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Modelling and Optimizing the Effect of pH on Remediation of Crude Oil Polluted Soil with Biochar Blend: RSM Approach

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research modelled the effect of pH on the remediation of crude oil-polluted soil using biochar blend. The biochar blends, PL-500, pW-500, and RS-400, were made by pyrolyzing poultry litter, pine wood, and rice straw at varied temperatures and times. The pH of the crude oil polluted soil was 4.72. Response surface experimental design mixed biochar to remediate total petroleum hydrocarbons (TPH). Following 30 days of bioremediation, 15g PL-500, 3g PW-500 and 6g RS-400, removed a maximum of 46% TPH. The experimental data were statistically modelled and optimized using design expert software and response surface methods. Analysis of variance (ANOVA) was used to determine the significance of each regression coefficient. Biochar blend

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improved soil pH to 6.9 following remediation. ANOVA indicated that PL-500 was significant for predicting TPH % degradation at p =0.0290, suggesting that its high pH, nutrient, and soil water conservation values made it more effective in remediating TPH. The quadratic model predicts $\%\mathrm{TPH} = 19.2 + 0.74\mathrm{A} - 7.2\mathrm{B} + 15\mathrm{C} + 0.016\mathrm{AB} + 0.13\mathrm{AC} + 0.71\mathrm{BC} + 0.0016\mathrm{A}^2 + 0.67\mathrm{B}^2 - 3.26\mathrm{C}^2$ with R^2 =0.8567. A model fit statistics were used to examine soil pH influence on TPH remediation. RSM study indicated a good positive association between statistical model and experiment with R^2 = 0.7612. The model fits experimental data and predicts that $\mathrm{Soil\ pH} = 6 + 0.065\mathrm{A} - 0.14\mathrm{B} + 0.19\mathrm{C} - 0.0055\mathrm{AB} - 0.0060\mathrm{AC} + 0.041\mathrm{BC} + 0.0013\mathrm{A}^2 + 0.012\mathrm{B}^2 - 0.061\mathrm{C}^2$. Remediation requires soil pH and biochar's alkalinity raised soil pH to 6.9, which promoted hydrocarbon-utilizing bacteria.

Keywords: Biochar blend; pH effect; bioremediation; crude oil polluted soil; RSM modeling and optimization.

1. INTRODUCTION

Nigeria has frequent crude oil spills; in the previous 50 years, an estimated 10–13 million tons of oil have been released into the environment, with more than 77% of that oil not being recovered [1]. The leaks are caused by sabotage, oil exploration operations, equipment failure, pipeline corrosion, and tanker accidents. Nigeria's oil deposits are located in the Niger Delta area, where the majority of oil exploration is conducted. As a result, an oil spill alters the characteristics and functions of the soil, rendering it unfit for biological processes [2]. The quality of the soil is continually damaged by spills because certain hydrocarbons in crude oil have high lipophilicity, which increases their bioaccumulating activity in an aquatic habitat [3]. The majority of the time, remediation techniques are straightforward and cheap, failing to take into consideration the complexity of the many contaminated media. Environmental contamination in the Niger Delta has been the subject of several studies, yet the issues persist, and nothing is done to clean it up [4]. The contaminant's and the soil's chemical, physical, and biological characteristics all have a role in the remediation technique selection [5]. It has been discovered that the physicochemical and thermal approaches are pricy and time-consuming [6]. Due to its simplicity, cost-effectiveness, and environmental friendliness, bioremediation has emerged as the most attractive technique [7,8]. Using microorganisms, the remediation process known as bioremediation converts harmful compounds into less dangerous or nontoxic forms [5]. The presence of pollutants, bacteria that consume the contaminants, enough oxygen, acceptable soil moisture, the proper temperature, nutrients to enable microbial development, and a suitable pH are, nonetheless, essential requirements for efficient bioremediation [6].

With the benefits of a large specific surface area and high porosity, biochar is a commonly employed microbial-immobilization carrier [9]. Natural biomass resources like sawdust and agricultural waste are used to make biochar, which offers microorganisms a conducive habitat. Moreover, biochar may increase the interface between microorganisms and pollutants by having a high potential for adsorbing petroleum contaminants. For instance, it has been demonstrated that biochar, which is created during the pyrolysis of biomass, can improve soil physical properties (aggregate stability, porosity, aeration, and water holding capacity), increase soil pH and cation exchange capacity, and productivity, as well as adsorb hydrophobic organics [10]. Results from studies on the use of biochar in bioremediation have been erratic. Garca-Delgado et al. [11] showed that the addition of biochar had no appreciable impact on the rate of deterioration. In contrast, biochar considerably enhanced degradation rates by around 20%, according to Qin et al. [12]. Response Surface Methodology (RSM) has gained popularity in the optimization of operating parameters in integrated systems, which is necessary to better understand the impact of process parameters in remediation [13]. RSM is a statistical and computational method that uses factorial design to plan tests, fit models, and identify the best circumstances for a goal response [14]. It is often used to enhance and grasp the functionality of novel, complicated systems with ambiguous processes [15]. RSM, however, is especially used in circumstances where a number of input factors may have an impact on a performance indicator or quality feature of the product or process [16]. Moreover, RSM quantifies the connection between the computed response surfaces and the controlled input parameters. Recently, Taguchi orthogonal design and Box-Behnken design were used as optimization strategies for the biodegradation of crude oil-contaminated soil [17,18]. The use of biochar in soil remediation to remove organic and inorganic contaminants and improve the bioremediation process has only been the subject of a small number of research.

The majority of studies, however, did not take the use of biochar blends into consideration; instead, they concentrated on using biochar made from a single biomass and on the use of factors that could be controlled to slow the degradation process, such as temperature, the presence of microorganisms, pH, moisture content, etc. Also, there are few studies on how to best bioremediate soil that has been contaminated by crude oil utilizing biochar produced by furnace-assisted pyrolysis. The purpose of this research is to examine the role of pH in bioremediation of TPH in acidic soil that has been contaminated by crude oil.

2. MATERIALS AND METHODS

2.1 Collection and Analysis of Polluted Soil Sample

A location in Kpuite, Tai LGA, Rivers State, Nigeria, has soil that had been contaminated by crude oil. The samples were taken from the contaminated site at a depth of 30 cm using a soil auger. They were then well mixed and homogenized before being placed into a sack bag. Standard techniques were used to assess the physicochemical characteristics of the contaminated soil, including measurement of temperature (°C), pH, nitrogen (%), potassium (EPA 30508), moisture content (%), electric conductivity (S/cm), cation-exchange capacity (CEC) [meq/100g], and phosphorus content (APHA 4500). Also, the total petroleum hydrocarbon (TPH) was calculated using the ISO 17025 technique, and the total heterotrophic bacteria (THB) (APHA 92158) and total heterotrophic fungal count (THF) were used to calculate the number of microorganisms present in the contaminated soil (APHA 9601B). By using the vapor phase approach, the hydrocarbon-using bacteria (HUB) and fungus (HUF) were identified.

2.2 Biochar Production and Characterization

2.2.1 Biochar production

The biochar samples were produced utilizing an Electric Vulcan furnace (A130 model) with a gas expeller using a variety of feedstocks, including rice straw (RS), poultry litter (PL), and pine wood (PW). Slow pyrolysis was used for this, with the following residence periods and temperatures: RS = 400°C for 1 hour, PL = 500°C for 1 hour 30 minutes, and PW = 500°C for 2 hours. The pyrolyzed samples were cooled to room temperature after the completion of the residence period, stored, and given the designations RS-400, PL-500, and PW-500 depending on the pyrolysis temperature. The pyrolyzed PL-500 and RS-400 biochars were then put through a 2-mm filter, and pellets that stuck to the sieve were ground down until they fit through a 0.42-mm sieve, which is what is known as "dust." The samples of biochar made from the feedstocks are shown in Fig. 1.

2.2.2 Biochar characterization

Biochars were ground to a thickness of around 0.25 mm for characterization. The American Society for Testing and Materials (ASTM) D1293 guidelines were used to measure the pH of each biochar, and the ASTM D 3176 guidelines were used to calculate the ash and nitrogen concentrations. The gravimetric technique was used to calculate the moisture content.

Fig. 1. Biochar produced from (a) poultry litter (b) rice straw and (c) pine wood

2.3 Design of Experiment for Bioremediation

The bioremediation experiment was carried out using Design-Expert Software version 13 and Box-Behnken Design (BBD) and Response Surface Methodology (RSM). With the aim of maximizing the response, RSM is a common mathematical and statistical approach for analyzing and modeling a process in which the response of interest is impacted by a number of factors [19]. It is a useful technique because it makes it possible to assess how various factors and their interactions affect one or more response variables [20]. The process parameters (PL-500, RS-400, and PW-500) are known as the independent variables, while the response (TPH) is referred to as the dependent variable. Understanding and evaluating the impact of various factors and how they combine to produce the answer is the major benefit of employing RSM (s). Thus, it is seen as a suitable strategy to optimize a process having one or more answers [21].

The three components (PL-500, RS-400, and PW-500(g)) are observable, hence the response surface may be expressed using Equation 1 as follows:

$$Y = f\,(X_1, X_2, X_3) \tag{1}$$

The goal is to optimize the response variable Y (TPH),

Where; X_1 = PL-500 (g), X_2 = RS-400 (g) and X_3 = PW-500 (g).

The independent variables are assumed to be continuous and sensitive to little experimental error. Typically, a second-order model is used to provide a fair approximation of the functional connection between independent variables and the response surface, as shown in Equation 2:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i{}^2 + \sum_i \sum_j \beta_{ij} X_i X_j + \varepsilon \tag{2}$$

Where: ε is the random error,

In matrix form, Equation 2 can be expressed in Equation 3 as:

$$Y = \beta X + \varepsilon \tag{3}$$

Equation (3) was solved in design expert application using RSM method and the experimental range is presented in Table 1.

The independent variables are the factors (PL-500, RS-400, and PW-500) influencing the remediation of total petroleum hydrocarbons (TPH). Equation (4) thus depicts the empirical expression [19,20,22-24] as:

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{2} \beta_{ij} X_i X_j \tag{4}$$

Where Y is the response; β_0 is a constant term; $\sum_{i=1}^{2} \beta_i$ is the summation of the coefficient of linear terms; $\sum_{i=1}^{2} \beta_{ii}$ is summation of quadratic terms; $\sum_{i=1}^{2} \sum_{j=i+1}^{2} \beta_{ij}$ is the summation of the coefficient of interaction terms; $X_i X_j$ are independent variables.

The nutrient-rich chicken litter, rice straw, and pine wood were employed as the three unique independent variables in the Box-Behnken factorial experimental design. Three levels (1, 0, +1) of each independent variable were investigated over the course of 17 experimental runs and one control. Based on investigations by Agarry & Ogunleye [25], who used pig dung and NPK fertilizer for bioremediation, the amounts were determined. As a result, the variables at three levels of RS -400 (2–6 g), PL–500 (5–15 g), and PW–500 (2-4 g) were optimized. The significance of each term in the fitted equations was determined using the statistical program Design Expert 13 (Stat-Ease Inc., Minneapolis, USA), and the goodness of fit was estimated for each example.

2.4 Bioremediation of TPH in Crude Oil Polluted Soil

400g of soil contaminated by crude oil was placed in a variety of plastic containers with the numbers 1 through 17 and a control. According to the RSM experimental range in Table 1, various amounts of PL-500 (5, 10, 15 g), RS-400 (2, 4, 6 g), and PW-500 (2, 3, 4 g) were added to the crude oil-polluted soil in each container. According to the findings of Agarry & Ogunleye [26], this range was chosen. Also, 400g of the soil that had been contaminated by crude oil was taken from the homogenized section and utilized as a control sample. The control sample didn't include any biochar. A suitable amount of aeration was achieved by mixing the soil, and the water holding capacity was adjusted by moistening the soil with more water.

Table 1. Experimental range and levels of the variables

Factors	High (+1)	Medium (0)	Low (-1)
PL-500 [A] g	15	10	5
RS-400 [B] g	6	4	2
PW-500 [C] g	4	3	2

The microcosms, which were made of plastic, were then maintained in a wooden greenhouse, and incubated at room temperature (which ranged from 27 to 33^{o}C). To ensure that nutrients and bacteria were evenly distributed, each plastic container (diameter: 15 cm; height: 8 cm) was thoroughly mixed. Plastic saucers were employed to stop water loss from under the containers in order to maintain a reasonable level of soil salt. All microcosms were manually mixed twice a week for the duration of the 30-day experiment in order to increase oxygenation and keep them wet. Following 30 days, the total petroleum hydrocarbon content (TPH) of the repaired soil was measured in order to assess the efficiency of the crude oil removal procedure. The soil that had been polluted by crude oil but hadn't been treated was also looked at as a control.

2.5 Analysis of Total Petroleum Hydrocarbon (TPH)

20 grams of each soil sample were taken from the microcosms after the 30-day bioremediation period, dried at room temperature for 72 hours, and then tested for TPH using the FLUORAT-02 analyzer and a fluorometric technique. Equation 5 was used to calculate the amount of total petroleum hydrocarbons that had deteriorated.

$$\%\text{TPH degradation} = \frac{C_0 - C_f}{C_0} \times 100 \quad (5)$$

Where C_0 = initial TPH concentration in soil (g/kg) and C_f = final concentration of TPH in bioremediated soil (g/kg).

2.6 RSM Modeling and Optimization

The results of the bioremediation experiment were used to anticipate the response variable (%TPH) using the RSM model in design expert software. An analysis of variance (ANOVA) was performed, and the P-value was used to determine the significance of each regression coefficient. The ideal parameters were verified by repeating the experiment under the ideal conditions. Based on this result, a fit summary table was made to evaluate the ideal model for the optimization project. During the RSM optimization process, only the response variable (%TPH) and the independent variables (PL-500 (g), RS-400 (g), and PW-500 (g)) were optimized. The approach for RSM optimization is shown in full in Fig. 2.

2.7 Model Performance Evaluation

A technique that heavily rely on performance evaluation is predictive modeling. Using the appropriate metrics, the efficacy of a predictive model is assessed and contrasted. For a certain prediction model, the appropriate metrics must be selected in order to provide an accurate result. In order to evaluate the model's performance and accuracy, equations (6) and (7) were utilized to determine the mean square error (MSE) and mean absolute percentage error (MAPE) as functions of error.

$$\text{MSE} = \frac{1}{N}\sum_{n=1}^{N}(\text{Experimental} - \text{Predicted})^2 \quad (6)$$

$$\text{MAPE} = \frac{1}{N}\sum_{n=1}^{N}\left(\frac{|\text{Experimental} - \text{Predicted}|}{\text{Experimental}}\right) \times 100 \quad (7)$$

Root mean square error (RMSE), which is more beneficial when there are significant mistakes, in order to get the RMSE, Equation 8 from Tanarslan et al. [27] was used:

$$\text{RMSE} = \sqrt{\frac{1}{N}\sum_{j=1}^{N}(\text{Experimental} - \text{Predicted})^2} \quad (8)$$

3. RESULTS AND DISCUSSION

3.1 Soil Properties Prior to Remediation

Table 2 displays the findings of the soil's crude oil contamination before treatment. According to the findings, the contaminated soil is not salty since the electrical conductivity (EC) value (73.4 S/cm) was below the WHO norm of 400 S/cm and did not exceed the threshold limit of 2000 S/cm [28,29]. The demonstrates that there is no salinity problem with the soil prior to treatment. According to Qin et al. [30], salinity may have a major effect on the bioremediation of petroleum hydrocarbons. Since high salinity inhibits microbial growth, biodegradation may proceed more slowly [31]. The crude oil-polluted soil, on the other hand, had an acidic pH (4.7), a temperature of 28.5 (°C), a moisture content of 21.49 (%), and a cation exchange capacity of 6.2 (meq/100g), while the potassium, total nitrogen, and phosphorus concentrations contents were 0.3, 0.7 and 21 (%) respectively, indicating a deficient and unbalanced nutrition of the soil. There were discovered to be 5.8 (10^3 cfu/g) and 5.7 (10^2 cfu/g) bacteria overall that are heterotrophic and consume hydrocarbons, respectively. So, if biochar encourages bacterial growth, this leads to a potential bioremediation strategy. Yet, the variety of hydrocarbon-using fungus ranged from 0.3 to 1.3 (102 cfu/g). The high moisture content of the soil that has been contaminated with crude oil was the explanation for the difference in cfu/g of the different species, ranging from 0.3 to 5.8 cfu/g, which suggests a bigger concentration of bacteria that consume hydrocarbons (HUF) (Table 2). It is promising if biochar promotes the growth of bacteria throughout the bioremediation process.

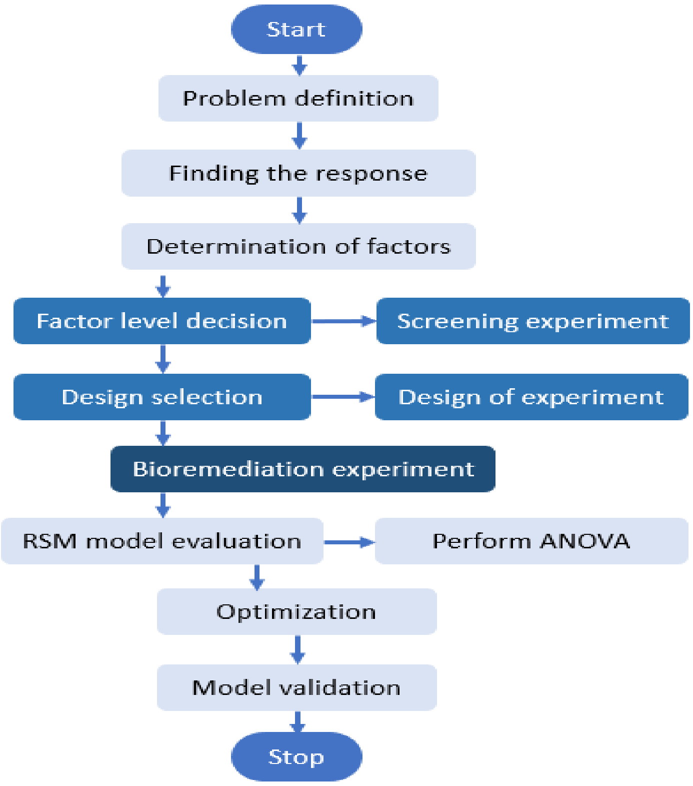

Fig. 2. Procedure for RSM modeling and optimization

Table 2. Results of soil properties prior to bioremediation

Physicochemical properties	**Value**
pH	4.7
Temperature (℃)	28.5
Moisture Content (%)	21.5
Electrical Conductivity ($\mu S/cm$)	73.4
Cation Exchange Capacity (meq/100g)	6.2
Total Nitrogen (%)	0.7
Potassium	0.3
Total Phosphorus	21.0
Total Petroleum Hydrocarbon (TPH) content (mg/kg)	1405
Microbial properties	
Total Heterotrophic Bacteria (10^3cfu/g)	5.8
Total Heterotrophic Fungi Count (10^2cfu/g)	1.3
Hydrocarbon Utilizing Bacteria (10^2cfu/g)	5.7
Hydrocarbon Utilizing Fungi (10^2cfu/g)	0.3

3.2 Physicochemical Properties of Feedstock and Biochar

The results of the physicochemical examination of feedstock and biochar are shown in Table 3. The pH of the pine wood (PW) feedstock was 3.53, and the initial moisture content was 12.33 (%). The bioremediation of acidic soil using PW may not be favorable due to the acidic feedstock. As indicated in Table 3, rice straw (RS) has a higher pH of 7.22 whereas poultry litter (PL) has a more acidic pH of 4.92. The pH of PW-500 (3.6) remained acidic after pyrolysis, whereas the pH of RS-400 and PL-500 rose to 8.23 and 7.160, respectively. The pH of RS-400 and PL-500 would blend together when the biochar was added, raising the pH of PW-500. Thus, because of the pH of the blended biochar, bioremediation would now be feasible. Also, the three types of biochar had ash concentrations of 18.96, 5.48, and 25.6 (%) for PW-500, RS-400, and PL-500, respectively, while the nitrogen content varied from 0.12 to 0.15 (%). The moisture contents for PW-500, RS-400, and PL-500 were 1.24, 1.577, and 43.89%, respectively. These show how the physicochemical properties of the feedstocks were affected by the pyrolysis temperature and residence duration. Increased pyrolysis temperature, according to Chatterjee et al. [32], resulted in greater C and ash concentrations, reduced N contents, and increased pore volume and micro surface area.

3.3 Bioremediation Experimental Result

Table 4 displays the findings of a 30-day bioremediation experiment using different biochar blends, which included 17 experimental runs and 1 control. The pH served as the basis for the experimental analysis whereas the decrease in TPH concentration of the contaminated soil served as the experimental basis. For all of the different blended biochar proportions, a substantial drop in TPH was seen after the 30-day period. The pH of each of the different ratios, nevertheless, ranged from 6.26 to 6.91. Statistical analysis and a response surface methodology (RSM) were used to further discover the best biochar mix for degradation.

3.4 RSM Model and Statistical Analysis

The experimental data was assessed for its ability to fit into a statistical model in order to create a statistical model to effectively predict and simulate the best degradation of TPH by biochar blend (quadratic equation). The coefficients of the model are denoted by the constant term, A, B, and C (linear coefficients for independent variables), AB, AC, and BC (interaction term coefficient), and A^2, B^2, and C^2 (quadratic term coefficient). Correlation coefficient (R^2), adjusted determination coefficient (Adj-R^2), and enough precision are used to assess a model's suitability. A model is considered acceptable when its p-value is 0.05 and unfit when its p-value is more than 0.05. To evaluate if differences between means were statistically significant, analysis of variance (ANOVA) was utilized.

The experimental data from experimental design (Table 4) were fitted to a second-order polynomial regression model in Equation 9, containing three linear, three interaction terms, and three quadratic terms [25,26,33]. This allowed us to derive the model equation for TPH removal from the crude oil contaminated soil using biochar blend. The final equation is therefore expressed as follows in terms of the real elements (PL-500, RS-400, and PW-500):

$$\%\mathrm{TPH} = 19.2 + 0.74\mathrm{A} - 7.2\mathrm{B} + 15\mathrm{C} + 0.016\mathrm{AB} + 0.13\mathrm{AC} + 0.71\mathrm{BC} + 0.0016\mathrm{A}^2 + 0.67\mathrm{B}^2 - 3.26\mathrm{C}^2 \quad (9)$$

Where:

A = PL-500 (g), B = RS-400 (g) and C = PW-500 (g)

In the first instance of statistical analysis for TPH (%) degradation, this quadratic model's R^2 value of 0.8542, which is greater than that of other polynomial models, was significant. According to Myers and Montgomery [34], there are other metrics that may be used to assess model accuracy in addition to R^2 value, including modified R^2, anticipated R^2, and prediction error sum of squares. As an additional verification of the model's efficacy, these parameters are employed. Adjusted R^2 is 0.6668 in this investigation, projected R^2 is -0.3094, and Adeq precision is 8.6241.

Only PL-500 is significant for forecasting TPH (%) deterioration at p = 0.0290, according to an ANOVA analysis of Table 5, and not all process parameters have a meaningful effect on the model. That indicates that PL-500 is more effective at getting rid of TPH because of its unique properties, including a high pH value, high nutritional value, and a reputation for protecting soil water content. In addition, the high pH value of the PL char caused the pH of the soil to increase from 4.72 to 6.9. Only PL char is relevant in the model, which may be due to a combination of the number of process factors at the design stage or the baseline characteristics of the soil. When used independently, the process variables were considered based on how successfully they eliminated %TPH from oil-polluted soil.

Table 3. Physicochemical characteristics of biomass and biochar

Properties of biomass	**PW**	**RS**	**PL**
pH	3.530	7.220	4.920
Moisture content (%)	12.33	12.20	91.16
Properties of biochar	**PW char**	**RS char**	**PL char**
pH	3.600	8.23	7.160
Moisture content (%)	1.240	1.577	43.89
Ash content (%)	18.96	5.58	25.86
Total nitrogen (%)	0.130	0.15	0.120

**PW (Pine Wood), RS (Rice Straw) and PL (Poultry Litter)*

Table 4. Factors and residual TPH after 30-days bioremediation period

Exp. runs	**PL biochar (g) [A]**	**RS biochar (g) [B]**	**PW biochar (g) [C]**	**TPH (%)**	**Soil pH**
1	10	2	2	39.9	6.7
2	15	2	3	43.9	6.9
3	10	6	2	38.1	6.7
4	10	4	3	35.6	6.5
5	10	4	3	35.3	6.5
6	15	4	4	42.8	6.8
7	5	6	3	35.4	6.5
8	10	4	3	34.6	6.5
9	10	6	4	36.0	6.6
10	5	2	3	33.3	6.3
11	10	4	3	39.3	6.6
12	15	6	3	46.7	6.9
13	10	4	3	41.0	6.8
14	5	4	2	26.4	6.3
15	10	2	4	32.2	6.3
16	15	4	2	38.6	6.8
17	5	4	4	27.9	6.4
Control	-	-	-	*11.3*	*4.3*

Table 5. ANOVA for quadratic model for %TPH removal

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	**Remarks**
Model	389.99	9	43.33	4.56	0.0290	significant
A-PL biochar	300.73	1	300.73	31.63	0.0008	Significant
B-RS biochar	6.20	1	6.20	0.6522	0.4459	Not significant
C-PW biochar	2.06	1	2.06	0.2167	0.6557	Not significant
AB	0.0979	1	0.0979	0.0103	0.9220	
AC	1.76	1	1.76	0.1853	0.6798	
BC	8.13	1	8.13	0.8546	0.3860	
A^2	0.0035	1	0.0035	0.0004	0.9852	
B^2	29.80	1	29.80	3.13	0.1199	
C^2	44.87	1	44.87	4.72	0.0664	
Residual	66.55	7	9.51			
Lack of Fit	34.20	3	11.40	1.41	0.3630	not significant
Pure Error	32.35	4	8.09			
Cor Total	456.55	16				

The f-value of 4.56 indicates the model's statistical significance. This magnitude of an f-value has a 2.90% chance of being noise-related. Nonetheless, model terms are significant when the p-value is less than 0.05. In this case, Factor "A" (PL biochar) is a crucial model variable. Nevertheless, values over 0.1 imply that the model terms are not important. If there are many extraneous model terms, model reduction (removing elements like PW char as it negatively affects TPH degradation) may help the model. Also, the 1.41 f-value of the lack of fit shows that it is not significant when compared to the pure error.

3.5 The Effect of pH in TPH Remediation of Acidic Soil

Since it is a sign of numerous soil processes, soil pH is essential to understanding soil systems. It offers important information on the availability of the exchangeable cations as well as indicating whether the soil is basic, neutral, or acidic. Plant nutrient availability and microbial responses in soils are controlled by soil pH. Table 6 presents the statistical study of the role of soil pH in TPH removal from acidic soil that has been contaminated with crude oil. The model is significant at p=0.0274 according to the ANOVA results, and PL-500 is also a significant term (p=0.0007). All 17 samples, with the exception of the control sample, in which biochar was not added, showed an elevation in pH. This demonstrates the biochar blend's strong and advantageous impact on pH. With the help of PL-500 and RS-400 (g) in the mix, soil pH raised from 4.720 to 6.9 as a result. As shown by the F-value of 4.66, the model is significant (Table 6). The likelihood of noise producing an F-value this big is just 2.74%. Model terms are significant when their p-values are less than 0.05. In this instance, the model word "A" is important. Values larger than 0.1000, however, suggest that the model terms are not important. The lack of fit is not significant in comparison to the pure error, according to the lack of fit's f-value of 1.49. A significant lack of fit F-value has a 34.50% likelihood of being the result of noise. Such small misfit, however, is advantageous.

The fit statistics was used to assess the appropriateness of the model in order to develop the model equation for the influence of soil pH on TPH elimination. This was calculated using the RSM analysis experimental design-expert 13 program, and the results showed that the coefficient of variation was 1.87%, with a standard deviation of 0.1171, mean value of 6.5900, and R^2 = 0.7612, adjusted R^2 = 0.7061, projected R^2 = -0.5658, Adeq accuracy = 11.227, and covariance (CV) = 1.7800, or 17.8%, were the correlation coefficient results (ideal). These findings demonstrate a high and favorable correlation between the statistical model and the experiment. The model may be used to make predictions since it matches the experimental data. Equation 10 provides the final model equation for predicting soil pH in terms of real variables.

$$\text{Soil pH} = 6 + 0.065\text{A} - 0.14\text{B} + 0.19\text{C} - 0.0055\text{AB} - 0.0060\text{AC} + 0.041\text{BC} + 0.0013\text{A}^2 + 0.012\text{B}^2 - 0.061\text{C}^2 \quad (10)$$

Where:

A = PL-500 (g), B = RS-400 (g) and C = PW-500 (g).

Table 6. ANOVA for quadratic model for soil pH

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remarks
Model	0.6393	9	0.0710	4.66	0.0274	significant
A-PL biochar	0.5000	1	0.5000	32.79	0.0007	Significant
B-RS biochar	0.0300	1	0.0300	1.97	0.2034	Not significant
C-PW biochar	0.0378	1	0.0378	2.48	0.1593	Not significant
AB	0.0121	1	0.0121	0.7936	0.4026	
AC	0.0036	1	0.0036	0.2361	0.6419	
BC	0.0272	1	0.0272	1.79	0.2233	
A^2	0.0041	1	0.0041	0.2697	0.6195	
B^2	0.0100	1	0.0100	0.6563	0.4445	
C^2	0.0158	1	0.0158	1.04	0.3426	
Residual	0.1067	7	0.0152			
Lack of Fit	0.0563	3	0.0188	1.49	0.3450	Not significant
Pure Error	0.0504	4	0.0126			
Cor Total	0.7460	16				

You can forecast the reaction (soil pH) for any given level of each element using the Equation (10) in terms of real factors (PL-500 (g), RS-400 (g), and PW-500 (g)). Here, the levels for each component should be stated in their original units. It is not advised to use Equation 10 to determine the relative importance of each component since the coefficients are scaled to take into account the units of each element and the intercept is not at the center of the design space.

The 2D contour plot (g) in Fig. 3 shows the impact of interaction between PL-500 and RS-400. The positive effects of PL-500 and RS-400 interactions on the biodegradation process are shown in this graph (Fig. 3a). The PL and PW characters do not interact with one another. Nevertheless, it was discovered that although RS-400 levels decreased, PL-500 (g) levels increased and the pH of the soil increased (Fig. 3b). Similar to this, it was found that raising PL-500 (g) at a constant PW-500 (g) increased soil pH as seen in the 2D response surface plots (Fig. 3c). The interaction between PW-500 (g) and PL-500 (g) and its impact on soil pH are shown in a 3D surface plot in Fig. 3d.

Moreover, Fig. 4a's 2D contour plot demonstrates how PW-500 (g) and RS-400 are related (g). PW-500 and RS-400 are both helpful to the biodegradation process, however this figure demonstrates that they do not interact. Nevertheless, as shown in the 3D response surface plot in Fig. 4b, it was discovered that a rise in PW-500 (g) at a fixed RS-400 (g) resulted in a decreased soil pH value. The experimental, anticipated, and residual soil pH values after bioremediation are shown in Table 7. In contrast to the contaminated soil's original pH of 4.720, it is clear that the control sample, to which no biochar mix was applied, had the lowest pH (4.26).

Moreover, Fig. 5 shows how soil pH substantially influences TPH breakdown and how more TPH is eliminated the higher the pH. The variation in pH value after remediation is due to the high pH in PL-500 and RS-400 of the biochar mix at various experimental levels from the response surface approach design.

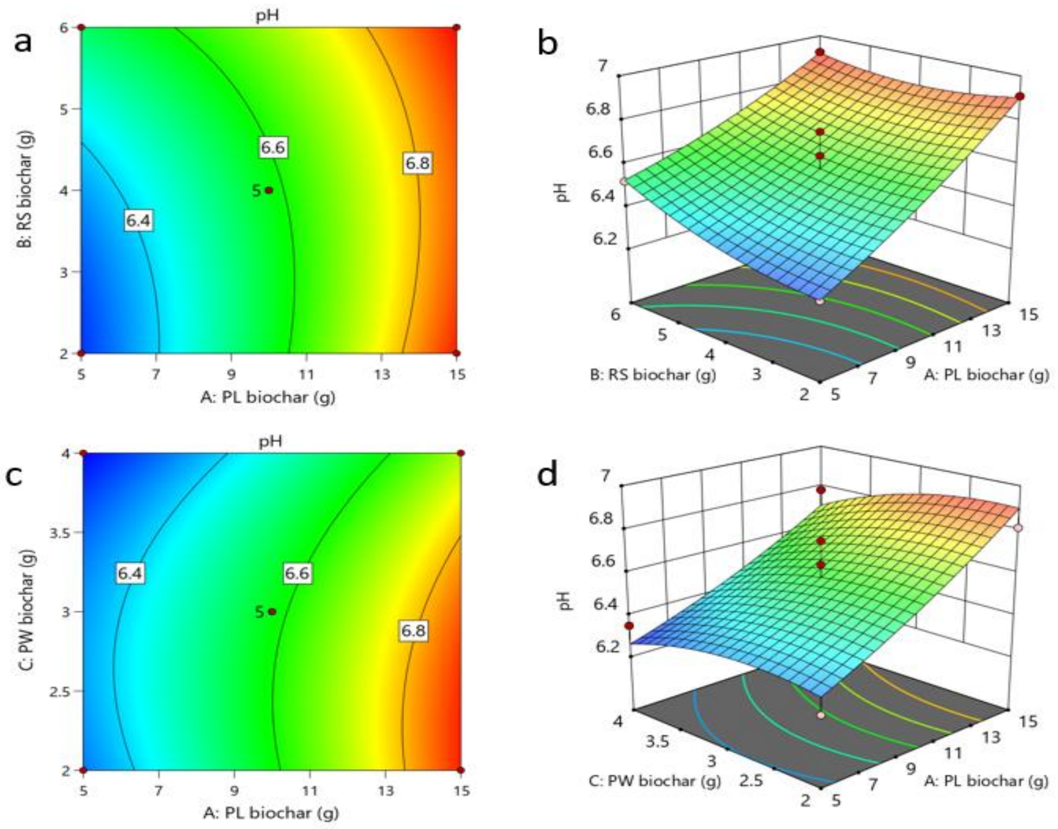

Fig. 3. 2D contour and 3D surface plots. (a – b) shows interaction effect of RS-400 and PL-500 on Soil pH during TPH degradation while (c – d) shows interaction effect of PL-500 and PW-500 on Soil pH during TPH degradation

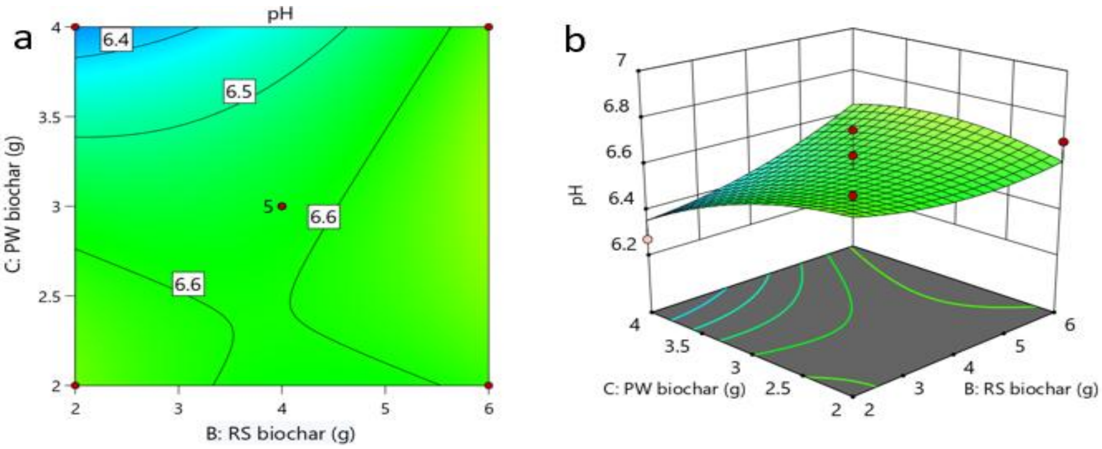

Fig. 4. 2D contour and 3D surface plots showing interaction effect of RS-400 and PW-500 on soil pH during TPH degradation

Table 7. Experimental, predicted and residuals for soil pH after bioremediation

Runs	Experimental soil pH	Predicted soil pH	Residual
1	6.7	6.7	0.08
2	6.9	6.9	0.01
3	6.7	6.6	0.09
4	6.5	6.6	-0.07
5	6.5	6.6	-0.07
6	6.8	6.7	0.08
7	6.5	6.5	-0.01
8	6.5	6.6	-0.09
9	6.6	6.6	-0.08
10	6.3	6.3	-0.00
11	6.6	6.6	0.06
12	6.9	6.9	0.00
13	6.8	6.6	0.17
14	6.3	6.3	-0.08
15	6.3	6.4	-0.09
16	6.8	6.9	-0.09
17	6.4	6.3	0.09
Control	*4.6*	-	

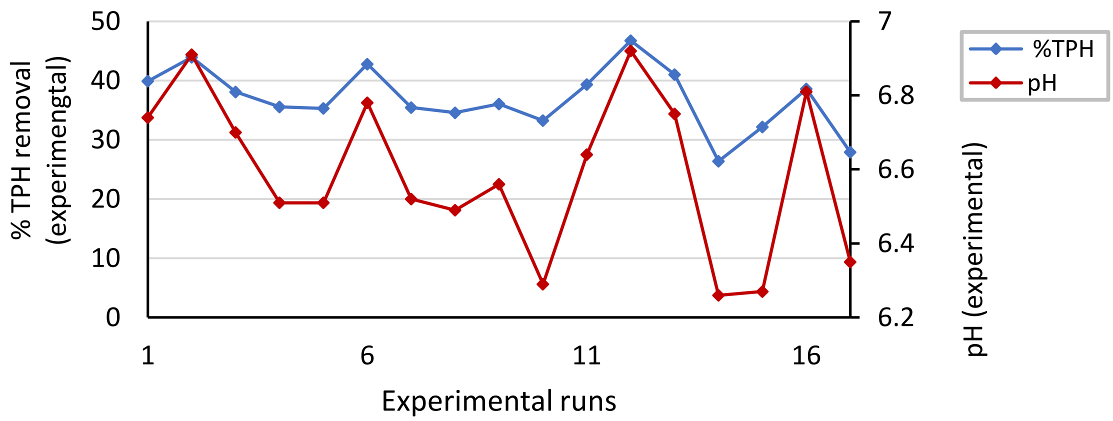

Fig. 5. The effect of soil pH on TPH degradation at various experimental levels

3.6 Response Surface Numerical Optimization of TPH Degradation

A numerical optimization method based on the desire function was utilized to determine the real-world optimal conditions for the TPH bioremediation process. In this experiment, anticipated values and actual findings were contrasted. Analysis of variance (ANOVA) was performed as described in Table 5, and the p-value was used to determine the significance of each regression coefficient (0.0260). The optimal parameters were obtained by repeating the experiment under perfect conditions, which is congruent with the conclusions of Olatunji et al [20]. Based on this result, a fit summary table was made to evaluate the ideal model for the optimization project. The fit summary findings demonstrate that quadratic models may effectively represent the examined responses (TPH and pH). This result is based on the sequential p-value of the quadratic model, which is 0.05 and validates the findings of Olatunji et al. [20]. The RSM optimization procedure was finished by maximizing PL-500, RS-400, and TPH. The findings would match those shown in the ramp plot for ideal conditions in Fig. 6 if we maximize PL-500 and TPH (%) and subsequently decrease PW-500. This would provide the strongest case for biodegradation.

In contrast, PL-500 is maximized when soil pH is used as the response variable while PW-500 and RS-400, among other process variables, are held constant. Fig. 7 shows the findings of the ramp plot, which are as follows. Figs. 8 and 9 illustrate the attractiveness of the optimization and interaction plots of ideal soil pH points.

3.7 Statistical Model Validation

Figs. 6 and 7 provide a summary of the best conditions for maximum %TPH degradation and the impact of soil pH after 30 days as determined from the response surface. Nonetheless, Equations 6 to 8 were used to calculate the statistical model's validation. The validation graphs, which represent the correlation between experimental and expected values of TPH and soil pH, are also shown in Fig. 10. The actual and projected values of TPH degradation and pH were found to be strongly and positively correlated, indicating a substantial association between the biochar mix and the two response variables, TPH and pH, respectively. Table 8 summarizes the model validation results. Fig. 11 also provides a comparison between the experimental and projected soil pH values.

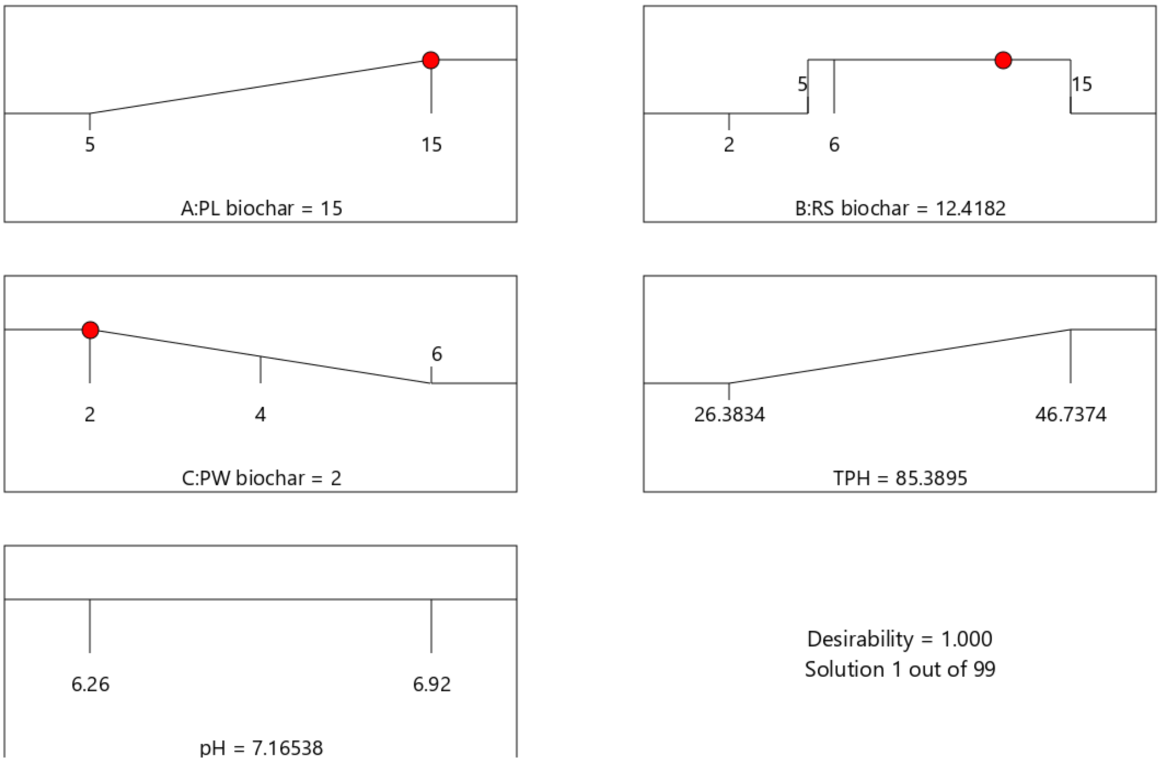

Fig. 6. Ramp plot of optimal conditions for TPH degradation

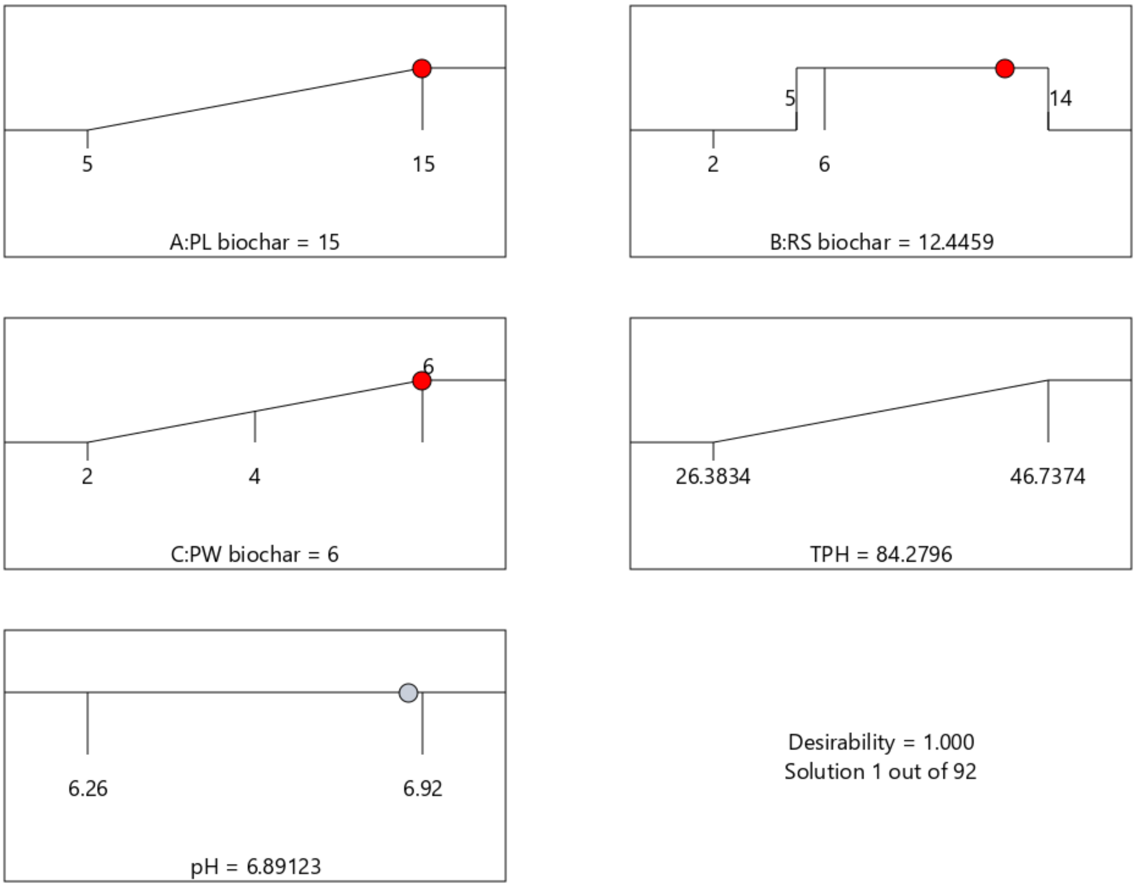

Fig. 7. Ramp plot of optimal conditions for soil pH on TPH degradation

Since it is a sign of numerous soil processes, soil pH is a crucial factor in understanding soil systems. It reveals the availability of exchangeable cations as well as the soil's acidity, neutrality, or basicity. The pH of the soil controls microbial activity and nutrient availability. In the present investigation, the soil pH before restoration was discovered to be 4.72, suggesting an acidic state. This shows how highly acidic the ecological zone is. Acidic soil may lead to phosphate fixation, which lowers the capacity of bacteria to fix atmospheric nitrogen. Strong acid is a sign that the soils of the research environment contain particular metals including zinc, iron, manganese, and aluminum. The high precipitation is to blame for the soil's acidity, which is characteristic of southern Nigerian soils and makes most of the basic cations in the soil drain away [35]. Yet, the pH of the soil rose to a high of 6.9 following 30 days of remediation with a biochar mix. This result is in line with those of Lawson et al. [36] and Zhang et al. [37], who discovered that the pH of soil was higher in biochar-only or biochar-plus-nutrient treatments than in nutrient-only or control treatments.

Yet, the alkaline nature of biochar and the presence of negatively charged functional groups on the biochar surface that bind H+ from the soil solution cause the pH to rise following biochar application [38,39]. Bacteria that consume hydrocarbons multiply when soil pH rises [40]. Similar to this, Zhang et al. [37] found that biochar increases soil characteristics including pH, microorganisms, CEC, and others. The fact that the pH increased in this investigation supports the fact that the biochar mix was responsible for the change in pH since the remediated soil sample is acidic (pH=4.72). Because of this improvement, adding PL-500 is better than adding PW-500, which has a lower pH value of = 3.6. All of these benefits are a result of the elemental makeup of biochar, which has the ability to directly alter the chemical characteristics of soil and provide a chemically active surface capable of altering nutrient dynamics and catalyzing beneficial reactions. Moreover, the porous nature of biochar and its substantial surface area help to alter the physical and chemical composition of soil. This quality is particularly beneficial in acidic soils since raising the pH makes essential plant components like calcium, potassium, and phosphorus more soluble. [41] Contrarily, since biochar application may produce an excessive rise in soil pH, it may be harmful to alkaline soils [42]. More so than biochar blends with a larger proportion of PL char, Ducey et al. [43] research showed that biochar blends with a higher percentage of pine wood result in lower soil pH. This backs up the conclusions of the present research. Ducey et al. [43] employed a biochar mix, albeit not for bioremediation, to enhance soil nutrients and the microbial population [44-46].

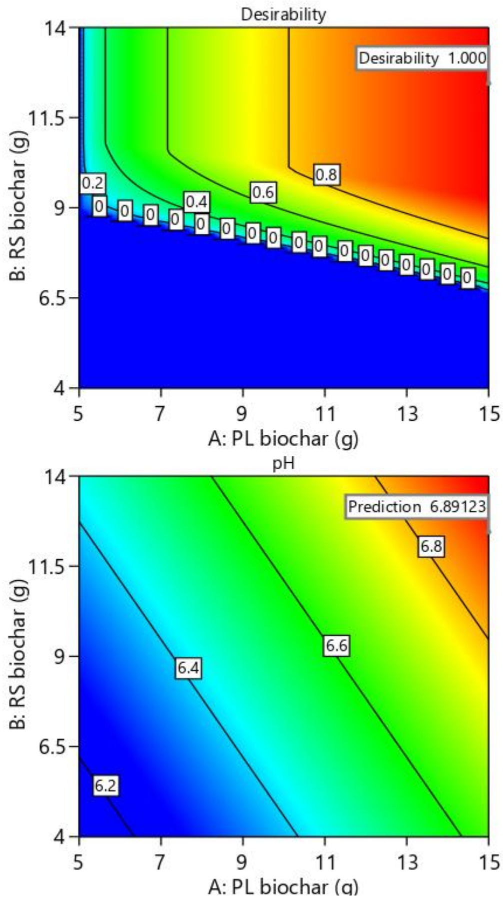

Fig. 8. Desirability plots for optimization of soil pH

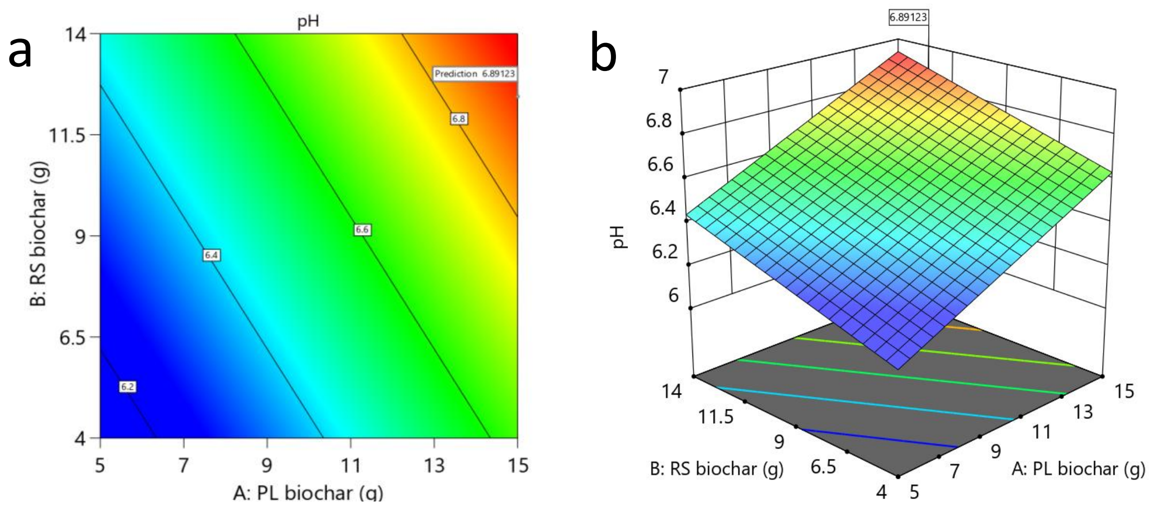

Fig. 9. 2D contour and 3D surface plots for optimal conditions for soil pH effect

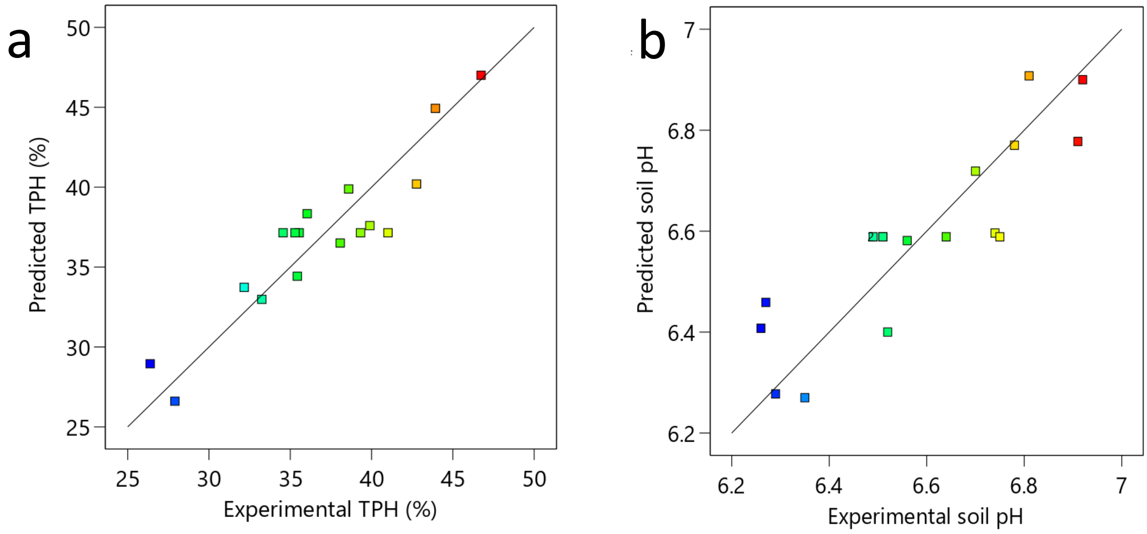

Fig 10. The relationship between experimental and predicted values of %TPH removal and soil pH

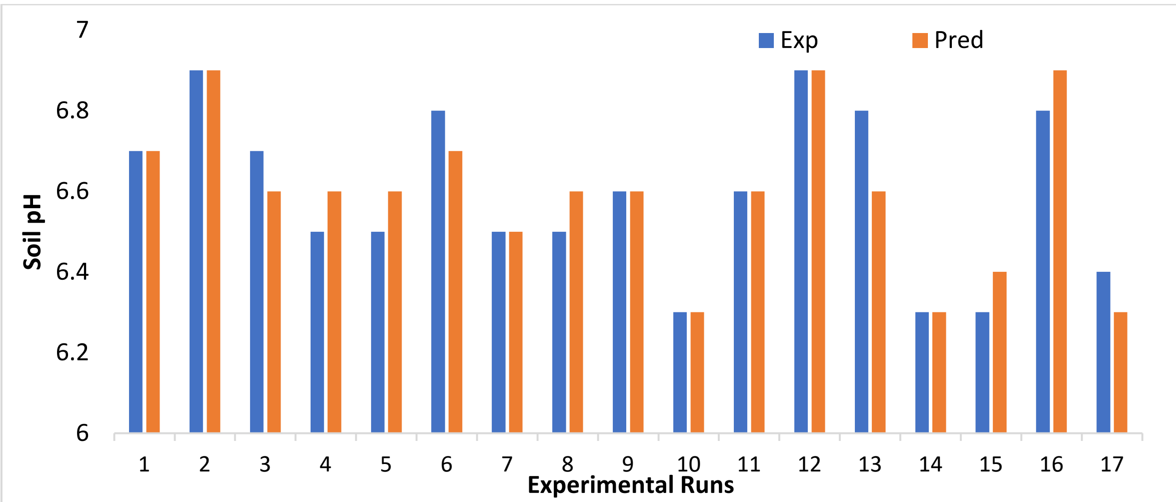

Fig. 11. The comparison between experimental and predicted soil pH during TPH remediation

Table 8. Summary of error computation

Statistical error analysis	%TPH	pH
R^2	0.854	0.857
MSE	3.900	0.006
MAPE (%)	4.900	1.019
RMSE	1.800	0.067

4. CONCLUSION

In the current study, it was shown that the pH of biochar-blend (PL and RS biochar > 7) effectively removed 46.75% of TPH from the soil that had been polluted with crude oil. After a 30-day cleanup period, the pH level rose as a result of this. The addition of biochar-blend, mostly composed of PL and RS biochar, raised the soil pH from 4.72 to 6.9 therefore improving the degradation process or making the soil amenable for bioremediation. While several writers have reported on various aspects that affect the breakdown of hydrocarbons, the interactions of these contaminants with different properties of biochar are often what regulate the removal processes. The identification of the underlying mechanisms of the adsorption process is required for assessing the effectiveness of the removal of contaminants by biochar. The features and yield of biochar are primarily categorized by the characteristics of the feedstock and the production environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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