were prepared through hand picking of stones, dirt, and sticks, air-dried and then sieved with a 2 mm sieve. The soils were homogenized and characterized by physicochemical and microbial parameters according to the established standards. The physical and chemical parameters evaluated include total organic carbon, total nitrogen, total phosphorus, pH, texture, and moisture content.

Okoh et al, [39] previously analyzed soil from these same locations and obtained a soil classified as clayey with 19.73% sand, 25.69% silt and 54.58% clay and a mangrove soil analyzed as 26.76% sand, 47.37% silt, and 35.90% clay and identified as silty loam.

Soil particle size analysis was performed using the Boyoucous hydrometer method, and soil textures were confirmed using the soil texture triangle. Organic carbon content was determined by the titration method, the total nitrogen content was determined by the Kjeldahl digestion, and steam distillation method, potassium content and available phosphorous were determined as described by Brandt [40]. Residual total petroleum hydrocarbon (TPH) was estimated according to Mishra et al, [41] by taking out ten grams of a contaminated soil sample from each microcosm and extracting the total petroleum hydrocarbon (TPH) was extracted (liquid-liquid extraction) from it with 40 ml of chloroform. The extract was dried at room temperature. Residual TPH was measured gravimetrically by reading absorbance at 400nm using visible range spectrophotometer and comparing the results a prepared standard plot.

Table 2-1: Soil Parameters and methodologies for Estimation

S/No	ANALYSIS	METHODOLOGY	REFERENCES
1	pH	ISO DIS 10390 (2002)	[40]
2	TPH (mg Kg^{-1} soil)	EPA 8015 : 2003 ISO 16703: 2001	[42]
3	C:N:P (mg kg^{-1}soil)	ISO 11466:1995	[43]
4	Moisture Content	The Gravimetric Methods	[44]
5	Water Holding Capacity	ASTM-D7367	

Table 2-2: Parameter Characterization of the contaminated soils.

S/No	Parameter	Clay Soil	Mangrove soil
1	Soil Moisture Content (%)	4.65	4.26
2	Organic Carbon (%)	3.64	4.86
3	Nitrogen (N) (g/kg)	1.28	1.16
4	Phosphorus (P) (g/kg)	0.02	0.016
5	Potassium (K) (g/kg)	1.81	1.72
6	Total Petroleum Hydrocarbon (g/kg)	70	70
7	Water Holding Capacity	36.05	22.7
8	pH	5.74	6.36
9	Texture classification	Clayey	Silty Loam

2.2 Experimental setup and Bioremediation experiment

The laboratory study was conducted using plastic buckets in the open atmosphere under a shade and incubated for 60 days. The independent variables were investigated at three levels, and the design includes 12 unique runs, 3 replicate center points, and 1 control as shown in tables 2.4. The sampled soils were spiked with 70g/kg of crude oil, homogenized and spread outside to allow for the evaporation of volatile components. Each treatment bucket had one kilogram (1kg) of contaminated soil and some measure of NPK (20:10:10) fertilizer (100 – 160) g/kg of soil, maintained at a pH (5 – 8) and moisture contents of (50 – 60% soil water holding capacity) according to the design. The soil used as a control was not amended with a stimulator.

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The content in all treatment buckets was mixed every week to achieve an even distribution of heat and moisture to obtain an ideal condition for microbial activities and even out the creation of hotspot within the unit where contamination may persist. The moisture and pH content of each microcosm was adjusted every week by sprinkling with water and by adding $Ca(OH)_2$ or ACID as the case may be. Biodegradation activity was assessed by following the loss of hydrocarbon contaminant in the soil through monitoring the total petroleum hydrocarbon (TPH) remaining at each time interval [45]. Samples for GC analysis were collected every 20 days for the residual Total Petroleum Hydrocarbon contents determined as hexane-extractable material according to the standard method [42]. Tables 3-1 show the remains of TPH on the 60th day of the experiment.

Table 2-3: Experimental Range and Design levels of the variables

Dependent Factor		Range of Variables		
		Low	Medium	High
Soil Nutrient (NPK) (kg/kg)	X_1	0.1	0.13	0.16
pH of soil	X_2	5	6.5	8
Soil Moisture content % WHC	X_3	40	50	60

X1= NPK fertilizer (kg/kg soil), X2 = Soil pH, X3 = Soil Moisture content (%WHC) (g/kg Soil)

Table 2-4: Coded and Uncoded Box-Behnken Design for the Three Independent soil Variables

Run no	X1	X2	X3	NPK kg/kg Soil	PH	Moisture for Clay Soil g/kg	Moisture for Mangrove Soil g/kg
1	-1	-1	0	0.1	5	0.12	0.19
2	1	-1	0	0.16	5	0.12	0.19
3	-1	1	0	0.1	8	0.12	0.19
4	1	1	0	0.16	8	0.12	0.19
5	-1	0	-1	0.1	6.5	0.1	0.15
6	1	0	-1	0.16	6.5	0.1	0.15
7	-1	0	1	0.1	6.5	0.14	0.23
8	1	0	1	0.16	6.5	0.14	0.23
9	0	-1	-1	0.13	5	0.1	0.15
10	0	1	-1	0.13	8	0.1	0.15
11	0	-1	1	0.13	5	0.14	0.23
12	0	1	1	0.13	8	0.14	0.23
13	0	0	0	0.13	6.5	0.12	0.19
14	0	0	0	0.13	6.5	0.12	0.19
15	0	0	0	0.13	6.5	0.12	0.19
16	Control for clay soil			0.00	5.74	0.042	
17	Control for mangrove soil			0.00	6.36		0.047

X1=Coded value for Nutrient, X2 = coded value for pH and X3 = coded value for Moisture

3 Results and Discussion

3.1 Characterization of Soils

The physical and chemical analysis of the soils was conducted by "Manual of soil analysis: Assessing and monitoring bioremediation" [46]. The Results as summarized in table 3.1 texturally separated the sampled soils. The measured values were found not optimal for the pH, and moisture range reported for bioremediation processes by Sarkar, [47] and need to be amended for optimum degradation of contaminants.

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3.2 Crude Oil Biodegradation Studies and Statistical Analysis

3.2.1 Natural Attenuation versus Enhanced Remediation

After incubation for 60 days under atmospheric condition and treatments as per the Box-Behnken experimental design matrix. Observation shows that the residual TPH in each experimental unit decreased. The observed and predicted TPH degradation level in each plastic bucket is as presented in table 3-2.

Table 3-1: Results for Residual %TPH in contaminated Mangrove and Clay Soils soil

RUN No	X1	X2	X3	Akenfa Clay Soil		Ekowe Mangrove Soil	
				Experimental	Predicted	Experimental	Predicted
1	-1	-1	0	58.24	58.75	26.87	26.63
2	+1	-1	0	49.98	49.69	25.53	25.88
3	-1	+1	0	57.37	57.66	27.06	26.71
4	+1	+1	0	43.02	42.51	23.61	23.85
5	-1	0	-1	56.24	55.54	21.38	21.68
6	+1	0	-1	46.50	46.61	23.92	23.62
7	-1	0	+1	54.76	54.65	24.94	25.23
8	+1	0	+1	38.67	39.37	19.99	19.69
9	0	-1	-1	51.28	51.46	22.50	22.44
10	0	+1	-1	49.54	49.94	21.31	21.36
11	0	-1	+1	50.41	50.01	22.20	22.15
12	0	+1	+1	43.45	43.27	21.22	21.28
13	0	0	0	47.19	47.73	19.86	19.86
14	0	0	0	48.37	47.73	20.07	19.86
15	0	0	0	47.63	47.73	19.66	19.86
Control				**71.52**		**63.57**	

Figure 3.1 compares the residual TPH in treated mangrove and clay soils with the pot subjected to natural attenuation during the experimental 60 days remediation period. The rate of decay of TPH is shown to be slower in the clay soil, and the results indicate that an accelerated degradation of hydrocarbons occurred in all contaminated soils enhanced with inorganic fertilizer, which was consistent with other studies [47, 48]. The experimental run containing mangrove soils recorded the lowest residual TPH in soil at the end of the remediation period. The result confirms the fact that mangrove soil is rich in features that aid biodegradation such as organic matters, minerals, and exchangeable bases than clay soil [28]. The buckets containing clay soils showed higher values for residual TPH. Clay soil particles have narrow microspores of inaccessible apertures and surrounded by the soil particles that slow the degradability of hydrocarbon in them.

Figure 3-1: Comparing the decay of TPH in treated mangrove and clay soils with natural attenuation

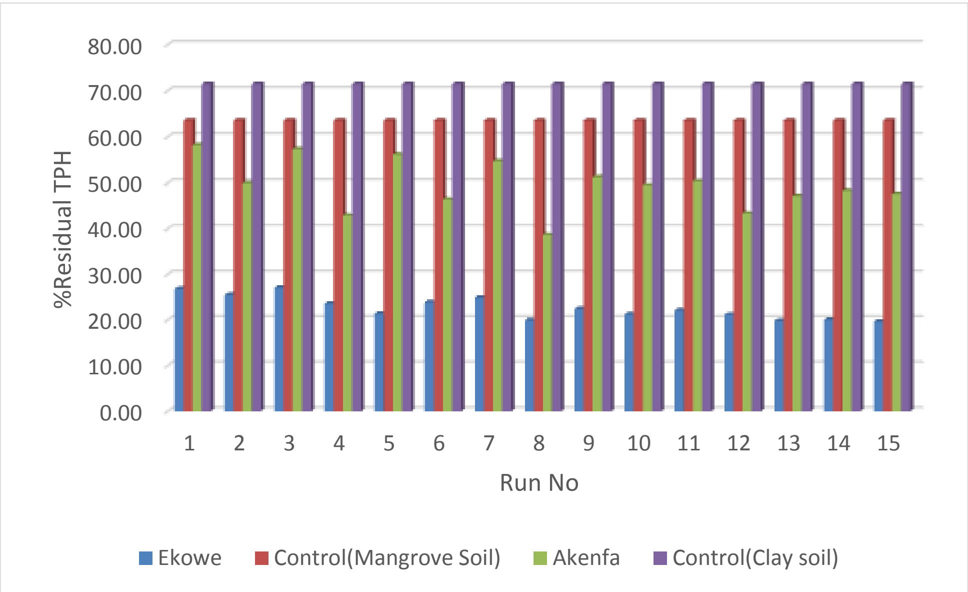

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Table 3-2 shows the experimental results of the Box–Behnken design experiments. Fifteen experiments were performed to investigate effects of the Soil nutrient (x1), pH (x2) and soil moisture (x3) along with their interaction on the degree of TPH removal (y1) from the mangrove and clay soils. The significance of the fit of the second-order polynomial for the %TPH removal in the soils was assessed by carrying out an analysis of variance (ANOVA). The results of the ANOVA and the fit statistics are shown in Tables 3-2 and 3-3.

Table 3-2: ANOVA Response for %TPH Clay/Mangrove Quadratic Model

SOURCE	SUM OF SQUARES		DF	MEAN SQUARE		F-VALUE		P-VALUE		CODED FACTOR	
	Clay	Mangrove		Clay	Mangrove	Clay	Mangrove	Clay	Mangrove	Clay	Mangrove
Model	427.85	88.64	9	47.54	9.85	85.13	60.32	< 0.0001	0.000	52.3	80.14
A-Nutrient	293.30	6.48	1	293.30	6.48	525.23	39.69	< 0.0001	0.0015	6.05	0.90
B-pH	34.16	1.90	1	34.16	1.90	61.16	11.64	0.0005	0.0190	2.07	0.49
C-Moisture	33.09	0.0722	1	33.09	0.0722	59.25	0.4422	0.0006	0.5355	2.03	0.09
AB	9.27	1.11	1	9.27	1.11	16.60	6.82	0.0096	0.0476	1.52	0.53
AC	10.08	14.03	1	10.08	14.03	18.05	85.90	0.0081	0.0002	1.59	1.87
BC	6.81	0.011	1	6.81	0.011	12.20	0.0675	0.017	0.8053	1.30	-0.05
A^2	21.22	40.87	1	21.22	40.87	38.01	250.33	0.0016	< 0.0001	-2.40	-3.33
B^2	15.14	24.52	1	15.14	24.52	27.11	150.19	0.0034	< 0.0001	-2.02	-2.58
C^2	4.35	1.48	1	4.35	1.48	7.78	9.06	0.0384	0.0298	1.08	0.633
Residual	2.79	0.8164	5	0.5584	0.1633						
Lack of Fit	2.08	0.7323	3	0.6937	0.2441	1.95	5.81	0.3566	0.1504		
Pure Error	0.7112	0.0841	2	0.3556	0.0420						
Cor Total	430.64	89.45	14								

P- Value < 0.05 is assumed significant

Table 3-3: Soil fit Statistics

	Clay Soil	Mangrove Soil
Std. Dev.	0.7473	0.4041
Mean	50.49	77.33
C.V. %	1.48	0.5226
R^2	0.9935	0.9909
Adjusted R^2	0.9818	0.9744
Predicted R^2	0.9190	0.8669

The significance of each soil coefficient was determined by p-value. The **Model F-value** of 60.32 for the Mangrove soil and 85.13 with p-values of < 0.0001 for Clay soil implies that the models were significant and had only a 0.01% chance of occurring due to noise. **For each soil parameters P-values,** less than 0.05 indicate model terms are significant while values greater than 0.1000 shows that the model terms were not significant. For the mangrove soil, the factors A, B, AB, AC, A^2, B^2, C^2 were significant model terms while A, B, C, AB, AC, BC, A^2, B, and C^2 were significant model terms for the clay soil. The ANOVA, in this case, confirms the adequacy of the quadratic model for the soils. The coefficient of determination (R^2) defined as the ratio of the explained variation to the total variation, and it is measures of goodness of fit were 0.9935 for the clay soil and 0.9909 for the mangrove soil. According to Raymond et al. a well-fitted model should yield an R^2 of at least 0.8 [38]. The R^2 shows that the models adequately represented the relationship among the variables investigated. Putting each of the regression terms into the second order quadratic equation and by eliminating from the model the terms that are not very significant to produce the following model equation for the soils.

%TPH (Clay Soil) = 52.27 + 6.06A + 2.07B + 2.03C + 1.52AB + 1.59AC + 1.31BC - 2.40A2 - 2.03B^2 + 1.08C2 ....3.2

%TPH (Mangrove) = 80.14 + 0.9A + 0.49B + 0.53AB + 1.87AC - 3.33A^2 – 2.58B^2 + 0.63C^2 .... 3.3

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The coefficient represents the expected change in %TPH per unit change in factor value when other remaining factors are held constant while the intercept is the overall average response of all the runs for a particular soil sample. The equation in terms of these coded factors can be used to make predictions about the response (%TPH Reduction) for any given levels of each element. The relative impact of the factors on the response was ascertained by comparing the factor coefficients. Since the Coefficients Table is laid in terms of coded factors we can make inferences about the relative effects on %TPH removal. For instance, observed that the linear coefficient for NPK fertilizer (A) for the Clay soil is +6.05 in the %TPH removal equation for clay soil is much higher than every other coefficients in the clay soil equation. The implication is that for the region studied the linear influence of nutrients (NPK fertilizer) on the %TPH removal of crude oil from a clay soil environment was more positive and significant than every other variable. Similarly, the interactive effect of Nutrient and Soil moisture (AC) affected the removal rate of crude oil in the mangrove soil more than the other variable. The coefficients are shown in table 3-4 along with their relative significance for comparison.

Table 3-4: Comparing Coefficients for Mangrove and Clay Soil

Soil	Interactive Coefficients			Linear Coefficients			Quadratic Coefficients		
	AB	AC	BC	A	B	C	A^2	B^2	C^2
Akenfa (Clay)	+1.52	+1.59	+1.30	+6.05	+2.07	2.03	-2.40	-2.02	+1.08
P- Value (Clay)	0.0096	0.0081	0.0174	< 0.0001	0.0005	0.0006	0.0016	0.0034	0.0384
Ekowe (Mangrove)	+0.5275	+1.87	-0.053	0.90	0.488	0.095	-3.33	-2.58	0.633
P- Value(Mangrove)	0.0476	0.0002	0.8053	0.0015	0.0190	0.5355	< 0.0001	< 0.0001	0.0298

Where A= nutrient, B = pH and C = moisture

3.3 Optimizing the Soil Parameters

The major research reported in this work is the evaluation and comparing the optimum parameter values that will maximize the removal of hydrocarbon pollutants (TPH) from the contaminated mangrove and clay soils through the numerical optimization of the soil factors with default to the parameter extremes. The highest observed %TPH removal in each soil was set as the lowest desirability and 100%TPH removals as the highest theoretical acceptable limit in order not to come short of the potential optimum. The Design Expert 11 software was employed to optimize and maximize %TPH removal, a threshold of 100 as the upper limit and 80 and 54 as the lower limits were set as the desirability to achieve the degradation objectives. Table 3.5 and figure 3.1 gave the best factor settings and desirability prediction for the soils.

Table 3-5: Best Factor Selection That Gave the Best Degradation Responses

Soil	NPK Fertilizer	Moisture	pH	%TPH	Desirability	
Ekowe Mangrove Soil	0.143	0.23	6.698	81.484	0.316	Selected
Akenfa Clay Soil	0.160	0.140	8.000	63.502	0.373	Selected

3.4 Discussion of the Results

The following points are deduced from the results by comparing the two remediation models developed for the crude oil contaminated soils affected by the selected combinations of predictor's variables. The results indicate that an accelerated degradation of hydrocarbons occurred in all the soils enhanced with inorganic fertilizer. The percentage removal of TPH is seen to be lower in the clay soil, and, which was consistent with other studies [47, 48]. The experimental run containing mangrove soils recorded the highest %TPH removal at the end of the remediation period.

This result is a confirmation of the reports that mangrove soil is rich in features that aid biodegradation such as organic matters, moderate clay contents and water holding capacity, minerals, and exchangeable bases than clay soil [28, 29]. The higher residual TPH values in the clay soils could be explained by clay soil particles having narrow microspores of inaccessible apertures which may not permit the passage of nutrient and oxygen and surrounded by the soil particles that may slow the degradability of hydrocarbon in them.

3.4.1 Effect of Nutrients Addition (Biostimulation)

Observation from table 3.2, taking runs 1 and 2 and runs 3 and 4 and as shown in Table 3.3, the results of ANOVA denote that the soil nutrient has a significant influence on the %TPH removal in clay soil (p-value < 0.0001) and mangrove soil (P<0.0015). The result for both soils indicated a remarkable increment in the %TPH reduction as the quantity of NPK fertilizer is increased. Observing table 3-4, shows that by maintaining the clay soil moisture content at its maximum water holding capacity and at a pH of about 6.8 while increasing the nutrient will bring about increment in the %TPH removal. Similarly, at a pH of around 6.8 and a maximum moisture content for the mangrove soil, raising the level of nutrient to about 0.14kg/kg soil gives the optimum %TPH removal for the mangrove soil. The indication is that nutrient addition to both soils comprehensively enhanced the level of crude oil degradation in the soils. The result suggests that nutrient is the major factor that limits the rate of deterioration in the soils, but the degree of influence is more pronounced in the clay soil than in the mangrove. Other researchers such as Sang-Hwan, Chorom,

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Ayotamuno, Borrensen et al, [49, 50, 51, 52, 34] made a similar observation. However, a look at table 3-2, runs 5 and runs 6 and figure 3.4b and c for the mangrove soil suggests that a nutrient level of 0.14kg/kg as the maximum beyond which %TPH will decrease. Similar observations were made by authors such as Park and Lynch [53, 54]. The result was a confirmation of similar observation by Vinas et al and Bento et al to the inhibiting effect of nutrient addition in contaminated soil remediation [55, 56]. This result amplified the significant difference in the level of crude oil degradation in clay and mangrove soils when subjected to the same conditions.

Razende RP et al, Giasi & Morelli concluded that the observed difference in degradation operation is weaker in clay soil than the mangrove soil because of the mass transfer difficulty of contaminants in clayey soils [57, 58].

3.4.2 Effect of Moisture on the Rate and Extent of Bioremediation.

The relations between moisture content and degradation rate of crude oil in the experimental soils is visualized by considering table 3-4 and run numbers 6 and 8, 9 and 11 that shows a steady increase in the level of %TPH removal from contaminated soils as the moisture contents increases. Literature has it that an increase in the soil moisture content will primarily boost the activities of the soil microbes because of the rapid increase in their motility and nutrient bioavailability [59]. The degradation rate with respect to variation in moisture contents seems linear in both soils, and the impact on the extent of biodegradation was appreciably high and significant. The most significant effect was observed in the clay soil while the mangrove soil showed a slight dependence on the moisture content. Runs 9 and 11 show a limiting effect of moisture content on the level of %TPH removal in the mangrove soil observed at increased pH and high nutrient. Harper et al. made a similar observation and suggested that increasing the moisture level beyond a critical level have a limiting effect on biodegradation rate, a view confirmed by Borresen et al. [60, 34]. Sabate et al. and Ali Akbar also reported that adjusting the moisture level at 40% of soil WHC had enhancing effect on bioremediation of a creosote-contaminated soil, whereas amendment with nutrients showed the inhibiting impact on the rate of biodegradation [59, 61].

3.4.3 Effect of pH on Soil Remediation

By considering experiment Runs No.1 and 3 comparing it to Runs No 2 and 4 in table 3-2 and table 3-4. The runs had the same conditions but with different pH. Compared to other parameters, pH was a unique one that had no observable linear relationship with %TPH change. Both mangrove and clay soils demonstrated the highest rate of TPH removal when pH was high approaching the alkaline range. From the data increasing the pH of the soils exerted more influence on %TPH removal in the clay soil (7%) than the mangrove soil (1.92%). Dibble and Bartha,[20] observed maximum degradation rate at pH 7.8 in oil sludge samples, beyond which the rate of degradation decreased. In the present study, similar results were obtained, Mangrove soils showed the maximum %TPH removal at pH 6.7 while it was pH = 8 that was optimal for the clay soil. The optimal %TPH removal level of 85% and 60% was observed for mangrove and clay soil at these pH levels respectively. In the mangrove soil, the optimal rate occurred at pH = 6.7 beyond which a decreasing trend was observed. The results indicated that extreme pH is inhibitory to microbes involved in the microbial degradation processes in mangrove soil.

3.4.4 .Interactive effects of Soil Factors on the Optimization Equations

The relationships and the tendency of the soil factors to influence the %TPH degradation efficiency are visualized as shown in table 3-4 by looking at the interactive effects of two variables within their studied ranges, on the %TPH degradation while keeping the third variable fixed at the zero levels. The shape of the resultant contour plot indicates the natures and extents of the interactions between factors. An elliptical contour plot shows a prominent interaction, whereas a negligible effect appears as a circular contour plot.

Observing table 3.5 and figures 3-4, %TPH reduction was influenced positively by interactions between all the soil factors in clay soil while the interaction between nutrient and moisture that influenced %TPH removal in the mangrove soil. The graphical representation of the responses are shown in Figures 3.4 will help us to picture the effect of parameter interactions on the percent TPH removal from the contaminated soils.

3.4.5 Effect of soil texture on the degree of degradation

One of the objectives of this work was to determine the influence of soil texture on the %TPH removal in soils. Recall that the experimental pots were subjected to the same treatment under the same conditions, the only variant being the soil textural class. The textural type of the soil was, therefore, the limiting factor in the %TPH removal of contaminants from the soils. After 60 days of experimentation under the same amendment and atmospheric conditions, a maximum of 81.5% TPH and 63.5% TPH removal was achieved for the mangrove and clay soils respectively. The difference in these figures can only be accounted for by the soil textural classes.

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4 Conclusion

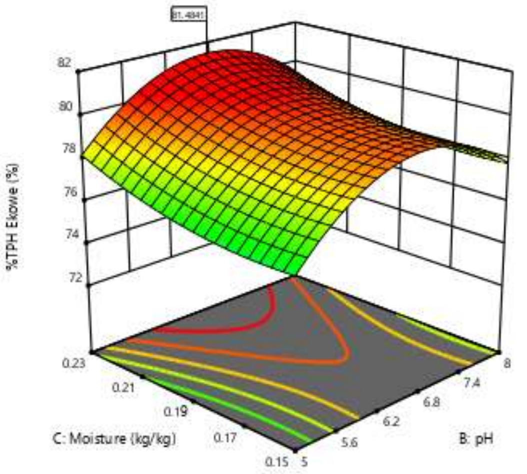
Results of analysis revealed that at the optimum, the biodegradation of petroleum hydrocarbons was greater in the mangrove soil than in clay soil with the mangrove soil showing marked decrease in TPH concentrations of over 80% of the original TPH concentration whereas, the TPH in clay soil was degraded to about 60%, compared to the control 36.5% for mangrove and 28.5% for clay soils respectively. An indication that the addition of nutrient and moisture tremendously influenced the %TPH removal process in the soils.

Within the limit of experimental error the optimum parameters setting that maximized the %TPH removal for the mangrove soil was NPK Fertilizer 0.143kg/kg soil Moisture 0.23kg/kg soil and a pH of approximately 6.7 while it was 0.160kg/kg soil and moisture content of 0.140kg/kg soil and a pH of 8.0 for clay soil. Keeping these parameters at the optimum values is critical for a successful bioremediation treatment. There is a possibility that this work may be one of the first instances where comparative remediation study of crude oil contaminated Mangrove and Clay soils was examined. By considering the impact of a combination of soil factors (Nutrient, pH, Moisture) in a detailed manner as done, this work had several limitations as the models' predictive accuracy was less than desired due to glaring weaknesses which include the quality of the data set and possible lack of quadratic relationship between the %TPH reduction and the independent variables. There could probably be some other important factors not accounted for in the models.

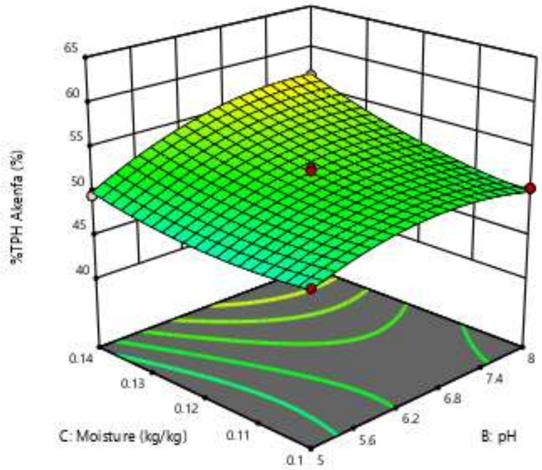
Table 4-6: Interaction effect of process variables on %TPH Removal in the Soils

Mangrove Soil

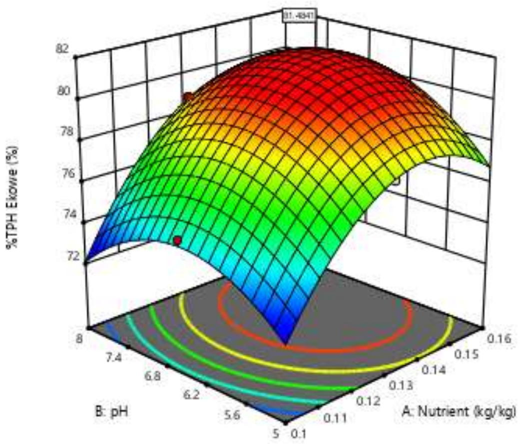
Clay Soil



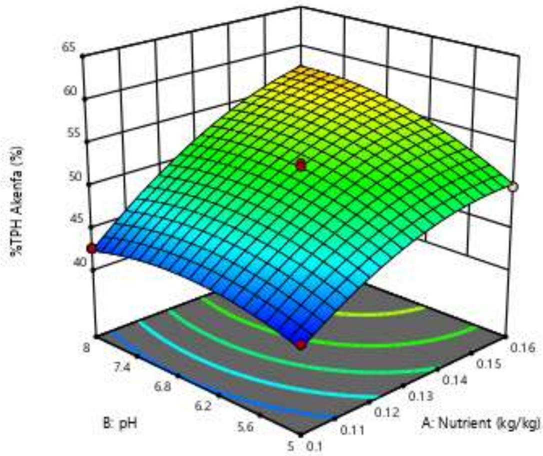
(a) pH and Moisture



(a) pH and Moisture



(b) pH and Nutrient



(b) pH and Nutrient

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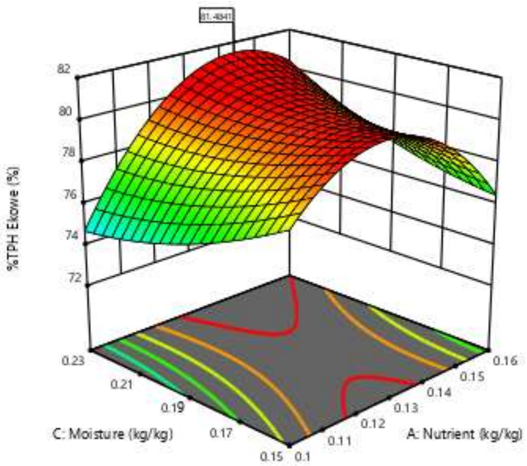
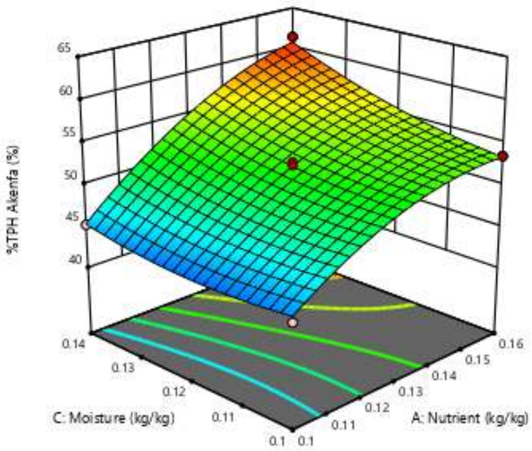


(c) Nutrient and Moisture



(c) Nutrient and Moisture

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CONFLICT OF INTEREST

We, the authors declare that we have no conflict of interest concerning this paper and its publication.

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