Modeling the Degradation of Total Petroleum Hydrocarbon in Soil using Mushroom

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Abstract

Experimental investigation was carried out to examine the performance of mushroom on the degradation of total petroleum hydrocarbon in soil in crude oil polluted soil in Niger Delta Area of Nigeria. The result obtained illustrates the effect of soil characteristics on the performance of mushroom and the effectiveness on total petroleum hydrocarbon degradation. The physiochemical parameters considered during the investigation include, Total Petroleum Hydrocarbon (TPH) moisture content, organic carbon, total Nitrate, electrical conductivity, nitrate content ($N0_3$), available phosphorus, soil pH, of the soil samples. The parameters obtained were used as indices for evaluating the levels of pollution and remediation. The experiment set up was monitored for a period of 90 days in a hatch bioreactor; it is seen that the TPH concentration decrease with time. A mathematical model of bioremediation of (TPHs) in soil has been developed to predict the remediation rate during the bioremediation of the contaminated soil. Three species of mushroom substrate were used as the remediating agent. The result obtained reviewed that mushroom are capable of degradation Total Petroleum Hydrocarbon in soil environment.

Keywords - *Degradation, petroleum hydrocarbon, soil, mushroom, modeling.*

I. INTRODUCTION

It is widely known that the exploration and exploitation of crude oil in Nigeria have been in existence since 1956. Following the discovering of crude oil, oil spillage resulting from industrial activities and pipeline vandalization have a concern in the Niger Delta area of Nigeria where the bulk of the oil is produced. The soil and groundwater of oil exploration and production zones are frequently contaminated especially in the Niger Delta area. Crude oil contains petroleum in hydrocarbons, which consist of three major groups of compounds. These are alkane (paraffin's), alkenes (olefins) and aromatics. Total Petroleum Hydrocarbon (TPH) is a term used to describe a large family of several hundreds of chemical compounds that originally come from crude oil (Gustatson, 2007). There are many different chemicals in crude oil and other petroleum products, therefore, it is not practical to measure each one separately. Nevertheless, it is useful to measure the amount of TPH at a site (ATSDR, 1999).

The production of crude oil and the use of chemicals in technologically advanced societies provoke the release of many toxic/hazardous substances in the soil, aquatic environment, but during bioremediation process (environmental friendly substances are released to the environment (Nano, Borroni, & Rota, 2003 & Maki Sasaki &Haramaya, 2005). Such materials could promote degradation at low concentration. In the past, the risk associated or connected to hazardous substances was evaluated using chemical analysis. Nevertheless, chemical analysis by itself can only identify and quantify pollutants when the analysis in question is known; moreover, synergic and antagonistic effects between contaminated substances are not always established (Jobson Mclauglin, Cook & Westlake, 1974 &Bartha, 1986, Alexander, 1994, Nance, 2002).

However, a more precise assay to ascertain the degradation of Total Petroleum Hydrocarbon in a reactor can be applied using a biological test based on the exposure of the contaminants into microbial attack (Atlas &Bartha, 1973). Such contact can display Redox reaction thereby increasing the microbial activity as well as increased the degradation rate of Total Petroleum Hydrocarbon in a bioreactor.

Biological remediation, a process defined as the use of micro-organism or plants to detoxity or remove organic and inorganic xenobiotic compounds from the environment is a remediation option that offers green technology solution to the problem of environmental degradation. This process relies microbial enzymatic activities to transform or degrade the contaminants in the environment (Philip et. al, 2005). Bioremediation techniques allow the evaluation of substrate degradation, as well as microbial growth rate. It employs mushroom which is like many bacterial osmotically sensitive growing rapidly to feed on substracte using carbon as energy source (Adams & Stauber, 2004).

Research conducted, which examine some parameters such as biomass, growth and several

effects caused by low concentration of pollutant reveal its importance on improving the bioremediation process. The aim of this study was to determine the biochemical properties of the mushroom substrates affecting the rate of degradation of crude oil contaminated soil.

II. MATERIALS AND METHOD

A. Sample Collection

Soil Sampling: The soil sample were collected from the agric farm Rivers State University of Science and Technology, Nkpolu, Port Harcourt. The soil samples were collected with hand trowel and transferred into plastic container (sample bottles). The samples were then transported to the department of soil science laboratory in Rivers State University of Science and Technology, Nkpolu, Port Harcourt, for further analysis to be conducted on the soil characteristics.

Crude oil collection: The crude oil for this experiment were obtained from Nigeria National Petroleum Company (NNPC) in Port Harcourt, Rivers State. The mushrooms used were bought from Dihomat Farm, Rivers State University of Science and Technology Nkpolu, Port Harcourt.

Experimental Design: The soil were divided into six treatment sample cells in six different container (bucket). The different sample were coded as WF-O to WF-5. Cell WF.O was the control volume, i.e. did not receive any treatment, whereas cells WF-1, WF-2, WF – 3, WF – 4, and WF – 5 were marked to receive 1000g, 900g, 800g, 750g, 700g of mushroom respectively during the remediation period.

Table 2.1:

CELLS	DESCRIPTION
WF-0	Addition of 5kg of soil, 240ml of crude oil.
WF-1	Addition of 5kg of soil, 240ml of crude oil, 1000g of mushroom substrate (saprophytic), 0.75 litre of H_20.
WF-2	Addition of 5kg of soil, 240mlof crude oil, 900g of mushroom substrate (saprophytic), 0.75 litre of H_20.
WF-3	Addition of 5kg of soil, 240mlof crude oil, 800g of mushroom substrate (parasitic), 0.75 litre of H_20.
WF-4	Addition of 5kg of soil, 240mlof crude oil, 750g of mushroom substrate (parasitic), 0.75 litre of H_20.
WF-5	Addition of 5kg of soil, 240mlof crude oil, soil 700g of mushroom substrate (symbiotic), 0.75 litre of H_20.

Microbial sampling: The soil were later transported in the department of micro-biology in Rivers State University of Science and Technology, Nkpolu, Port Harcourt for the purpose of isolation, identification and characterization of possible microorganisms, present in the soil.

B. Soil Analysis

Prior to analysis, soil samples were collected in batches. The soils were stirred properly and transferred into well labeled polyethylene bags, using a sterile knife. Care was taken to clean up the sampling knife with mentholated spirit before introducing into each soil. Each sample was collected in the triplicate and sent to laboratories. After sampling, the soil samples were air dried and crushed. The crushed soil samples were then passed through a 2mm sieve and collected into clean well-labeled polyethylene bags, the physicochemical parameters include;

C. Analysis of Total Petroleum Hydrocarbon

Three days after pollution, each set bottles for analysis of Total Petroleum Hydrocarbon (TPH) before addition of the mushroom and water. 10g of each sample was taken and put into sample bottles labeled WF-0 to WF-5. 80ml of chloroform was measured and added to each sample and the sample was tightly closed and thoroughly shaken for proper mixing of contents. The mixtures in the bottles were left to stand for 2 days to allow for complete extraction of the crude oil by the chloroform. On the 4^{th} day, each of the samples was decanted; the clear liquid was transferred to fresh sample bottles and the volume made up to 60ml using chloroform. The UV-VIS spectrophotometer was standardized using chloroform for the blank, with wavelength set at 290nm.

The absorbance of sample was measured immediately after completion of the last step and the digital readout of the instrument recorded.

Soil pH and Electrical Conductivity (EC)

The hydrogen ions concentrations of the soil samples were determined using the pH electrical conductivity meter (pH meter). To achieve this, 10g homogenized soil sample (pounded in a soil mortar and sieved through a 2mm sieve was weighed and put in a pH cup and the addition of 25ml of dionized water followed suit. This then resulted into soil: water concentration of 1:2. The mixture was stirred for 1 hour and reading was taken. The pH meter was already calibrated using a buffer solution of 4 and 7 (Clark 1992, Bates 1994, Black, 1995). The readings were then taken by inserting the probe of the pH/EC meter into solution (soil solution). The EC of the soil samples were measured in micro Siemens/cm (µs/cm). The probe of the electrode was washed after each reading for accurate results and to avoid cross-contamination.

D. Moisture Content (M.C)

This was determined using the oven drying method. In this method, 20g of wet soil (W_1) were put into an aluminum foil and placed in an oven to dry at 105°C. After 24 hours, the soil samples in the oven were removed and reweighed. The dry weight, therefore, become an index for determining the moisture content of the soil sample. The final weight (W_2) of each sample is recorded using an electronic weighing balance.

E. Total Organic Content (TOC)

To determine the TOC, 250mg of air-dried soil sample were taken in 250ml conical flasks, and 5ml of 1M potassium dichromate solution was added. Therefore, 10ml concentrated sulfuric acid was added gradually and the contents were allowed to incubate for 30 minutes at room temperature. Then 100ml of de-ionised water, 5 ml of concentrated phosphoric acid, 0.1g of dry sodium fluoride, and 0.5 ml of diphenylamine indictor were added sequentially. The contents of the flask were titrated against 0.5M ferrous ammonium sulfate. The end point was noticed as dull green through turbid blue to brilliant green. Distilled water blank was run simultaneously, and the TOC was calculated as described by Hooda and Kaur (1999).

TOC (mg/g soil) = 6.791/W (1-T_1/T_2) x 100

Where;

T_1 = Volume of titrant used against samples (ml)

T_2 = Volume of titrant used against distilled water blank (ml)

Total Nitrogen: Total nitrogen in the soil was determined by a process called the kjeldaw digestion and distillation method. 10g of soil containing about 10mg N was weighed accurately in a dry 500ml kjeldahl flask. 20m of distilled water was added and the flask was swirled for a few minutes and kept standing for about 30 minutes. 11g of K_2S0_4 with catalyst mixture and 30ml conc. H_2S0_4 were added through a pipette. The flask was heated with care at low heat on the digestion stand, until the water was removed. The heating process continued until the digest cleared. The mixture was then allowed to boil for 5 hours and then left to cool, while slowly adding about 100ml of water to the flask.

Digest was carefully transferred into an Erlenmeyer flask. Digest was careful transferred into an erlenmyer flask. 50ml H_3B0_3 indicator. Solution was added into a 500ml Erlenmeyer flask and placed under the condenser in the distillation column. The kjldah flask was cleaned and the content of the Erlenmeyer flask was transferred into it. About 150ml of 10m NaoH was poured into the flask and attached immediately to the column. The condenser was kept cool by adequate water supply. NH_4^+ in the distillate was titrated with standard HCL or H0504 colour change at the end point was from green to pink.

Available Phosphorus: Available phosphorus in soil was measured by the Bray and Kurtz method. 2.85g **of soil** was weighed into a tube and 20ml of an extracting solution of NH_4 was added. The tube was shaken properly for 1 minute and the content filtered through a filter paper. 10ml aliquot of the sample as pipette into a 50ml volumetric flask with the addition of 10ml distilled water. 4ml of a reagent prepared from dissolving 1.056 of ascorbic acid in 200ml of a mixture of 12g of ammonium molybdate (NH_4) M024, $4H_20$) in 250ml distilled water, was added and allowed for 15 minutes to enable colour development. The absorbance of the standard was measured on a spectrophotometer at 660mH. A graph of absorbance versus concentration was plotted on a graph paper. The phosphorus concentration was finally determined from the curve obtained.

3.5.1 Model Development

The degradation of non-conservative substance is usually modeled as a first-order reaction: it is assumed that the rate of loss of substance is proportional to the amount of substance that is present (Gilbert and Masters, 2006).

Considering a steady state system with non-conservative pollutant, many contaminants undergo biochemical reaction at a rate sufficient to treat them as a non-conservative substance. From Michael'smenten's equation

$$X + Y + Z \xrightarrow{k_m} P + Q + S$$

Where,

X = Soil under consideration
Y = TPH
Z = Mushroom substrate
K = Rate constant
P = Gases
Q = Heat and
S = New biomass (The mass of biological material contained in a system)

Applying the mass balance principle

Mass flux in+ Net rate of degradation = Mass Accumulation – mass flux out due to biochemical reaction (2.1)

Mass flux in

$$\left(mass \,/\, time\right) = Qin \left(volume \,/\, time\right) X \; Cin \left(mass \,/\, volume\right) \quad (2.2)$$

Where

Q = volumetric flow in;

Cin = pollutant concentration

Net rate of degradation due to biochemical reaction: Production of a pollutant by a biochemical reaction is usually described in terms of concentration. Thus, multiplying the chemical rate of change of concentration by the volume (v) gives:

$$\frac{Vdc}{dt} \qquad (2.3)$$

$$\text{Net rate of reaction} = \frac{Vdc}{dt} \qquad (2.4)$$

$$\text{New rate of reaction } \frac{Vdc}{dt} = -VKC \qquad (2.5)$$

Where K = degradation constant

Accumulation rate

Using concentration units of (mass/volume), the total pollutant mass in the soil is equal to concentration C, multiplied by volume v, of reactor (soil).

Thus, accumulation rate is

$$\frac{d(CV)}{dt} = \frac{Vdc}{dt} \qquad (2.6)$$

For a non-conservative substance like TPH and for batch process, non-steady state conditions results as concentration is bound to change.
This implies that mass accumulation rate is non zero.

Mass flux out

$$\text{Mass flux out } \left(mass/time\right) = C_{tank} \; X \; Q_{out} \qquad (2.7)$$

Where

C_{tank} = pollutant concentration in reactor $\left(mass/volume\right)$

Q_{out} flow out $\left(mass/time\right)$

Substituting all parameters into equation (1) gives

$$Q_{in} \; X \; C_{in} - VKC = \frac{Vdc}{dt} + C_{tank} \; X \; Q_{out} \qquad (2.8)$$

But since the operation is a batch process, we assume that

$Q_{in} = Q_{out} = 0$

Substituting we get

$$0 \text{ x } C_{in} - VKC = \frac{Vdc}{dt} + C_{tank} \text{ x } 0 \qquad (2.9)$$

$$= -VKC = \frac{Vdc}{dt} \qquad (2.10)$$

Dividing both side by V yields

$$\frac{dc}{dt} = -KC \qquad (2.11)$$

Separating the variables, we get

$$\frac{dc}{c} = -K\,dt \qquad (2.12)$$

Integrating both sides yields

$$\int_{co}^{c} \frac{dc}{c} = -K \int_{o}^{t} dt \qquad (2.13)$$

$$In\left(\frac{C_o}{C_o}\right) = -kt \qquad (2.14)$$

F. Exponentiation both sides yields

$$\frac{C}{C_O} = e^{-kt}$$

$$\therefore \; C_{(t)} = C_O \, e^{-kt} \qquad (2.15)$$

Where

C (t) = concentration of TPH at time, t

C_O = Initial concentration of TPH at t = 0

K = TPH degradation constant.

III. RESULTS

After ten weeks of remediation, all the soil samples showed comparable and considerable depletion in Total Petroleum Hydrocarbon levels. The initial values of Total Petroleum Hydrocarbon TPH for samples WF-0, WF-1, WF-2, WF-3, WF-4, and Wf-5 are almost equal, because each sample was contaminated with the same amount of crude oil each sample showed reduction in Total Petroleum Hydrocarbon TPH, including sample WF-0, to which no mushroom substrates was added. The particle size analyses of the soil before treatment showed that the soil texture is silty clay (see table 3.1).

The soil physiochemical characteristics such as TPH, PH, Total Nitrogen, Available phosphorus, moisture content, Electrical conductivity, organic carbon, SO_0 $N0_3$, throughout the period of remediation are presented in table 3.2 to 3.7. the soil P^H values ranged from 6.24 to 6.80, for the control soil, while the other samples ranged from 6.24 to 7.00 also the moisture content increased from 10% to 14% which makes the environmental condition favourable for bioremediation Rowell (1977). Table 3.10 shows that the degradation rate constant using 1000g of mushroom and the correlation coefficient between time in (weeks) and the residual concentration of Total Petroleum Hydrocarbon TPH indicates that, there is a strong relationship between the two variables. The residual concentration of Total Petroleum Hydrocarbon TPH after 10 weeks of remediation is shown in table 3.8 while the soil P^H after 10 weeks of remediation is shown in 3.9.

The control soil sample seems to be lower than the other three because no remediating agent was used in in the control sample, it was observed under a natural condition. From table 3.10, it shows that parasitic mushroom can degrade Total Petroleum Hydrocarbon TPH faster than the other species used. The P-values indicates that there is no significant difference between the experimental result and calculated values of the residual concentration of Total Petroleum Hydrocarbon TPH.

The calculated values of the residual concentration of Total Petroleum Hydrocarbon TPH where obtained from the mathematical model developed. MATLAB was used to compute the calculated values of the Total Petroleum Hydrocarbon TPH using an initial concentration of 68.75mg/kg and the rate constant (km) from the linear graphs of fig. 3.8 to 3.11. The ANOVA result on table 3.15 show that there is no significant difference in the residual concentration of Total Petroleum Hydrocarbon TPH between the experiment carried out and the mathematical model developed.

Although the control sample has a lower degradation rate because no mushroom was added. The P-values from the ANOVA result on table 3.15 reflects a strong indication that the model is valid and can be used to predict bioremediation of Total Petroleum Hydrocarbon TPH at any given time using mushroom, also, the rate of degradation was found to be 0.2558, 0.2512, 0.2634 and 0.1435 for saprophytic, symbiotic and parasitic and the control sample as shown in table 3.10.

The model equation was used to compute the residual TPH by using an initial concentration of 68.78mg/kg and the rate constants (km) from the linear graphs in figure 4.1, 4.2, 4.3 and 4.4. The mathematical model computation and one-way ANOVA with 95% confidence interval between calculated and experimental values was carried out using MATLAB.

The sharp increase in soil organic carbon result to crude oil contamination, this is in accordance with the evidence of Jobson et al., (1974) that oil spills result in significant increase in percent organic carbon because crude oil is a mixture of carbon and hydrogen. In the cause of remediation treatment, organic carbon dropped to near background conditions during the remediation treatment. One would have expected an increase in the Total Nitrogen (N) of various cells but on the contrary this decreased with decrease in the period of remediation. The reductions in Total Petroleum Hydrocarbon (TPH) with continuous utilization of mushroom imply that the nitrogenous nutrients supplied provided a favourable environment for Total Petroleum Hydrocarbon (TPH) degradation (Ogboghodo et al., 2001).

There was no significant percentage reduction of Total Petroleum Hydrocarbon (TPH) in all the cells after two (2) weeks of remediation. After the two (2) weeks of remediation the percentage reduction of Total Petroleum Hydrocarbon TPH was 8%, 10%, 11%, 10%, 10%, 11%. For cells WF-0, WF-1, WF-2, WF-3, WF-4 and WF-5 respectively.

There was a marked increase in percentage reduction of TPH in all the cells including the control, after four to ten weeks of remediation. The percentage of reduction after four weeks of remediation were 48%, 75%, 71%, 73%, 70% and 68% while for ten weeks of remediation were 68%, 95%, 94%, 92%, 94%, and 91% for cells WF-0, WF-1, WF-2, WF-3, WF-4 and WF-5 respectively (see Table 4.16). The results indicate that the mushroom applied increased the degradation of the TPH in the cells.

The tables below show the soil characteristics before and after contamination with TPH.

Table 3.1: Initial Assessment of Soil

Percentage (%)				pH 1 : 2.5	EC µ/cm	Percentage (%)		C/N Ratio
Sand	Silt	Clay	Moisture			Organic C	Total N	
13.7±0.5	41±0.2	4.5±0.5	14±1	4.65±0.1	29±2	0.18±0.02	0.62±0.3	0.4±0.01

Results represent the means ± standard deviation of three replicates

The soil parameters from the initial assessment indicate that the soil to remediate is acidic with a mean pH value of 4.65 and the moisture content was found to be 14% which is not suitable environmental condition for bioremediation (Greene et al., 2000).

Table 3.2: Physio-chemical characteristic of soil after 3 days of contamination

Sample Code	pH 1:2	TPH Mg/kg	Elect. Cond. µ/cm	Moist content % by mass	Org. Carb. %	NO_3	PO_4	SO_4	Total N %
WF-O	6.24	68.78	15	13±2	3.36	4.85	0.55	3.46	6.63
WF-1	6.34	65.60	20	14±1	3.38	4.46	0.50	4.00	6.40
WF-2	6.30	70.50	22	11±1	3.35	4.20	0.42	3.65	5.72
WF-3	6.33	68.80	19	12±2	3.28	3.88	0.47	3.80	6.38
WF-4	6.35	66.50	28	14±1	3.40	4.75	0.64	4.20	5.86
WF-5	6.29	67.64	17	10±2	3.36	4.60	0.54	3.50	6.00

Table 3.3Physio-chemical characteristic of soil after 2 weeks of remediation.

Sample Code	pH 1:2	TPH Mg/kg	Elect. Cond. µ/cm	Moist content % by mass	Org. Carb. %	NO_3	PO_4	SO_4	Total N %
WF-O	6.32	60.50	020	14±1	3.58	5.00	0.68	4.00	6.70
WF-1	6.79	58.80	883	12±2	3.40	4.86	0.55	4.55	6.80
WF-2	6.72	57.74	960	13±1	3.45	4.50	0.48	4.21	5.98
WF-3	6.54	58.85	980	11±1	3.38	4.20	0.52	4.35	6.50
WF-4	6.51	58.70	650	13±1	3.50	5.08	0.68	4.78	5.94
WF-5	6.57	57.50	220	12±1	3.46	5.00	0.67	4.30	6.40

Table 3.4: Physio-chemical characteristic of soil after 4 weeks of remediation.

Sample Code	pH 1:2	TPH Mg/kg	Elect. Cond. µ/cm	Moist content % by mass	Org. Carb. %	NO_3	PO_4	SO_4	Total N %
WF-O	6.78	22.70	94.80	15±1	5.65	3.65	0.56	3.68	7.56
WF-1	6.85	16.75	99.60	14±1	4.57	4.20	0.37	3.76	4.76
WF-2	6.75	17.88	99.70	16±1	4.38	4.65	0.40	3.40	4.96
WF-3	6.85	17.76	98.50	14±1	4.73	4.58	0.46	3.36	4.85
WF-4	6.90	18.28	98.60	13±1	5.00	4.88	0.42	3.56	4.36
WF-5	6.87	19.60	110.50	16±1	4.80	5.00	0.38	3.8	3.87

Table 3.5: Physio-chemical characteristic of soil after 6 weeks of remediation.

Sample Code	pH 1:2	TPH Mg/kg	Elect. Cond. µ/cm	Moist content % by mass	Org. Carb. %	NO_3	PO_4	SO_4	Total N %
WF-O	6.80	20.80	95.76	14±1	6.86	3.80	0.48	4.00	5.48
WF-1	6.76	10.54	75.60	15±2	3.87	3.60	0.30	3.54	4.00
WF-2	6.80	11.67	78.50	16±1	4.00	3.78	0.38	3.20	3.76
WF-3	6.84	11.58	104.50	14±1	4.30	3.85	0.40	3.15	3.50
WF-4	6.85	10.65	80.00	13±1	4.50	3.90	0.44	3.28	3.80
WF-5	6.78	10.48	76.00	16±2	4.00	4.60	0.36	3.30	4.00

3.6: Physio-chemical characteristic of soil after 8 weeks of remediation.

Sample Code	pH 1:2	TPH Mg/kg	Elect. Cond. µ/cm	Moist content % by mass	Org. Carb. %	NO_3	PO_4	SO_4	Total N %
WF-O	6.80	19.54	74.00	14±1	4.30	4.87	0.34	3.40	3.78
WF-1	7.00	8.00	70.50	13±1	3.32	3.30	0.35	3.20	3.60
WF-2	6.98	9.30	68.50	12±1	3.56	3.43	0.34	3.00	3.40
WF-3	6.95	8.50	75.00	10±2	3.85	3.56	0.38	3.00	3.20
WF-4	6.88	8.30	74.50	15±1	3.60	3.55	0.40	3.10	3.45
WF-5	7.00	9.20	65.00	14±1	3.75	4.30	0.35	3.00	3.40

Table 3.7: Physio-chemical characteristic of soil after 10 weeks of remediation.

Sample Code	pH 1:2	TPH Mg/kg	Elect. Cond. µ/cm	Moist content % by mass	Org. Carb. %	NO_3	PO_4	SO_4	Total N %
WF-O	**6.26**	**18.50**	**71.00**	**13±1**	**4.20**	**4.78**	**0.31**	**3.40**	**3.67**
WF-1	**6.96**	**6.00**	**69.50**	**14±1**	**3.22**	**3.20**	**0.36**	**3.20**	**3.50**
WF-2	**6.78**	**6.30**	**68.30**	**12±2**	**3.46**	**3.30**	**0.33**	**3.00**	**3.40**
WF-3	**6.88**	**7.10**	**70.00**	**14±1**	**3.65**	**3.46**	**0.31**	**3.10**	**3.20**
WF-4	**6.98**	**6.20**	**66.50**	**13±1**	**3.32**	**3.35**	**0.35**	**3.20**	**3.35**
WF-5	**7.00**	**8.00**	**64.50**	**14±1**	**3.36**	**3.80**	**0.32**	**3.00**	**3.30**

Physiochemical characteristics of soil after 10 weeks of remediation using 1000g of mushroom substrate and a control sample.

Table 3.8: Residual concentration of TPH after 10 weeks of remediation

Time (weeks)	Concentration of TPH (mg/kg)			
	Saprophytic	*Parasitic*	*Symbiotic*	*Control*
0	68.78	68.78	68.78	68.78
2	58.74	58.70	57.50	60.50
4	17.88	18.28	19.60	22.70
6	11.67	10.65	10.48	20.80
8	9.30	8.30	9.20	19.54
10	6.30	6.20	8.00	18.50

Table 3.9: Soil pH after 10 weeks of remediation

Time (weeks)	pH (1:2)			
	Saprophytic	*Parasitic*	*Symbiotic*	*Control*
0	6.30	6.35	6.29	6.24
2	6.12	6.51	6.57	6.32
4	6.75	6.90	6.87	6.78
6	6.80	6.85	6.78	6.80

8	6.98	6.88	7.00	6.80
10	6.78	6.98	7.00	6.26

The pH values on table 4.9 above shows that there is no significant change or variation of pH in the different samples. Therefore, the pH tends toward neutral which indicates a favourable condition for biodegradation.

A. Determination of rate of reaction k_m

The rate constants where calculated by linearizing the model equation and plotting ln [TPH] against time for saprophytic, parasitic, symbiotic and the control sample respectively.
Linearizing Eq. 2.15 gives

$$\text{In}\left[C_{(t)}\right] = In\left[C_o e^{-k t}\right]$$

$$\text{In}\ C_{(t)} = In\ C_o + (-kt)$$

In

$$C_{(t)} = -Kt + In\ C_o \quad (3.1)$$

Now, comparing Eq. 3.1 with the general linear equation
y= mx + c

Where,

y = In C

m = gradient of the graph = K

x = t (time) and

C = Intercept of the graph= In C_0

The graphs below shows that there is a strong correlation between time and concentration and also, concentration reduces as time increases which is an indication of degradation with respect to time. The negative sign in the linearized equation depicts degradation, loss of TPH with respect to time.

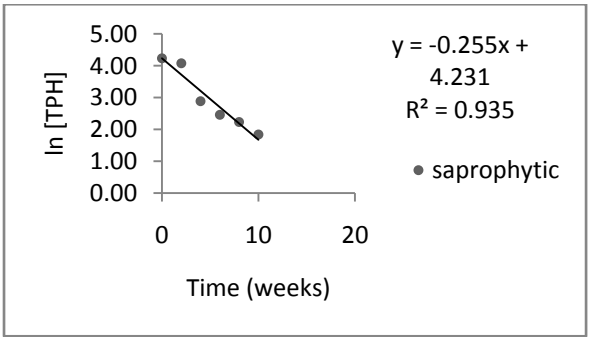


Figure 3.1: The graph ln [TPH] vs Time for saprophytic mushroom

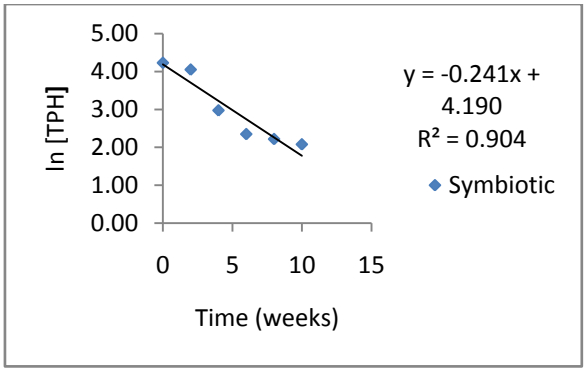


Figure 3.2: The graph of ln [TPH] vs Time for symbiotic mushroom

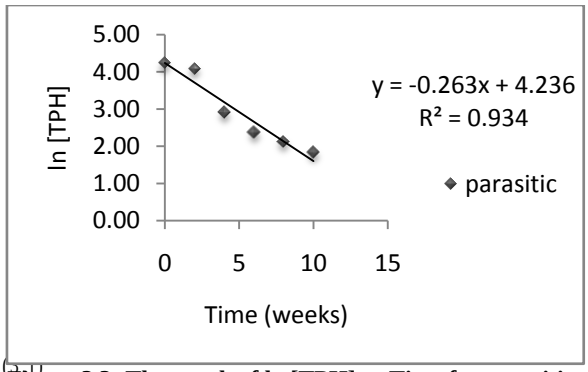


Figure 3.3: The graph of ln [TPH] vs Time for parasitic mushroom

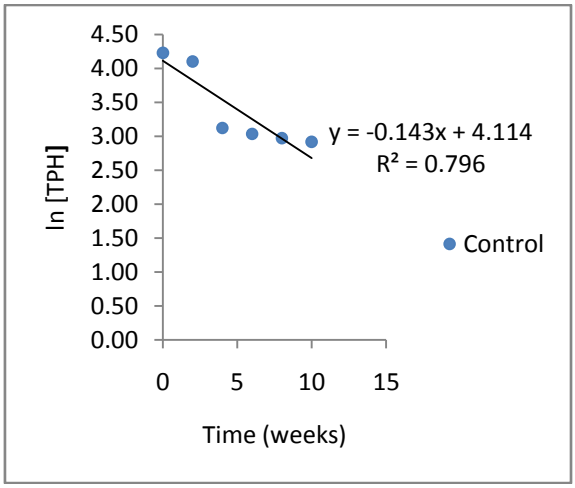


Figure 3.4: The graph of ln [TPH] vs Time for the control sample

Table 3.10: Rate constant, k_m and correlation coefficient between Time and TPH concentration

Mushroom substrate (1000g)	Rate constant, k_m (day^{-1})	R^2
Saprophytic	0.2558	0.9352
Parasitic	0.2634	0.9342
Symbiotic	0.2512	0.9045
Control	0.1435	0.7963

The table, 4.10 above shows the degradation rate constants using 1000g of mushroom substrate and the correlation coefficient between Time in (weeks) and the residual concentration of TPH indicates that there is a strong relationship between the two variables. The control soil sample seems to be lower than the other three because no remediating agent was used in the control sample, it was observed under a natural condition. From 4.10 it shows that parasitic mushroom can degrade TPH faster than the other species used.

B. Analysis of Results

The model equation was used to compute the residual TPH by using an initial concentration of 68.78 mg/L and the rate constants (k_m) from the linear graphs in figure 4.1, 4.2, 4.3 and 4.4. The mathematical model computation and One- way ANOVA with 95% confidence interval between calculated and experimental values was carried out using MATLAB.

ANOVA Table 3.11:Calculated and experimental values using saprophytic mushroom

Source	SS	dfMS	F	Prob>F	
Columns		6.594	1	6.594	0.00985
210.9229					
Error	6692.98	10	669.298		
Total	6699.574		11		

p =0.9229

ANOVA Table 312: Calculated and experimental values using symbiotic mushroom

Source	SS	dfMS	F	Prob>F	
Columns		5.2622	1	5.2622	0.0080551
0.93026					
Error	6532.8211	10	653.282		
Total	6538.0833		11		

p =0.9303

ANOVA Table 3.13: Calculated and experimental values using Parasitic mushroom

Source	SS	df	MS	F	Prob>F
Columns		8.4548	1	8.4548	0.01243
0.91343					
Error	6801.6534	10	680.1653		
Total	6810.1082	11			

p =0.9134

ANOVA Table 3.14: Calculated and experimental values using Parasitic mushroom

Source	SS	df	MS	F	Prob>F
Columns	20.273	1	20.273	0.044097	
0.83789					
Error	4597.3655	10	459.7366		
Total	4617.6385	11			

p =0.8379

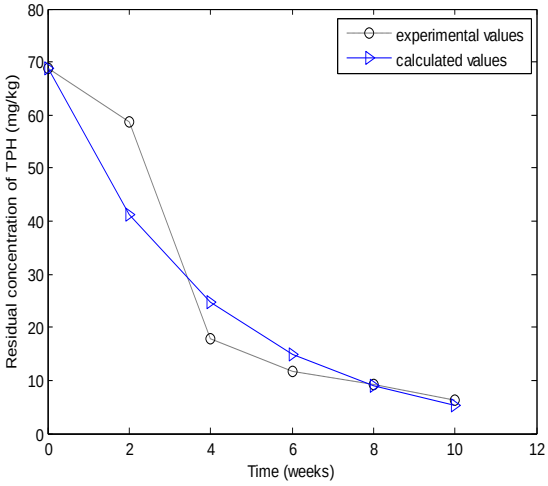


Figure 3.4: A graph of experimental and calculated values of TPH for saprophytic mushroom

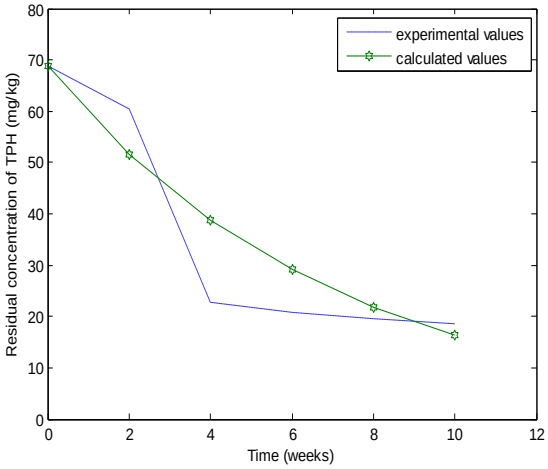


Figure 3.5:A graph of experimental and calculated values of TPH for control sample

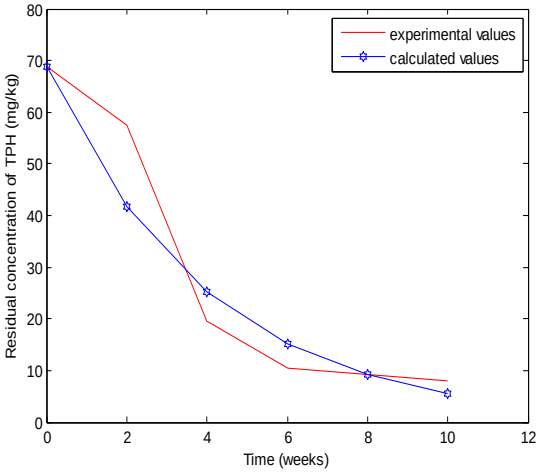


Figure 3.7: A graph of experimental and calculated values of TPH for symbiotic Mushroom

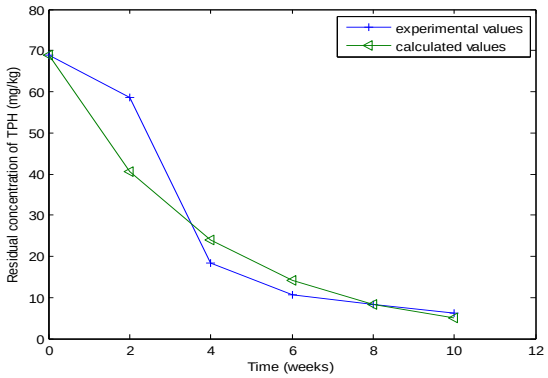


Figure 3.6: A graph of experimental and calculated values of TPH for parasitic mushroom

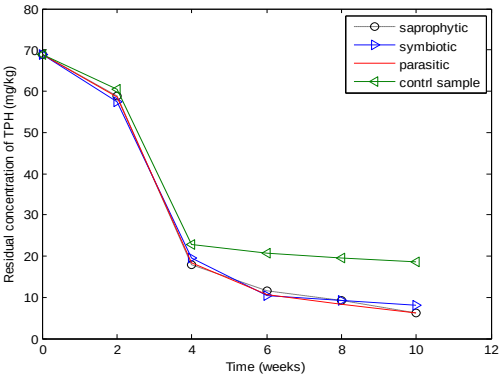


Figure 3.8: Experimental values of TPH showing the effects of the different samples.

The figure above shows the effects of the remediating samples used; from Fig. 4.9 the remediation in the control sample is significantly different from the other three species between week 4 and week 10 and between week 2 and week 4 the degradation is very high for all the samples, this a strong indication that the mushroom species where very active within that period.

Table 3.15: Analysis of variance between experimental and calculated values of TPH

Mushroom substrate	P-value
Saprophytic	0.9229
Symbiotic	0.9303
Parasitic	0.9134
Control	0.8379

The table 4.8 above shows the probability values of the various between experimental and calculated values for the various mushroom species used for the remediation and the control sample (which was remediated under natural condition with any remediating agent). The P-values indicates that there is no significant difference between the experimental result and calculated values of the residual concentration of TPH.

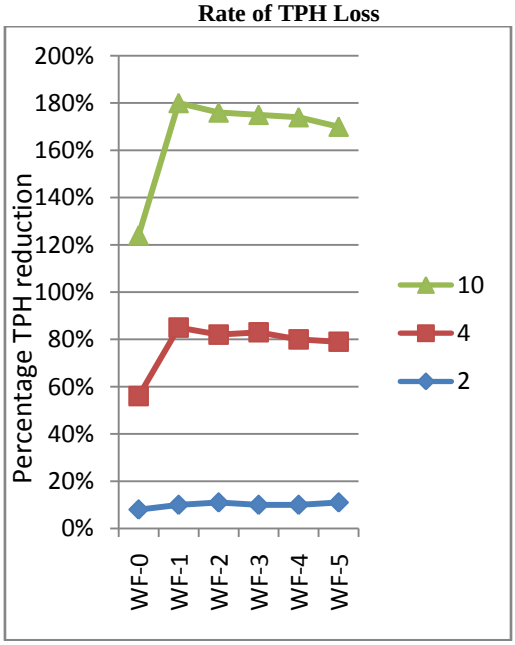
The calculated values of the residual concentration of TPH where obtained from the mathematical model developed. MATLAB was used to compute the calculated values of the TPH using an initial concentration of 68.78mg/kg and the rate constant (k_m) from the linear graphs on fig. 4.1-4.4 The ANOVA result on Table 4.15 Show that there is no significant difference in the residual concentration of TPH between the experiment carried out and the mathematical model developed. Although the control sample has a lower degradation rate because no mushroom was added.

The P-values from the ANOVA result on Table 4.15 reflects a strong indication that the model is valid and can be used to predict Bioremediation of TPH at any given time using mushroom substrate. Also, the rate of degradation was found to be 0.2558, 0.2512, 0.2634 and 0.1435 for saprophytic, symbiotic and parasitic and the control sample as shown in table 4.10. It shows that parasitic mushroom can degrade TPH faster than the other species. The correlation coefficients on table 4.10 show that there is a strong relationship between time and concentration and the degradation of TPH is dependent on time because the concentration TPH reduces as time increases.

Table 3.16: Percentage TPH Reduction

Cell	Sampling period week		
	2	4	10
WF -0	8%	48%	68%
WF -1	10%	75%	95%
WF -2	11%	71%	94%
WF -3	10%	73%	92%
WF -4	10%	70%	94%
WF -5	11%	68%	91%

Rate of TPH Loss



IV. CONCLUSION

Crude Oil has been a very important source of economic growth to the Nigerian economy due to its importance in energy and industrial utilization. These realizations have become more pronounced in the last decade, leading to more extensive exploration and exploitation for more oil reserves. This in turn has led to extensive pollution of the environment. Bioremediation which exploits the biodegradative abilities of micro-organisms has been endorsed as the preferred alternative in the long term restoration of areas polluted with petroleum hydrocarbons due to its cost efficiency and environmental friendliness.

In conclusion, this study which was aimed at accessing the effect of crude oil on soil physicochemical and microbiological properties and to evaluate the biodegradability of Total Petroleum Hydrocarbons by mushroom species in the laboratory, and to analyze the rates of substrate utilization by these micro-organisms, has its focus o the Niger Delta environment which has suffered immensely from the adverse effects of crude oil.

This study on microbial bioremediation is recommended for industries whose activities lead to the pollution of the environment by crude oil, as it is a long-term remediation scheme which is environmentally responsive.

The study is also recommended as a tool for accomplishing good and effective bioremediation project i.e it helps in good engineering design, good field implementation, and good engineering project management. Obtaining the rate of substrate uptake with time is useful as it provides information on the expected time of completion of the bioremediation scheme.

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