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The Influence of Biostimulator in the Remediation of Petroleum Sludge Polluted Clay Soil: *The Concept of Moringa Application*

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Abstract— *The Bioremediation of Petroleum Sludge (PS) in a clay soil environment of the Niger Delta region of Nigeria using Moringa Seed oil extract (MO) as a Biostimulator (BS) has been investigated, with a view to studying the mitigation impact on the total petroleum hydrocarbon (TPH) content of the petroleum sludge. Two Bioreactors labeled R_1 (with MO treatment) and R_2 (control: No treatment) containing 3.0kg of clay soils were polluted with 300ml of petroleum sludge. 50ml of MO was added to R_1 as a biostimulator. R_2 received no form of treatment. Bioremediation extent monitoring was carried out bi-weekly by sampling of the bioreactors contents and analyzing for the individual petroleum hydrocarbon using a gas chromatography (GC). Analysis of the samples at two weeks intervals for a period of 12 weeks reveals that bioremediation occurred in the treatment reactor and the control reactor to which no biostimulator was added. Most of the Hydrocarbon degradation occurred within the first four weeks of the experiment. It was found that moringa seed oil extract was very effective and suitable for remediation of petroleum sludge polluted clay soils due to the high degradation rates of the individual hydrocarbons recorded in the biostimulated reactor against the low degradation rates of those of the control reactor with no form of bio-treatment.*

Keyword— Bioremediation, Biostimulation, Petroleum Sludge, Clay Soil, Moringa Seed Oil Extract.

I. INTRODUCTION

At Petroleum Refineries, flow stations and crude oil ocean terminals, petroleum is stored in various storage tanks before refining processing and shipment to various locations outside the shores of Nigeria. During this period of storage, petroleum sludge generation is inevitable due to the settlement of suspended solids. In addition to the grandiose manpower requirement and waste management issues associated with petroleum sludge handling, its presence in storage tanks reduces the oil storing capacities of the tanks with the increasing possibility of tank corrosion. Hence, the continual removal of the petroleum sludge from storage tanks becomes a necessity.

Nkeng and Nkwelang (2012) have stated that one of the major problems faced by the petroleum industries is the safe disposal of petroleum sludge in the environment since many of the constituent of petroleum sludge are toxic and carcinogenic. Petroleum sludge when improperly disposed to soil environment, alters the physical and chemical properties of the soil resulting to much changes in soil characteristics (Robertson et al., 2007; Kamalu et al., 2016). According to the finding of Al-Mutari (2008), the petroleum sludge contaminated soil may create nutrient deficiency, inhibit seed germination and cause restricted growth or demises of plants on contact.

Detoxification of petroleum sludge contaminated soils is therefore essential prior to soil re-use for agricultural and other purposes. Various physicochemical treatment techniques have been developed to clean up petroleum sludge polluted soils such as incineration, thermal desorption and chemical oxidation. In general, such treatments according to Alamri (2009) are very expensive, energy intensive and not sustainable with respect to their environmental impact which includes damage to the soil structures and toxicity issues associated with chemical additives. These limitations have been the basis of search for more economical and environmentally sound approaches to the clean up of petroleum sludge contaminated soils. Biological (Bioremediation) treatment of organic pollutants is a promising field of research which gives reliable, simple and cheap technologies over the

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chemical and physical process (Thayer, 1991; Kamalu et al., 2016).

Bioremediation has been defined as any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition (Ukpaka, 2007, Daugulis and McCracken, 2003; Leahy and Cohwell, 1990; Atlas, 1981; Adebuso *et al*., 2007; and Das and Mukherjee, 2007). Ukpaka (2012) in a study on the effects of functional parameters on the microbial characteristics in crude oil degradation has concluded that the use of microorganisms with appropriate metabolic capacities is the first step to achieving a successful bioremediation. In the present study, Ex-Situ biostimulation of the indigenous microbes by the addition of moringa seed oil extract was conducted to reclaim the petroleum sludge contaminated clay soil by evaluating its response to the degradation of individual hydrocarbons present in the petroleum sludge.

II. MATERIALS AND METHODS

MATERIALS:

The materials used includes: petroleum sludge (PS) obtained from Nigerian Agip Oil Company (NAOC) tank farm, Brass, Bayelsa State, Nigeria; clay soil collected from Rumuche-Emohua, Rivers State, Nigeria; Moringa seed oil extract purchased at Yenagoa Local Government Area Council office, Yenagoa, Bayelsa State, Nigeria; Glass beakers, measuring cylinder, weighing scale, mercury in glass thermometer; plastic buckets, trowel, masking tapes and gas chromatography (GC).

III. EXPERIMENTAL PROCEEDURE

Bioremediation mitigation experiment was conducted in two conic plastic buckets of seven litres capacity each. These containers served as the treatment reactors. 3.0kg of the experimental soil (clay) was measured into each of the treatment reactors labeled R_1 and R_2. 300ml of the petroleum sludge was measured into each of the plastic containers. Biostimulation method of Bioremediation was employed to enhance the microbial degradation of the hydrocarbon contaminants in the petroleum sludge polluted clay soils using moringa seed oil extract (MO) as the microbial biostimulant. 50.0ml of the moringa seed oil extract was measured out and poured into reactor R_1. Reactor R_2 which is the control received no Biostimulant. The contents of the reactors, after the addition of the biostimulant were properly stirred and transferred to a secluded hall away from sunlight and down pour, for a 90 day experimental period.

3.1 BIOREMEDIATION EXTENT MONITORING

The Bioremediation mitigation process was monitored by periodically analyzing samples of the petroleum sludge polluted clay soils undergoing remediation. Samples of the soils from the two reactors were collected at intervals of 15 days after proper mixing of the soils with trowel. The collected samples were analyzed for individual petroleum hydrocarbon content at SpringBoard Laboratories, Road 3, House 1, Udoka Housing Estate, Awka, Anambra State.

3.2 DETERMINATION OF INDIVIDUAL PETROLEUM HYDROCARBON IN THE SOIL SAMPLES

Reagents: Hexane, Acetonitrile

Apparatus: Buck 530 gas chromatography

Individual petroleum hydrocarbon presented in the PS polluted soil samples were extracted with 200ml of hexane. The mixture was separated using a separating funnel with the hexane layer concentrated in a rotary evaporator. 1ml of acetonitrile was added into a vial and placed into a Buck 530 gas chromatograph equipped with an on-column, automatic injector, electron capture detector, HP 88 capillary column (100m x 0.254m film thickness). The individual petroleum hydrocarbons present in the samples were then displayed in the visual unit of the gas chromatograph.

IV. RESULTS AND DISCUSSION

The results of the experiment with respect to the degradation of the individual petroleum hydrocarbons in the petroleum sludge polluted clay soil mitigation process are presented in tables 1, and 2 as shown in the appendix. From the experimental results, most of the petroleum hydrocarbons were not detected and so were skipped in tables 1 and 2, either because their concentrations were below the detection limits (<0.01mg/kg) of the gas chromatography used (Bulk 530 gas equipped with an on-column, automatic injector, electron capture detector) or they are not present in the petroleum sludge.

The profile of the bioremediation from the biostimulated reactor (R_1) and the control reactor (R_2) are shown in figures 1 and 2 below.

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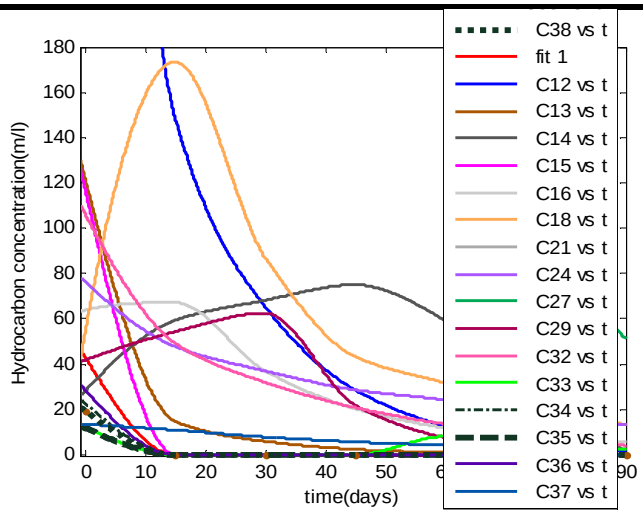

Fig.1: Hydrocarbon concentration bioremediation ersus time in days of bioremediation (biostimulated reactor).

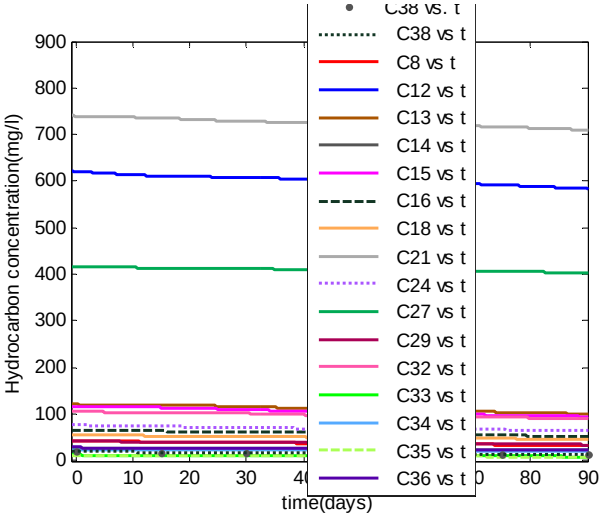

Fig.2: Hydrocarbon concentration bioremediation versus time in days of bioremediation (control).

The profiles from the biostimulated reactor (R_1) as depicted in figure 1 shows evidence of bioremediation of the individual petroleum hydrocarbon components of the petroleum sludge. Significant reductions from the initial concentration of the petroleum hydrocarbons were achieved. Most of the hydrocarbons degraded completely (100%) in the course of the mitigation experiment. It was observed from the profiles of most hydrocarbons such as C_{14}, C_{16}, and C_{18} increased in concentration. The sudden increase in concentrations of these hydrocarbons were due to fresh hydrocarbon formations due to the degradation of higher molecular weight hydrocarbons in the reactor, which also later degraded in the course of the experiment. The profiles shown in figure 2 are those of the control reactor with no biotreatment. From the profiles, slight degradations were recorded from the initial concentrations of the hydrocarbons. The slow rate of degradation is an indication that natural attenuation is not a suitable process for large scale bioremediation.

OCTANE (C_8)

Complete mineralization (100% degradation) of the octane component of the petroleum sludge occurred in reactor R_1, from the second week of the experiment. The complete degradation of the octane in the treatment reactor is attributed to the fact that the biostimulator (moringa seed oil extract) used contains certain nutrients in the form of phosphates and nitrates that are very important in the bioremediation process. The moringa seed oil extract supplies its nutrients to the soil, stimulating the indigenous microbes which in turn feed on the hydrocarbons present in the soil. The percentage degradation of octane in the control reactor (R_2) was 23.5934%. The slight reduction in the concentration of octane in the control reactor was due to the absence of a biostimulator in the degradation process. The result of the control reactor further validates the fact that bioremediation can proceed naturally (natural attenuation) without any form or soil amendment.

DODECANE (C_{12})

There was significant reduction in Dodecane in the treatment reactor (R_1) with the exception of the control reactor (R_2) which was only slightly reduced. From an initial concentration of 621.5731mg/l, Dodecane reduced to 1.9753mg/l (99.6822% degradation in the biostimulated reactor (R_1) and 583.8485 mg/l (6.0692% degradation) in the control reactor (R_2).

The significant reduction of the Dodecane fraction of the petroleum sludge in R_1 attests to the effectiveness of the bioremediation process using moringa seed oil extract as a biostimulator.

TRIDECANE (C_{13})

The initial concentration of the Tridecane detected was 120.9383mg/l before the biostimulation of the PS polluted clay soils. The final results obtained at the end of the 90 day experiment shows complete degradation (100%) of the Tridecane from the 75th day of the experiment in the treatment reactor, R_1, and 16.5999% (100.8626mg/l) reduction in the control reactor (R_2)

PENTADECANE (C_{15})

Pentadecane degraded completely in the treatment reactor (R_1) due to the activities of microorganisms (Hydrocarbon

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utilizing bacteria) being stimulated by the nutrient – rich biostimulator (moringa seed oil extract).

The degradation recorded in the control reactor (18.1369%) is insignificant in comparison to the complete mineralization recorded in the treatment reactor R_1 (biostimulated with moringa seed oil extract). This result is an indication that large scale bioremediation cannot be handled by natural attenuation.

HEXADECANE (C_{16})

Hexadecane increased from an initial concentration of 63.7382mg/l to 67.1647mg/l (5.3759% increase) within the first two weeks of the mitigation experiment in the treatment reactor (R_1). This increase in concentration is a function of the degradation of heavier ($>C_{16}$) molecular weight hydrocarbons in the treatment reactor. Response to microbial degradation of the Hexadecane component of the petroleum sludge was noticed on the 30^{th} day of the experiment, reducing from 67.1647mg/l to 36.9406mg/l. Subsequent bi-weekly results of sample analysis showed continual reduction of hexadecane. At the end of the 90 day experiment, Hexadecane has degraded to 5.3803mg/l (91.5588%) in the treatment reactor R_1. Within the experimental period, Hexadecane, degraded to 53.2825mg/l (16.4041% reduction) in the control reactor R_1. The slight reduction is an indication that natural attenuation is not an effective method of bioremediation.

OCTADECANE (C_{18})

The reduction in concentration of octadecane within the 90 day experimental period in the treatment reactor (R_1) was 13.4185mg/l from initial concentration of 55.1684mg/l (75.6772% reduction). Tremendous increase in concentration of octadecane occurred in the first two weeks of the experiment in R_1 as was observed on the 15^{th} day (173.390mg/l). This increase in concentration is accounted for by the breakdown of higher molecular weight hydrocarbons ($>C_{18}$) in the treatment reactor. From the 30^{th} day, result of analysis showed degradation of octadecane. The percentage reduction of the octadecane in the control reactor (R_2) was 19.5764%. This value is low compared to that of the treatment reactor R_1, due to the non-application of a biostimulator.

HENEICOSANE (C_{21})

The results obtained from the bioremediation experiment for Heneicosane degradation in the petroleum sludge polluted clay soil shows that biostimulation is a useful tool in microbial degradation of hydrocarbons. The total concentration of Heneicosane measured prior to the commencement of the experiment was 740.2861mg/l. At the end of the 90 day experiment, the initial concentration of Heneicosane has reduced to 220.2769mg/l in the treatment reactor (R_1) and 710.4419mg/l in the control reactor (R_2). The above results accounts for the effectiveness of the bioremediation process and the efficiency of the moringa seed oil extract applied in stimulating the indigenous microbes in the polluted soils. R1 remediation by 70.2 and R_2 4.0314%.

TETRACOSANE (C_{24})

Analysis of samples revealed that bioremediation of Tetracosane occurred in both reactors R_1, (with a biostimulator) and R_2 (without stimulator). Percentage reduction of Tetracosane in the treatment reactor, R_1 was 82.5096% and 16.1966% in the control reactor, R_2. The result of the treatment reactor in comparison to the control reactor compliments the fact that moringa seed oil extract is an effective biostimulator and suited for bioremediation of petroleum sludge.

HEPTACOSANE (C_{27})

Heptacosane component of the petroleum sludge was very high at initial measurement compared to other hydrocarbon components present in the petroleum sludge. In the course of the bioremediation mitigation, heptacosane reduced from an initial concentration of 416.7177mg/l to 5.1362mg/l in the treatment reactor R_1, and 403.1791mg/l in the control reactor, R_2. The high rate of degradation of the Heptacosane component in the treatment reactor (R_1) was due to the high metabolic activities of the resident microorganisms stimulated by moringa seed oil extract. The low reduction (3.2489%) in the control reactor was because of natural attenuation (without stimulation).

NONACOSANE (C_{29})

Complete mineralization of the Nonacosane component of the petroleum sludge was achieved in the treatment reactor, R_1 (100% degradation) in the last two weeks of the experiment. Nonacosane increased in concentration in the first four weeks of the experiment. This increase in concentration was caused by the degradation of heavier hydrocarbons ($>C_{29}$) in the treatment reactor, R_1. Further analysis of samples gave results with indication of occurrence of bioremediation from the 45^{th} day to a complete mineralization of the Nonacosane at the end of the experiment. The control experiment R_2 recorded 17.3961% degradation within the 90 day remediation period. The high degradation rate of C_{29} in the MO stimulated reactor was due to the enhancement of C_{29} bioavailability by moringa seed oil extract.

DOTRIACONTANE (C_{32})

Dotriacontane fraction of the petroleum sludge showed grandiose reduction in concentration in the course of the

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mitigation. A percentage reduction of 96.2134 % took place in the treatment reactor, R_1, and only 14.9019% in the control reactor, R_2, which was unaided with bionutrient carriers. This results shows the effectiveness of the impact of moringa stimulator in accelerating petroleum sludge degradation.

TRITRIACONTANE (C_{33})

Tritriacontane was degraded completely (100%) in the treatment reactor, R_1 within the first two weeks of the remediation, with slight reduction of 27.2231% in the control reactor, R_2. Complete mimeralization of the Tritriacontane compoinent of the petroleum sludge was maintained till the 6^{th} week of the remediation. At the 8^{th} week, there was fresh formation (8.0749mg/l) of Tritriacontane in the treatment reactor, due to degradation of higher molecular weight components (>C_{33}) in the reactor. The formed Tritriacontane was significantly degraded to 2.4451mg/l within the last four weeks of the experiment, corresponding to 69.7197% degradation of the formed tritriacontane component.

Within the same experimental time, the reduction in the control reactor (R_2) was minimal (27.2231%) compared to the reactor with a biostimulator. The low degradation rate recorded in the control reactor is an indicator of the unreliability of natural attenuation in petroleum sludge bioremediation.

TETRATRIACONTANE (C_{34})

TPH analysis of samples of the petroleum sludge polluted clay bioremediation reveals 100% (complete) degradation for Tetratriacontane component in the moringa stimulated bioreactor (R_1). Complete degradation of the C_{34} component within the first two weeks of the experiment gives credibility to the adequacy of the use of moringa seed oil extract in the mitigation of petroleum sludge polluted clay soils of the Niger Delta Environment. In the control reactor, R_2, which was not biostimulated, the C_{34} component degraded to 19.5301mg/l from an initial concentration of 22.8800mg/l, representing 14.6412% degradation within the 90 day experimental period.

PENTATRIACONTANE (C_{35})

Pentatriacontane (C_{35}) degraded completely (100%) from an initial concentration of 11.7860mg/l within the period of the experimental investigation in the biostimulated reactor, R_1 observed that the moringa seed oil extract in addition to being a microbial stimulant was responsible for the bioavailability of the heavier substrates to microbial consumption. In comparison to the non-MO stimulated bioreactor, R_2, a total of 23.1724% within the experimental period. The low degradation was due to the non – enhancement of the bioavailability of the heavier substrates in the control reactor to microbial consumption as in the MO stimulated reactor.

HEXATRIACONTANE (C_{36})

Initial concentration of the C_{36} component of the petroleum sludge was 28.3650mg/l prior to the bioremediation mitigation experiment within the period of the experimental investigation, results of sample analysis indicates occurrence of bioremediation in the reacted (R_1) and untreated (R_2) reactor. In the treated reactor (with MO stimulation), C_{36} degraded completely (100%). While in the untreated reacted, C_{36} component slightly degraded to 24.5848mg/l (13.3270% degradation), the low degradation rate was as a result of the fact that the remediation in the control reactor proceeded on natural attenuation without any form substrate bioavailability enhancement and microbial stimulator.

HEPTATIACONTE (C_{37})

Heptatriacontane (C_{37}), a heavier component of the petroleum degraded slightly, from an initial concentration of 13.4670mg/l to 10.8788mg/l in the MO treated reactor, R_1, within the first two weeks of the experiment. This initial slow rate of degradation was due to the time taken for the C_{37} degrading microbes to adapt in the reactor, consuming C_{37} as a new source of energy. By the end of the 90 day experimental period, results of sample analysis show 100% degradation of the C_{37} component in the moringa treated bioreactor, R_1.

The untreated reactor, R_2 (without addition of MO) on record 17.0944% degradation of the C_{37} component, reducing to 11.1649mg/l from an initial concentration of 13.4570mg/l. the low degradation rate of C_{37} component in the control reactor validates the claim that natural attenuation cannot effectively remediate hydrocarbon polluted environments within a short period as in the experimental investigation period.

OCTATRIACONTANE (C38)

Initial petroleum sludge characterization prior to commencement of bioremediation mitigation gave octatriacontane initial concentration as 19.2760mg/l. Results of sample analysis indicated that bioremediation occurred in moringa stimulated bioreactor (R_1) as well as the control reactor (R_2) without any form of biostimulation. Percentage reduction of octatriacontane component in the MO treated reactor was 100% and 26.5454% in the control reactor. The results of the treated reactor in comparison with the untreated reactor shows the reliability of biostimulation in achieving effective bioremediation of petroleum sludge in a record time.

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V. CONCLUSION

From the experimental study, it is concluded that the reduction in the total Petroleum Hydrocarbon (TPH) in the course of the remediation was due to the presence of TPH–degrading microbes in the polluted soil, whose growth and degrading capabilities were a direct function of the effectiveness of the biostimulator (moringa seed oil extract) applied in the experimental process. The moringa seed oil extract(biostimulator) was responsible for the enhancement of substrates and microbial activation in the treated reactor. The results of the individual petroleum hydrocarbon degradation in the control reactor, R_2, in comparison to those of the treatment reactor, R_1, were very low varying between 11% to 27% (percentage reduction). The result from the control reactor is an indicator that bioremediation can occur naturally without any form of soil amendment. The drawback to the natural attenuation is that the rate of natural bioremediation is very slow. Hence, large scale in-situ or ex-situ bioremediation cannot be reliably and successfully carried out in a record time.

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APPENDIX 1

Table.1: TPH BIOREMEDIATION EXTENT FOR PS POLLUTED CLAY SOIL IN R_1 (WITH THE ADDITION OF MORINGA SEED OIL EXTRACT)

Parameters (Mg/L)		**TIME (DAYS)**						
		0	**15**	**30**	**45**	**60**	**75**	**90**
Octane	C8	42.7640	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Dodecane	C12	621.5731	147.8045	65.0339	28.6150	12.7154	5.5399	1.9753
Tridecane	C13	120.9383	14.7158	5.8863	2.3545	0.9418	0.0000	0.0000

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Tetradecane C14	28.2899	59.2994	68.1943	75.0137	59.2250	17.7675	2.6651
Pentadecane C15	116.1860	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Hexadecane C16	63.7382	67.1647	36.9406	20.3173	11.7168	6.1460	5.3803
Octadecane C18	55.1684	173.3906	86.6953	43.3477	31.6586	20.8369	13.4185
Heneicosane C21	740.2861	703.2965	555.6042	438.9273	346.7526	273.9345	220.2769
Tetracosane C24	76.5193	47.7428	36.9874	28.5955	23.0360	16.1662	13.3835
Heptacosane C27	416.7177	187.5274	93.7802	42.2754	18.9572	10.4976	5.1362
Nonacosane C29	41.8416	54.3140	62.4611	21.8613	7.6515	2.6780	0.0000
Dotriacintane C32	105.9294	49.2215	31.9937	20.7961	13.5175	8.7864	4.0111
Triacontane C33	12.3700	0.0000	0.0000	0.0000	8.0749	5.4337	2.4451
Tetratriacontane C34	22.8800	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Pentatriacontane C35	11.7860	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Hexatriacontane C36	28.3650	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Heptatriacontane C37	13.4670	10.8788	7.8327	5.6396	4.0605	1.9236	0.0000
Octatriacontane C38	19.2760	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table.2: TPH BIOREMEDIATION EXTENT FOR 12 PS POLLUTED CLAY SOIL IN R_2 (CONTROL: WITHOUT ADDITION OF MORINGA SEED OIL EXTRACT)

Parameters (Mg/l)	**TIME (DAYS)**						
	0	**15**	**30**	**45**	**60**	**75**	**90**
Octane C8	42.7640	40.6754	39.5026	37.0508	36.8355	34.4990	32.6745
Dodecane C12	621.5731	611.4850	608.1138	603.0853	598.0640	591.7980	583.8485
Tridecane C13	120.9383	119.4854	116.3640	110.9094	108.5590	104.6251	100.8626
Tetradecane C14	28.2899	28.1456	26.6129	25.9935	25.6369	23.8414	23.3651
Pentadecane C15	116.1860	114.4850	109.4592	104.9422	101.2067	97.9320	95.1134
Hexadecane C16	63.7382	63.4850	61.2598	60.3708	57.2781	54.2808	53.2825
Octadecane C18	55.1684	53.5861	52.4319	50.2023	49.7815	47.9298	44.3684
Heneicosane C21	740.2861	735.4850	728.5468	725.2148	721.7541	716.5787	710.4419
Tetracosane C24	76.5193	73.8970	70.4718	69.4482	68.6258	66.7319	64.1258
Heptacosane C27	416.7177	413.9485	411.8519	410.8292	409.7163	406.0806	403.191
Nonacosane C29	41.8416	40.4950	39.5198	38.2926	36.9633	36.2012	34.5628
Dotriacintane C32	105.9294	103.3850	101.8658	97.5359	96.8555	93.1513	90.1439
Triacontane C33	12.3700	11.8515	11.1121	10.6129	10.0718	9.5331	9.0025
Tetratriacontane C34	22.8800	22.6508	22.3650	21.9621	20.9338	20.1291	19.5301
Pentatriacontane C35	11.7860	11.0936	10.7490	10.4419	9.6122	9.1183	9.0549
Hexatriacontane C36	28.3650	27.7795	27.3131	25.9471	25.0052	24.9392	24.5848
Heptatriacontane C37	13.4670	13.4351	13.1835	12.8243	12.0194	11.5165	11.1649
Octatriacontane C38	19.2760	18.5401	18.0132	16.6269	15.0293	14.8599	14.1591