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Microbial Degradation and its Kinetics on Crude Oil Polluted Soil

Akpoveta O. Vincent[1], Egharevba Felix[2], Medjor O. Weltime[3], Osaro K. Ize-iyamu[4] and Enyemike E. Daniel[5]

[1,2,3,4]Department of Chemistry, Ambrose Ali University, P.M.B 14, Ekpoma, Edo state, NIGERIA
[5]Chemistry department, Northumbria University, Newcastle Upon Tyne, U.K

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Abstract

A pilot study was conducted on soil simulated with crude oil to examine the effects of the hydrocarbon on soil properties, the potentials of exploring soil indigenous microbes and determining suitable conditions for effective degradation of the contaminant as well as evaluating the kinetics of the process. Soil collected from Agbor area of the Niger Delta in southern Nigeria was artificially spiked with 10% brent crude and studied. Control soil, simulated soil and treated soil were all characterised for pH, electrical conductivity, total organic carbon and matter, total nitrogen and phosphorus, texture and heavy metals(Cd, Pb, Ni, V and Cr) using standard analytical methods to determine the effect of crude oil pollution on these properties. Total petroleum hydrocarbon (TPH) was determined by measuring the amount of parent contaminant left in the soil at intervals in order to establish the efficiency and kinetics if the bioremediation process. Crude oil utilizing bacteria and fungi were also determined using standard microbiological procedures. Crude oil pollution caused a reduction in pH, conductivity and phosphorus level with significant effect in the growth rate of soil heterotrophic microbes, but however did not show any negative effect on the other properties. Crude oil did not affect the levels of the metals in the soil since the simulated soil showed lower metal concentration than the control soil, except for the remediation process which caused an increase in the concentration of Ni and V due to contributions of these metals from the animal waste used. The rate of microbial degradation was found to be dependent on availability of nutrient source and pH, as high biodegradation rate occasioned by an increase in microbial population was favoured between pH 6.7-9.6. Suitable pH condition and nutrient availability will enhance speedy microbial transformation of contaminant. A remediation efficiency of 81.69% was obtained on the sixth week indicating the efficiency and effectiveness of the process. The biodegradation process followed first order with a rate constant of 0.035day^{-1}. Biodegradation isotherm was found to be minus unity expressing the opposite linear relationship between the concentration of the contaminant in the soil (C_s) and the concentration degraded by the microbes (C_d) at different time intervals for the remediation period.

Keywords: Biodegradation, crude oil, kinetics, total petroleum hydrocarbon, simulation.

Introduction

Incidence of environmental pollution due to high rate of petroleum related activities in the Niger Delta area of southern Nigeria and other oil producing areas of the world has been associated with frequent oil spills, especially through oil wells blow outs, tanker accidents, bunkering, rupture of pipelines and sabotage. Disasters arising from such incidence results in the discharge of crude oil into the environment affecting both soil, air and water bodies. This threatens human health and that of the organisms that are dependent on the soil[1]. Accidental release of hydrocarbons into the environment and its attendant detriments is not restricted to oil producing regions alone, but other areas which are also prone to the increasing risks and possibility of spills due to tanker accidents and leakage from ruptured pipelines networked across such areas.

Pollution of the soil with petroleum derivatives is often observed in municipal soils around industrial plants and in areas where petroleum and natural gas are obtained[2,3]. In recent times, the decontamination and clean up of hydrocarbon polluted sites has increasingly received attention and interest. Crude oil is a known source of energy and income in the world, but its introduction into the environment poses a lot of pollution problems as it distorts the soil's originality, thus leading to loss of agricultural land. Considering the large quantity of oil going into the Niger Delta environment, especially farmlands, and the fact that the inhabitants of these areas are subsistent farmers, and also the seemingly inevitable consequences of oil spill, the need to clean up crude oil contaminated sites has become a key environmental issue. Due to the abilities of certain microbes to mineralize hydrocarbon components into environmentally friendly species such as carbon dioxide and water, the potentiality of these microbes in breaking down hydrocarbons has gained growing attention in modern day research. It is not just enough to rely on soil microbes for microbial transformation of hydrocarbons without understanding the conditions that will be suitable for effective and optimum biodegradation. This therefore brings out the need for this study to identify the hydrocarbon utilizing microbes and determine the conditions that will enhance speedy degradation of the hydrocarbon since mere microbial degradation takes a very long time to achieve substantial contaminant depletion. The study will also be aimed at evaluating the effects of crude oil pollution on soil properties and determining the kinetics of the biodegradation process. Kinetic study of such type will help in calculating the time required for the contaminant to

be reduced to a particular level and in the possible design of biodegradation kinetic models.

Material and Methods

Samples Collection: Soil samples were collected with a soil auger at surface depth (0-15cm) from a virgin fallow land in the forest area of Agbor, Delta State in southern Nigeria, having no pollution history and devoid of hydrocarbon contamination. Crude oil with specific gravity of 0.818g/cm^3 was obtained from shell petroleum development company (SPDC) flow station in Kokori, Ethiope East local Government Area of Delta state. Cow dung was collected from a cow farm situated along Lagos-Asaba road in Agbor, Delta State. While pig and poultry droppings were respectively collected from the piggery and poultry house in the Agric unit of the College of Education Agbor, Delta State.

Sample Preparation, Simulation and Amendment: Soil was air dried for a period of one week in a clean well-ventilated laboratory, homogenised by grinding, and filtered by passing through a 2mm mesh sieve. 1kg of soil was each measured into two clean dry plastic containers and moistened to 20% water holding capacity with distilled water to ensure proper mixing with the contaminant. Simulation of the soil samples was done by measuring 100g of crude oil corresponding to 122.25ml from gravimetric measurement into the two containers containing 1kg of soil each. The individual mixtures were thoroughly mixed to achieve a 10% artificial contamination. 10% spiking was adopted to achieve severe contamination because beyond 3% concentration, oil has been reported to be increasingly deleterious to soil biota and crop growth[4]. The manure samples were sun dried for one week after which they were grinded, thoroughly mixed, sieved through a 2mm sieve to achieve uniform particle size and stored in neat polythene bag for use. 1kg of the mixed manure was added to one of the containers containing 1kg of crude oil simulated soil in a 1:1 ratio and thoroughly mixed to obtain homogeneity. The second container containing 1kg of crude oil simulated soil served as the control.

Microbiological Analysis of Fungi and Bacteria Utilizing Microbes: The indigenous soil microbes with hydrocarbon utilizing abilities were isolated, identified and their microbial population determined before and within intervals of the treatment process using selective enrichment techniques and standard bacteriological methods Bergey and Breed, Anon.[5,6] so as to monitor the progress of the bioremediation process. In order to isolate and enumerate both heterotrophic and hydrocarbon utilizing bacteria, bacteria enrichment process using modified mineral salt medium of Mills et al[7] was carried out. Gram staining reaction method of Stewart and Beswick[8] was adopted for the characterization of isolates**.** Citrate utilization test, oxidase test, lndole production test and Urease hydrolysis test were all performed using methods as described by Cruickshank *et. al* [9]. Isolation and enumeration of fungal isolates were carried out with Sabraund dextrose agar using the spread plate technique as described by Alpha[10]. Fungal isolates were identified using the method of Harrigan and McCane [11].

Soil Characterisation/Physicochemical Analysis: Soil physicochemical characteristics such as soil texture, pH, total organic carbon, total organic matter, Carbon/Nitrogen ratio, total nitrogen, total phosphorus, soil conductivity and heavy metals (V, Pb Ni, Cd, Cr) were determined before contamination, one week after contamination and one week after the bioremediation process. Soil pH was determined electrometrically following the procedure outlined by Mylavarapus and Kennelley[12]. Particle size analysis was done using bouyoucos hydrometer method Bouyoucos[13]. Total organic carbon and matter were determined by the wet dichromate acid oxidation method Nelson and Sommers[14]. Total Nitrogen was determined using the method of Radojevic and Bashkin[15]. Total Phosphorus was determined by Bray and Kurtz method Bray and Kurtz method[16]. Electrical conductivity was carried out as described by Chopra and Kanzer[17]. Heavy metals were determined by digesting the samples with concentrated mixtures of hydrofluoric, nitric and perchloric acid AOAC[18] so as to convert all the metals present in the sample into such a form that they can be analyzed by the atomic absorption spectrophotometer.

Determination of Total petroleum Hydrocarbon (TPH): 1g of the soil sample was dissolved in 10ml of hexane and shaken for ten minutes using a mechanical shaker. The solution was filtered using a whatman filter paper and the filtrate diluted by taking 1ml of the extract into 50ml of hexane. The absorbance of this solution was read at 460nm with HACH DR/2010 Spectrophotometer using n-hexane as blank. Total petroleum hydrocarbon was determined at weekly intervals for six weeks.

Quality Control: Procedural blanks and standard solutions were prepared and included to ensure analytical quality control so as to assure the accuracy and reproducibility of the results. Replicate analyses were carried out on the determination of TPH to yield a mean which will be used to determine trueness and also standard deviation of the mean to measure precision Stanton[19], Valcarcel[20].

Results and Discussion

Crude oil pollution on the soil caused a reduction in pH, conductivity and phosphorus content from 5.1 to 4.5, 191.7µS/cm to 174.0µS/cm and 6.1mg/kg to 4.3mg/kg respectively. The observed reduction in pH and conductivity was similar to the findings of Osuji and Nwoye[21]. A reduction in pH implies increased acidity which is a problem for agricultural soils because many metal cations are more soluble and available in the soil solution at very low pH including Cd, Cu, Hg, Ni, Pb, and Zn McBride[22]. The resulting increased acidity could be due to the fact that hydrocarbons contain many free cations causing them to have properties of a weak acid. Reduced conductivity could be due to the non polar nature of the crude oil bringing about reduced ionic movement in the soil. The reduction in phosphorus level could be due to

possible oxidation of free phosphorus in the soil to phosphates because hydrocarbons can act as electron acceptors or oxidizing agents due to the presence of oxygen in them thereby producing a reducing environment. Significant increase in pH(4.5 to 6.9) and conductivity(174.0µS/cm to 250.30µS/cm) were observed after the bioremediation process except for total phosphorus(4.3mg/kg to 4.4mg/kg) which showed no change. The observed increase in pH and conductivity was due to the bioremediation process which removed the contaminant and introduced some salts and ions from the animal manure since they are known to contain them. The rise in pH from acidity (4.5) to alkalinity (10.3) during the bioremediation process was due to the animal waste used because of its high content of cations like calcium, magnesium, phosphorus, potassium and nitrogenous nutrients. Soil properties such as total nitrogen(0.15 to 0.22 to 0.30mg/kg), organic carbon (2.34 to 5.93 to 6.75%) and organic matter(4.03 to 10.22 to 11.64%) increased on addition of the hydrocarbon to the soil and subsequently increased after the bioremediation process as seen in table 1. The observed increase on introduction of crude oil could be due to the fact that crude oil contains varying proportions of nitrogenous substances and is highly carbonaceous. An increase in such properties is deemed desirable since they are important soil parameters that are critically important in maintaining soil fertility. The increase observed after the bioremediation process may not be unconnected with the waste supplement used since it was found to contain higher amount of nitrogen, organic carbon and matter when compared to the soil as seen in table 1. Changes in C/N ratios of the simulated and treated soils followed the trend in total carbon and nitrogen changes due to increased carbon and nitrogen on hydrocarbon application and bioremediation process. Crude oil did not negatively affect these soil properties as seen from the results. Particle size analysis shows that the sand (83.10-83.31%), silt(1.22-1.44%) and clay(15.46-15.47%) fractions were all in the same range for the control, contaminated and bio-remediated soils. A classification of the soil based on the USDA textural class[23] shows that the soil is loamy sand (coarse textured soil) and its classification according to the soil taxonomy classes shows that it is typic paleudit. This shows that there was no effect on the soil texture.

Table-1
Results of nutrient analysis; soil physicochemical properties and heavy metals before, one week after simulation and after remediation

Parameters	Animal waste	Soil	Soil+Crude oil	Remediated soil
pH	7.9	5.1	4.5	6.9
Conductivity(µs/cm)	-	191.7	174.0	250.3
Nitrogen(mg/kg)	0.51	0.15	0.22	0.30
Phosphorus(mg/kg)	0.111	6.1	4.3	4.403
Organic Carbon(%)	7.53	2.34	5.93	6.75
Organic Matter(%)	12.98	4.03	10.22	11.64
C/N Ratio	14.76:1	15.6:1	32.94:1	22.5:1
Total petroleum Hydrocarbon (mg/kg)	-	8.64	1587.5	317.05
Lead(mg/kg)	0.382	<0.001	<0.001	<0.001
Cadmium(mg/kg)	<0.001	0.025	0.015	0.021
Nickel(mg/kg)	0.446	0.419	0.284	0.489
Vanadium(mg/kg)	0.812	0.792	0.537	0.978
Chromium(mg/kg)	<0.001	<0.001	<0.001	0.012
% Sand	-	83.31	83.11	83.10
% Silt	-	1.22	1.43	1.44
% Clay	-	15.47	15.46	15.46

Table-2
Rate of change of total petroleum hydrocarbon(TPH) with time for the bioremediation of crude oil simulated soil

Time (days)	TPH (range)	Mean	ln(C) TPH	Mean + SD	% decrease
0	1580-1595	1587.5	7.370	1587.5±7.5	0.00
7	691.86-717.39	704.63	6.558	704.63±12.77	55.61
14	665.06-671.44	668.25	6.505	668.25±3.19	57.91
21	521.20-528.40	524.80	6.263	524.80±3.6	66.94
28	450.80-455.60	453.20	6.116	453.20±2.4	71.45
35	340.60-342.75	341.68	5.834	341.68±1.1	78.48
42	290.50-290.90	290.70	5.672	290.7±0.2	81.69

The concentrations of cadmium, nickel and vanadium in the control soil were found to be 0.025mg/kg 0.419mg/kg and 0.792mg/kg respectively, but gave lower concentrations of 0.015mg/kg, 0.284mg/kg and 0.537mg/kg in the crude oil simulated soil, suggesting no hydrocarbon influence on the metals. Less availability of the metals in the contaminated soil accounts for the observed decreased concentration. Higher concentrations of 0.489mg/kg and 0.987mg/kg for Ni and V respectively; were obtained for the bio-remediated soil. The high concentration may likely be due to possible contributions from the animal waste since the waste was found to have a Ni and V concentration of 0.446mg/kg and 0.812mg/kg respectively. The increased concentration could also be due to the metals presenting themselves in forms that are highly available since it was asserted by Abeh et al.[24] that metal concentrations could be in forms that can easily be made available under favourable conditions. Cadmium concentration in the bio-remediated soil (0.021mg/kg) was in the same range with that reported for the crude contaminated soil suggesting no possible contribution from the waste since the waste had a concentration of <0.001mg/kg. Lead and Chromium had a concentration of <0.001mg/kg in the control, contaminated and bio-remediated soils except for chromium with a concentration of 0.012mg/kg in the bio-remediated soil indicating very low availability of the metals.

Microbiological analysis gave a total of twelve heterotrophic bacteria of which eight are hydrocarbon utilizers. The heterotrophic bacteria are Alcaligen spp, Bacillus spp, Micrococcus spp, Chromobacterium spp, Corynebacterium spp, Serratia spp, Pseudomonas spp, Cellulomonas spp, Proteus spp, Flavobacterium spp, Norcardia spp, and Alcaligen spp. The eight hydrocarbon degrading bacteria are Alcaligen spp, Bacillus spp, Chromobacterium spp, Corynbacterium spp, Pseusomonas spp, Aeromonas spp, Serratia spp and Flavobacterium spp. Five hydrocarbon degrading fungi were also isolated and identified, they are: tricodema spp, penicillinium spp, *Rhizopus spp,* fusarium spp and Aspergillus. Total heterotrophic micro-organisms for the unpolluted soil was found to be $820X10^5$cfu/g, but decreased to $590X10^5$cfu/g on addition of crude oil to the soil as seen in table 3. A further decrease from $590X10^5$cfu/g to $1.3X10^5$cfu/g was recorded within the six weeks (forty two days) period of remediation indicating the inability of the microbes to survive in the presence of high crude oil concentration. Hydrocarbon degrading bacteria increased within the first three weeks from $1.8X10^4$cfu/g to $5.6X10^4$cfu/g and then progressively decreased to $1.2X10^4$cfu/g within the fourth, fifth and sixth week (twenty eight, thirty fifth and fourty second day). Similarly, hydrocarbon degrading fungi increased within the first seven days(one week) from $2.16X10^4$cfu/g to $11.1X10^4$cfu/g and decreased progressively to $1.5X10^4$ within the next four weeks(fourteenth, twenty first, twenty eight, and thirty fifth day) but showed a slight increase to $2.4X10^4$cfu/g on the last week(forty second day). Bio-stimulation resulted in the significant increase in population of the hydrocarbon degrading bacteria and fungi in the first three and one week respectively because the wastes provided nutrients for increased cell growth and enhanced biodegradation rate. This explains why biodegradation was fastest within the first three weeks (twenty one days) giving a 66.94 percentage decrease in total petroleum hydrocarbon. The crude oil contaminated soil also supported rapid bacteria and fungi growth because the crude oil served as carbon and energy source for them.

Table-3

Bacterial, Fungal and Total Viable Count for the bioremediation of crude oil simulated soil amended with animal wastes

Time (days)	TVC UP(CFU/g)	TVC_{CR}	$THUB_{CR}$	TF_{CR}	pH
0	$820X10^5$	$590X10^5$	$1.8X10^4$	$2.16X10^4$	4.5
7	-	$110X10^5$	$2.2X10^4$	$11.1X10^4$	8.8
14	-	$2.48X10^5$	$1.34X0^4$	$5.0X10^4$	8.46
21	-	$16.6X10^5$	$5.6X10^4$	$4.4X10^4$	9.6
28	-	$1.04X10^5$	$4.0X10^4$	$2.0X10^4$	10.1
35	-	$2.3X10^5$	$2.7X10^4$	$1.5X10^4$	10.3
42	-	$1.3X10^5$	$1.2X10^4$	$2.4X10^4$	9.3

UP: Unpolluted soil sample + Amendment (Animal waste) only
CFU/g: Colony formation unit per gram
TVC_{CR}: Total viable count for soil sample polluted with crude oil + amendment (Animal waste)
$THUB_{CR}$: Total hydrocarbon utilizing bacteria for Soil sample polluted with crude oil + Animal waste
TF_{CR}: Total fungal count for soil sample polluted with crude oil + amendment (Animal waste)

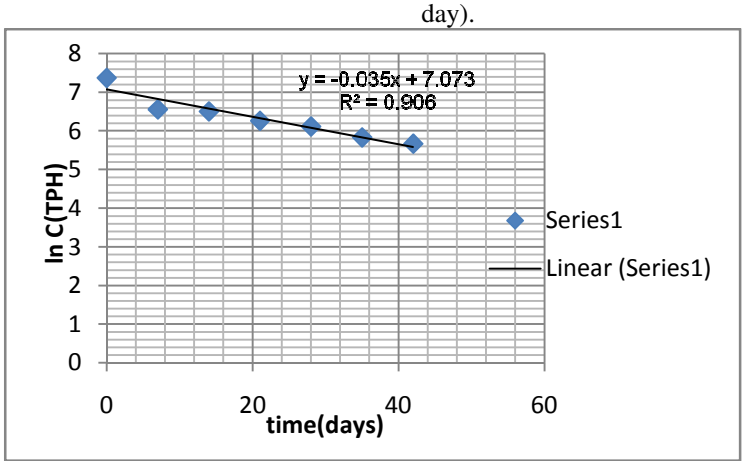
Table-4

Concentration of total petroleum hydrocarbon (TPH) in soil (Cs) and TPH biodegraded (Cd) for the crude oil simulated soil

Time (days)	0	7	14	21	28	35	42
C_s	1587.5	704.63	668.25	524.8	453.2	341.68	290.7
C_d	0	882.87	919.46	1062.7	1134.3	1245.82	1296.8

The reduction in population of the hydrocarbon degraders could be due to the fact that the organisms have exhausted the available nutrient supplies present in the system. It could also be that mineralization of hydrocarbons could have possibly resulted in the production of toxic metabolites which on introduction into the system reduces the growth phase of the microbes. The findings of Amadi and Odu[25] who reported an initial gradual increase in bacterial population following the application of petroleum hydrocarbon but a decline as the biodegradation progressed supports this explanation. It is seen from the results that the rate of biodegradation and microbial growth seems to be dependent on pH. Biodegradation was faster between pH 6.7-9.6 and recorded increased population growth within the first three weeks (twenty one days) but reduced significantly as pH increased. The pH of 6.7 was obtained as an average between the initial pH of the simulated soil and that on the first one week. The only possible explanation for the increase in population of hydrocarbon degrading fungi on the last week after a progressive decrease could be that low hydrocarbon concentration favours increased fungi growth for some fungi specie. Another reason could be that the decrease in pH from 10.3 on the fifth week (thirty fifth day) to 9.3 on the sixth week (fourty second day) provided a conducive environment for increased cell growth in some fungi specie.

Biodegradation and its Kinetics on the Crude Oil contaminated soil: A six weeks investigation on the biodegradation of crude oil in the contaminated soil reveals an 81.69% reduction in T.P.H on the sixth week (fourty second day) from an initial concentration of 1587.5mg/kg obtained from a 10% spiking. Biodegradation had increased rate within the first three weeks recording a 66.94% reduction, but the rate decreased significantly within the last three weeks. The TPH reduction resulted from the biodegradation process as only a minimal 2.6% reduction was achieved in the control under the same period. Concentration of total petroleum hydrocarbon (TPH) left in the soil at regularly spaced intervals and their natural logarithm were plotted against time as shown in figure 1 and 2 in order to analyse the kinetics for the biodegradation process. The biodegradation process followed first order kinetics since a plot of TPH concentration in soil against time gave an exponential curve and ln of TPH concentration against time was linear. Rate constant was found to be 0.035day^{-1}. The degradation pattern was similar to that reported by Peijun et al.[26]. Correlation analysis of R^2 for the crude oil biodegradation kinetics process was found to be 0.906, indicating linearity and positive correlations for the decrease in concentration as a function of time. However theoretical predictions using statistical forecast on the existing data's reveals a 99.6% T.P.H reduction to be achieved on the 9th week (63rd day).



Rate constant k equals 0.035 since –k=-0.035

Figure-1

Plot of ln C(TPH) against time for the bioremediation of crude oil simulated soil using bio-stimulation technique

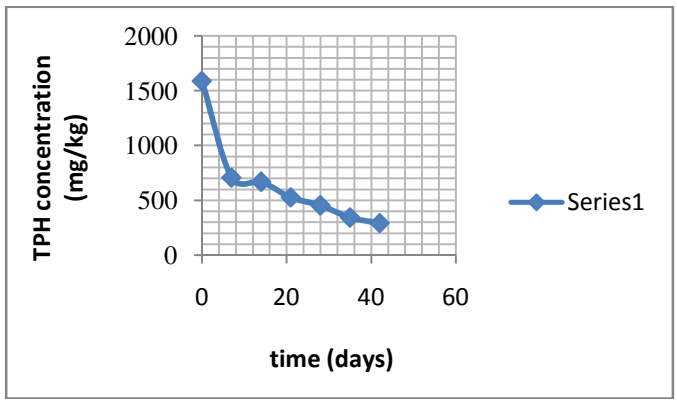


Figure-2

First order profile for the bioremediation of crude oil contaminated soil

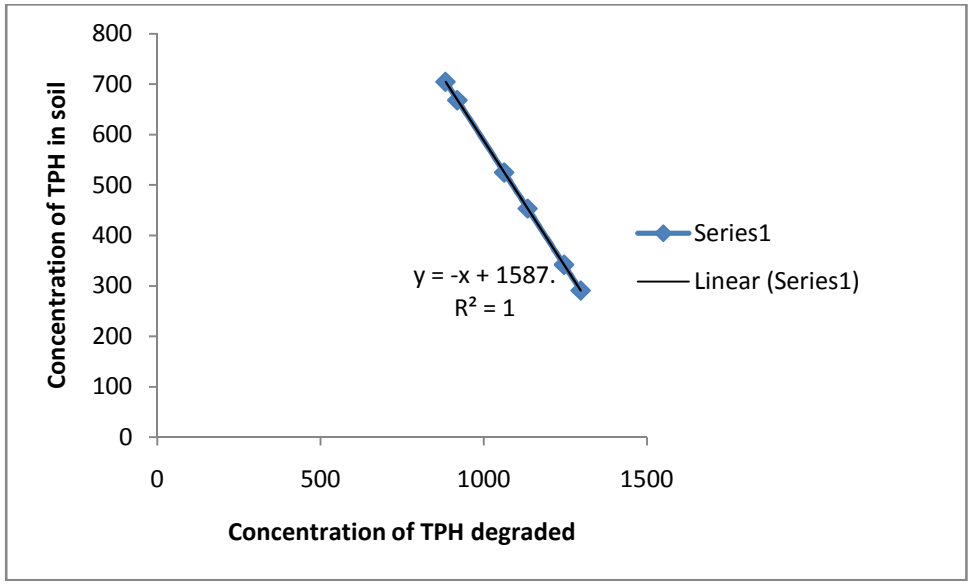


Figure-3
Linear degradation isotherm for the biodegradation of crude oil contaminated soil

Degradation isotherm for the bioremediation study: The biodegradation isotherm for the crude oil treated soil is shown in figure 3. Biodegradation isotherm at ambient temperature for the bioremediation study of the crude oil polluted soil was computed from the concentrations of total petroleum hydrocarbon degraded by the indigenous microbes and the residual concentration of total petroleum hydrocarbon left in the soil at each time. Concentration of total petroleum hydrocarbon degraded by the indigenous microbes (C_d) was computed by subtracting the amount of residual TPH (C_s) left after degradation from the initial concentration as presented in table 4, thus for each TPH determination with respect to time, there are two concentration values representing the concentration of TPH degraded by the indigenous microbes (C_d) and the residual concentration left in the soil after degradation (C_s). A plot of C_s versus C_d gave a straight line graph with the values of C_s and C_d linearly related. The line plotted through the points is known as the linear biodegradation isotherm and it is giving by $Kd = Cs/Cd$. The biodegradation isotherm was plotted to the same scale for comparison, and the K_d value calculated using a linear regression analysis as shown in the linear regression equation. The negative value of unity for k_d shows the opposing trend between C_s and C_d, which explains that as the concentration of the contaminant in the soil (C_s) is decreasing with time, the concentration degraded by the microbes (C_d) is increasing for the biodegradation study. The correlation coefficients (R^2=1) from the biodegradation isotherms indicates a very positive correlation between the degraded contaminant and the parent contaminant left in the soil during the degradation process at each time for the biodegradation experiment.

Conclusion

Crude oil effect on soil properties was evident on soil pH, conductivity, total phosphorus and microbial growth. However other properties such as soil texture, total nitrogen, organic carbon, organic matter, carbon/nitrogen ratio were unaffected. The findings of this study show that the rate of biodegradation depends majorly on soil pH and nutrient availability. It could be seen from the study that mere reliance on microbial transformation of the contaminant without providing suitable conditions for optimum and speedy degradation could take a very long time for significant remediation to be achieved. The minimal TPH depletion observed in the control soil justifies this view. As such, when conditions such as pH requirement and nutrient availability are taken into consideration during a bioremediation project, the rate of microbial degradation could be conveniently achieved within a much more shorter time than even what was obtained in this study since its kinetics have been established. The rigours of bioaugumenting contaminated soils for the purpose of bioremediation can be avoided and effective remediation still achieved if suitable conditions that will enhance indigenous microbial activities for optimum degradation are satisfied.

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