Modeling of *Azadirachta indica* leaves powder efficiency for the remediation of soil contaminated with crude oil

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A R T I C L E   I N F O

A B S T R A C T

A regression analysis model was developed to examine the reliability and the acceptability of the sun-dried and room-dried *Azadirachta indica* leaves for the remediation crude oil contaminated soil in Niger Deltal, Nigeria. An experimental approach was used to monitor the concentration of the total petroleum hydrocarbon (TPH) degradation that was enhanced in the presence of the microbes in the reactor. A regression analysis was performed to evaluate the reaction behavior microbes in reactor for the remediation of the TPH in the presence of sun-dried and room-dried *A. indica* leaves in sandy and loamy soils contaminated with 100 ml of crude oil. The physicochemical properties of control loamy soil was as; pH (6.75), electric conductivity (10.36 μS/cm), total oxygen (2.99%), total Nitrogen (0.091%), potassium (36.82942%), phosphorus (15.36%) and total bacteria (2.15×10^2 cfu/g). Whereas, these values were as pH (6.82), electric conductivity (21.48 μS/cm), total oxygen content (1.18%), total nitrogen (0.036%), potassium (24.03681%), phosphorus (5.18%) and total bacteria count (1.86×10^2 cfu/g) for sandy soil. The inoculant was prepared by blending sun-dried and room-dried *A. indica* leaves. The blended *A. indica* leaves in 50 to 100 g range were applied on the polluted soils for 35 days and significant reduction in contaminating agents was observed. The highest remediation was observed in soils treated with 100 g room dried *A. indica* leaves. A 96% remediation recorded in sandy soil treated with 100 g room dried *A. indica leaves* as TPH depleted from 35818.69 mg/kg to 1349.109 mg/kg and 92% reduction was observed in loamy soil treated with 100 g dried *A. indica* leaves and TPH depleted from 48508.92 mg/kg to 3977.739 mg/kg. The regression model developed was successfully employed to predict TPH remediation behavior, which can be applied to monitor remediation of contaminated soil.

Capsule Summary: A regression analysis model was developed to examine the remediation of soil contaminated with crude oil and developed model was successful in monitoring the TPH remediation of contaminated soil and could be used for monitoring the same for different radiating agents.

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INTRODUCTION

Over the years, the association of plants and endophytic bacteria has gained much attention and salience. These alliances are very vital in maintaining mutual interactions among plants and bacteria, and contribute to the ecological balance between them (Hardoim, 2008; Khan, 2013; Santoyo, 2016). Endophytes are organisms (bacteria or fungi) that lives inside plant tissues, innocuous to their hosts, and results from much studies have indicated their positive effects on plant growth and development (Gaiero, 2013; Khan, 2013; Weyens, 2009). Consequently, they add support to the plant's adaptive mechanism to biotic and abiotic stresses, promote the availability of nitrogen, phosphorus and iron, activate the production of plant hormones and protect the plants against pathogens (Abbamondi, 2016; Compant, 2005; Glick, 2007; Naveed, 2014; Qin, 2015; Santoyo, 2016). In addition, endophytes effectively degrade and detoxify contaminants thereby increasing the plant's tolerance to the contaminants. Also, they reduce plant stress by producing 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase (Barac, 2004; Gaiero, 2013; Huang, 2005; Ryan, 2008). Endophytic bacteria consequently, were seen to be of remarkable importance in effective phytoremediation programs (Barac, 2004; Weyens, 2009).

Phytoremediation is an eco-friendly method of decontaminating contaminated soils and underground waters, using plants and their endophytes. This technology is considered promising and economically feasible for the decontamination of organic contaminants from soils (Vangronsveld, 2009; Weyens, 2009a). The Niger-Delta region of Nigeria has been prone to significant soil contamination and accumulation of aliphatic and aromatic hydrocarbons in the environment due to non-regulated disposal of oil sludge, oil extraction, refining and leakage during storage and transportation. Intensive investigations on the neutralization of these organic pollutants have been done because they exert harmful effects on living organisms (Huang, 2005; Khan, 2013; Wang, 2008). Recent results propose that for a successful phytoremediation, the major element is combining plants with endurance for high levels of contaminants with plant-associated endophytes capable of breaking down pollutants (Germaine, 2009; Yousaf, 2011). Moreover, it is proven that plants growing in crude oil-polluted soil can selectively boost the widespread of suitable endophytes containing genes encoding for enzymes responsible for hydrocarbon degradation (Oliveira, 2014; Siciliano, 2001). Other Authors has shown that the numbers of endophytes with hydrocarbon degradation capabilities has increased as a result of the advent of hydrocarbon contamination (Barac, 2004; Oliveira, 2014; Taghavi, 2009). This implies that investigating the variety of the plant endophytic community is imperative for the selection of useful strains for phytoremediation of soils contaminated with organics hence improving plant adaptation and growth. Because of the wide range of symbiosis between the plant host and its endophytes, their potential has great imputation for biotechnological applications during phytoremediation (Khan, 2013; Li, 2012). The greater the plant's endurance for soil polluted heavily with crude oil, the more useful tool they are for phytoremediation purposes.

In the past years, a plethora of research was carried out on the mechanisms of endophyte associated phytoremediation of pollutants in soils and promising efficiency is reported. Discussing their basic mechanisms, we will present updated information as regards progress, obstacles and new directions in the field of endophyte assisted phytoremediation technology (Ukpaka and Wami, 2017; Ukpaka, 2017; Ukpaka, 2020; Ukpaka et al., 2020).

This research proved to be a huge contribution to crude oil bioremediation in the following ways: The method used in this experiment for crude oil bioremediation is cost-effective since the remedant is a widespread locally grown tree. This should counter previous methods proven to be very expensive. From the experiment, it is now easy to obtain the time required for the microbes to feed on the crude oil. Also, results from the experiment makes it easy to predict the actual quantity of *A. indica* leaves required to remediate a known quantity of crude oil. It was observed that *A. indica* leaves helps to replenish the natural NPK content of the crude oil polluted soil without adding fertilizers. It was also observed that *A. indica* leaves helps to stabilize the conductivity, pH, microbial activity and salinity of the crude oil contaminated soil.

Table 1: Schematic representation of the experimental set-up

Azadirachta indica leaves powder concentration (g)											
50		60		70		80		90		100	
Sandy	Loamy	Sandy	loamy	sandy	loamy	sandy	Loamy	sandy	loamy	sandy	loamy
SSD	LSD	SSD	LSD	SSD	LSD	SSD	LSD	SSD	LSD	SSD	LSD
SRD	LRD	SRD	LRD	SRD	LRD	SRD	LRD	SRD	LRD	SRD	LRD

Table 2: TPH as a function of exposure time in soil treated with 50 g of *A. indica* leaves powder

Time (days)	SSD (ppm)	LSD (ppm)	SRD (ppm)	LRD (ppm)
0	35818.69	48508.92	35818.69	48508.92
7	34778.7	46177.42	33374.06	45709.09
14	32381.02	43177.33	28375.86	40909.14
21	32181.96	40771.2	26363.67	40006.84
28	22880.75	32174.59	17669.36	29023.86
35	11654.06	19339.89	7263.492	14807.66

Table 3: TPH as a function of exposure time in soil treated with 60 g of *A. indica* leaves powder

Time (days)	SSD (ppm)	LSD (ppm)	SRD (ppm)	LRD (ppm)
0	35818.69	48508.92	35818.69	48508.92
7	32621.55	44507.49	32420.32	45045.42
14	29992.84	40451.23	27245.78	38915.67
21	26043.69	36210.31	23349.56	33751.86
28	17167.24	26417.93	14568.15	31049.03
34	15726.07	24199.65	13057.37	23276.2

Table 4: TPH as a function of exposure time in soil treated with 70 g of *A. indica* leaves powder

Time (days)	SSD (ppm)	LSD (ppm)	SRD (ppm)	LRD (ppm)
0	35818.69	48508.92	35818.69	48508.92
7	32497.46	44769.44	32153.41	43949.71
14	28423.68	39268.63	25530.19	35416.52
21	24597.45	33945.52	20000.24	30243.79
28	18097.89	28171.61	15193.53	24544.55
35	10499	20408.04	7862.638	15331.41

MATERIAL AND METHODS

Material and equipment

The soil samples and the *A. indica* leaves were obtained from a farm within Rivers State University vicinity. Materials that was used includes: sandy and loamy soil, crude oil (Bonny light), *A. indica* leaves, Melter weighing balance, beakers, pH meter for measuring decomposed sample, measuring cylinders, retort stands. Every 1 kg of soil was polluted with 100 ml of crude oil. Thereafter, the remedants which were obtained by sun-drying and room-drying the *Azadirachta Indica* leaves were added into the reactors in varying quantities from 50 g to 100 g to ascertain its effects on the contaminated soil for 35 days. Characteristics of the soil will be taken before and after application of the pollution reagent (Bonny light).

Sample preparation and experimental setup

Twenty-six (26) reactors were used in the experiment of which 2 were control experiment containing crude oil contaminated soils without bioremediant. The other 24 reactors contained crude oil contaminated soils thoroughly mixed with varying quantities of either sun-dried or room-dried blended *A. indica* leaves. The crude oil sample obtained from was investigated for its Poly Aromatic Hydrocarbon and Total Petroleum Hydrocarbon content. The sandy and loamy soils were weighed 1kg into thirteen places for each type of soil. Some of the *A. indica* leaves was sun-dried for 7 days and the others were room-dried for 14 days. Both the sun-dried and room-dried leaves were thoroughly blended. The weighed soil samples were contained in 26 reactors, 1 kg of soil to 1 reactor. Each 1 kg of soil sample was then contaminated with 100ml of crude by thorough mixing it

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Table 5: TPH as a function of exposure time in soil treated with 80 g of *A. indica* leaves powder

Time (days)	SSD (ppm)	LSD (ppm)	SRD (ppm)	LRD (ppm)
0	35818.69	48508.92	35818.69	48508.92
7	31919.62	42636.48	31883.73	43967.03
14	25683.22	34273.21	23390.61	33241.72
21	25357.28	34035.58	21921.3	28240.6
28	13013.31	18396.68	8296.345	17638.94
35	5632.847	4538.681	4538.681	12252.9

Table 6: TPH as a function of exposure time in in soil treated with 90 g of *A. indica* leaves powder

Time (days)	SSD (ppm)	LSD (ppm)	SRD (ppm)	LRD (ppm)
0	35818.69	48508.92	35818.69	48508.92
7	31717.64	42248.66	30441.2	40792.57
14	23616.36	32797.83	19934.21	28460.65
21	21268.04	28837.05	17744.23	21510.92
28	11085.56	19888.24	8432.991	13894.15
35	6513.655	8868.248	2198.7	6513.655

Table 7: TPH as a function of exposure time in soil treated with 100 g of *A. indica* leaves powder

Time (days)	SSD (ppm)	LSD (ppm)	SRD (ppm)	LRD (ppm)
0	35818.69	48508.92	35818.69	48508.92
7	29860.58	41159.9	27969.31	38315.02
14	19646.73	29005.54	15816.61	25273.57
21	12323.34	21844.79	9236.23	18698.77
28	6365.602	13771.45	3785.575	10236.94
35	1604.248	6079.484	1349.109	3977.739

with the soil. The blended sun-dried leaves were weighed ranging from 50g to 100g with one replicate for each measure. The room-dried leafs were weighed ranging from 50g to 100g with one replicate for each measure also. This summed up to 24 weighed blended leaf samples. The weighed leaf samples were systematically applied on 24 of the contaminated soil samples. Schematic representation of the experimental set up is shown in Table 1.

SSD represents the reactor containing sandy soil contaminated with crude oil and inoculated with blended sun-dried *A. indica* leaves. SRD is the same with SSD except that blended room-dried *A. indica* leaves were used instead of the sun-dried. To each type of soil contaminated with crude oil, quantities of either sun-dried or room-dried blended *A. indica* leaves ranging from 50 g to 100 g were added as illustrated in table 1. Each combination was thoroughly mixed to ensure uniformity of the components.

Before inoculation, the contaminated soils were analyzed for initial conditions like Total Hydrocarbon Content, initial microbial activity, conductivity, pH etc. Subsequently, the 24 experimental samples were analyzed every week for a period of 5 weeks. Within this period, 10ml of water was added to each of the 24 inoculated soil samples to aid easy movement of microbes.

Gas chromatographic analysis

The soil sample was poured into a 1 liter separation funnel. A 50 mL of methylene chloride was added to the sample bottle seal and shook for 30 seconds to rinse the inner surface. The solvent was transferred to the separation funnel and then sample was extracted by shaking the funnel for 2 minutes with periodic venting to release excess pressure.

The organic layer was allowed to separate from the water phase for a minimum of 10 minutes and then the methylene chloride was extracted in a 250 mL flask. A second 60mL volume of methylene chloride was added to the sample bottle. The separation funnel and the column were rinsed

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with 20 mL of the solvent into the extract. The extraction procedure was repeated a second time and combined with the other extract in an Erlenmeyer flask. The third extraction was performed in the same way. The combined extracts were poured through a drying column containing packed cotton wool, anhydrous sodium sulphate and silica. The extract was collected in the vial and concentrated by boiling it down with nitrogen gas to 1.0 mL. The remaining extract was mixed with 1.0 mL of the solvent and 1.0 μL was injected into the flame ionization detector gas chromatograph for the TPH analysis.

RESULTS AND DISCUSSION

The results obtained from this research work are presented in Tables and Figures. After 35 days of analyzing the soil samples at 7 days intervals, results were obtained. The results were then computed as presented in Table 2, 3, 4, 5, 6 and 7. The results presented in Table 2, 3, 4, 5, 6 and 7 were able to describe the effect of various quantity of *A. indica* leaves on Total Petroleum Hydrocarbon (TPH) degradation. Decrease in TPH was observed with increase in period of exposure for the various dosages.

SSD:	$y = 0.0165x^5 - 1.4148x^4 + 40.903x^3 + 472.57x^2 + 1600.8x + 35819$	$R^2 = 1$	(1)
LSD:	$y = 0.0083x^5 - 0.7222x^4 + 20.751x^3 - 237.69x^2 + 541.74x + 48509$	$R^2 = 1$	(2)
SRD:	$y = 0.0148x^5 - 1.2998x^4 + 39.156x^3 - 478.63x^2 + 1492.8x + 35819$	$R^2 = 1$	(3)
LRD:	$y = 0.0202x^5 - 1.7576x^4 + 51.963x^3 - 612.61x^2 + 1896.5x + 48509$	$R^2 = 1$	(4)
SSD:	$y = 0.0098x^5 - 0.718x^4 + 17.194x^3 - 159.6x^2 + 40.574x + 35819$	$R^2 = 1$	(5)
LSD:	$y = 0.0129x^5 - 0.9947x^4 + 25.897x^3 - 269.65x^2 + 357.13x + 48509$	$R^2 = 1$	(6)
SRD:	$y = 0.0149x^5 - 1.2018x^4 + 33.728x^3 - 390.77x^2 + 973.7x + 35819$	$R^2 = 1$	(7)
LRD:	$y = -0.049x^5 + 0.3044x^4 - 5.0435x^3 - 0.5995x^2 - 336.15x + 48509$	$R^2 = 1$	(8)
SSD:	$y = 0.0042x^5 - 0.3601x^4 + 10.5x^3 - 126.12x^2 + 7.4017x + 35819$	$R^2 = 1$	(9)
LSD:	$y = 0.008x^5 - 0.1021x^4 + 4.2227x^3 - 75.866x^2 - 1.7703x + 48509$	$R^2 = 1$	(10)
SRD:	$y = 0.0008x^5 - 0.1303x^4 + 6.5038x^3 - 126x^2 + 82.595x + 35819$	$R^2 = 1$	(11)
LRD:	$y = 0.006x^5 - 0.6154x^4 + 22.05x^3 - 323.42x^2 + 728.88x + 48509$	$R^2 = 1$	(12)
SSD:	$y = 0.0303x^5 - 2.5744x^4 + 75.031x^3 - 872.3x^2 + 2682.9x + 35819$	$R^2 = 1$	(13)
LSD:	$y = 0.0371x^5 - 3.1905x^4 + 93.695x^3 - 1089.6x^2 + 3202.6x + 48509$	$R^2 = 1$	(14)
SRD:	$y = 0.0357x^5 - 3.0315x^4 + 89.242x^3 - 1064.4x^2 + 3469.7x + 35819$	$R^2 = 1$	(15)
LRD:	$y = 0.0225x^5 - 1.978x^4 + 61.302x^3 - 787.74x^2 + 2486x + 48509$	$R^2 = 1$	(16)
SSD:	$y = 0.025x^5 - 2.1534x^4 + 64.585x^3 - 787x^2 + 2437.1x + 35819$	$R^2 = 1$	(17)
LSD:	$y = 0.0161x^5 - 1.4623x^4 + 45.864x^3 - 577.16x^2 + 1361.3x + 48509$	$R^2 = 1$	(18)
SRD:	$y = 0.027x^5 - 2.3936x^4 + 73.948x^3 - 923.35x^2 + 2827.8x + 35819$	$R^2 = 1$	(19)
LRD:	$y = 0.0114x^5 - 1.0767x^4 + 36.112x^3 - 494.79x^2 + 933.67x + 48509$	$R^2 = 1$	(20)
SSD:	$y = 0.005x^5 - 0.4985x^4 + 18.318x^3 - 282.71x^2 + 389.27x + 35819$	$R^2 = 1$	(21)
LSD:	$y = 0.0114x^5 - 1.0675x^4 + 35.686x^3 - 490.7x^2 + 975.33x + 48509$	$R^2 = 1$	(22)
SRD:	$y = 0.0102x^5 - 0.965x^4 + 32.79x^3 - 454.17x^2 + 757.54x + 35819$	$R^2 = 1$	(23)
LRD:	$y = 0.0149x^5 - 1.3517x^4 + 43.008x^3 - 545.41x^2 + 681.98x + 48509$	$R^2 = 1$	(24)

$y = 0.005(5)^5 - 0.4985(5)^4 + 18.318(5)^3 - 282.71(5)^2 + 389.27(5) + 35819 = 32832.04$	(25)
$y = 0.005(10)^5 - 0.4985(10)^4 + 18.318(10)^3 - 282.71(10)^2 + 389.27(10) + 35819 = 25273.7$	(26)
$y = 0.005(15)^5 - 0.4985(15)^4 + 18.318(15)^3 - 282.71(15)^2 + 389.27(15) + 35819 = 18431.860$	(27)
$y = 0.005(20)^5 - 0.4985(20)^4 + 18.318(20)^3 - 282.71(20)^2 + 389.27(20) + 35819 = 13304.4$	(28)
$y = 0.005(25)^5 - 0.4985(25)^4 + 18.318(25)^3 - 282.71(25)^2 + 389.27(25) + 35819 = 9177.3$	(29)
$y = 0.0114(5)^5 - 1.0675(5)^4 + 35.686(5)^3 - 490.7(5)^2 + 975.33(5) + 48509 = 44946.58$	(30)
$y = 0.0114(10)^5 - 1.0675(10)^4 + 35.686(10)^3 - 490.7(10)^2 + 975.33(10) + 48509 = 35337.3$	(31)
$y = 0.0114(15)^5 - 1.0675(15)^4 + 35.686(15)^3 - 490.7(15)^2 + 975.33(15) + 48509 = 27766.14$	(32)
$y = 0.0114(20)^5 - 1.0675(20)^4 + 35.686(20)^3 - 490.7(20)^2 + 975.33(20) + 48509 = 22855.6$	(33)
$y = 0.0114(25)^5 - 1.0675(25)^4 + 35.686(25)^3 - 490.7(25)^2 + 975.33(25) + 48509 = 18040.68$	(34)
$y = 0.0102(5)^5 - 0.965(5)^4 + 32.79(5)^3 - 454.17(5)^2 + 757.54(5) + 35819 = 42861.4$	(35)
$y = 0.0102(10)^5 - 0.965(10)^4 + 32.79(10)^3 - 454.17(10)^2 + 757.54(10) + 35819 = 22137.4$	(36)
$y = 0.0102(15)^5 - 0.965(15)^4 + 32.79(15)^3 - 454.17(15)^2 + 757.54(15) + 35819 = 14552.6$	(37)
$y = 0.0102(20)^5 - 0.965(20)^4 + 32.79(20)^3 - 454.17(20)^2 + 757.54(20) + 35819 = 9861.8$	(38)
$y = 0.0102(25)^5 - 0.965(25)^4 + 32.79(25)^3 - 454.17(25)^2 + 757.54(15) + 35819 = 5901.25$	(39)
$y = 0.0149(5)^5 - 1.3517(5)^4 + 43.008(5)^3 - 545.41(5)^2 + 681.98(5) + 48509 = 42861.4$	(40)
$y = 0.0149(10)^5 - 1.3517(10)^4 + 43.008(10)^3 - 545.41(10)^2 + 681.98(10) + 48509 = 31768.8$	(41)
$y = 0.0149(15)^5 - 1.3517(15)^4 + 43.008(15)^3 - 545.41(15)^2 + 681.98(15) + 48509 = 24058.33$	(42)
$y = 0.0149(20)^5 - 1.3517(20)^4 + 43.008(20)^3 - 545.41(20)^2 + 681.98(20) + 48509 = 19456.6$	(43)
$y = 0.0149(25)^5 - 1.3517(25)^4 + 43.008(25)^3 - 545.41(25)^2 + 681.98(25) + 48509 = 14177.25$	(44)
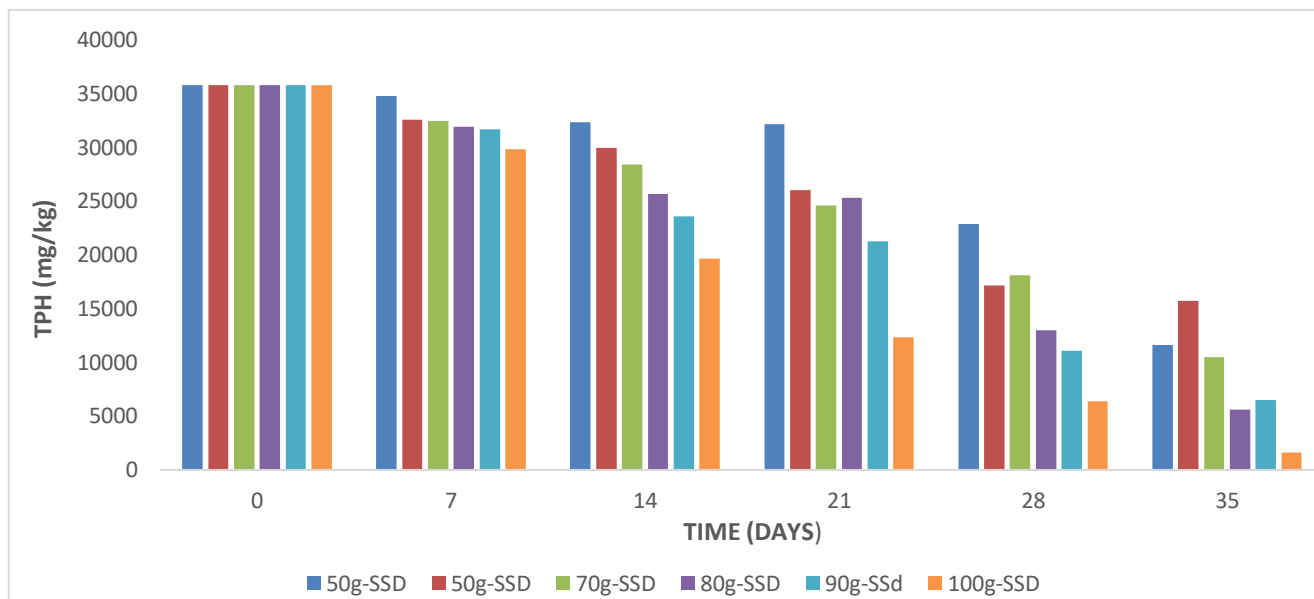
Fig. 1: TPH remediation in sandy soils using varying quantities of blended sun dried *A. indica leaves*
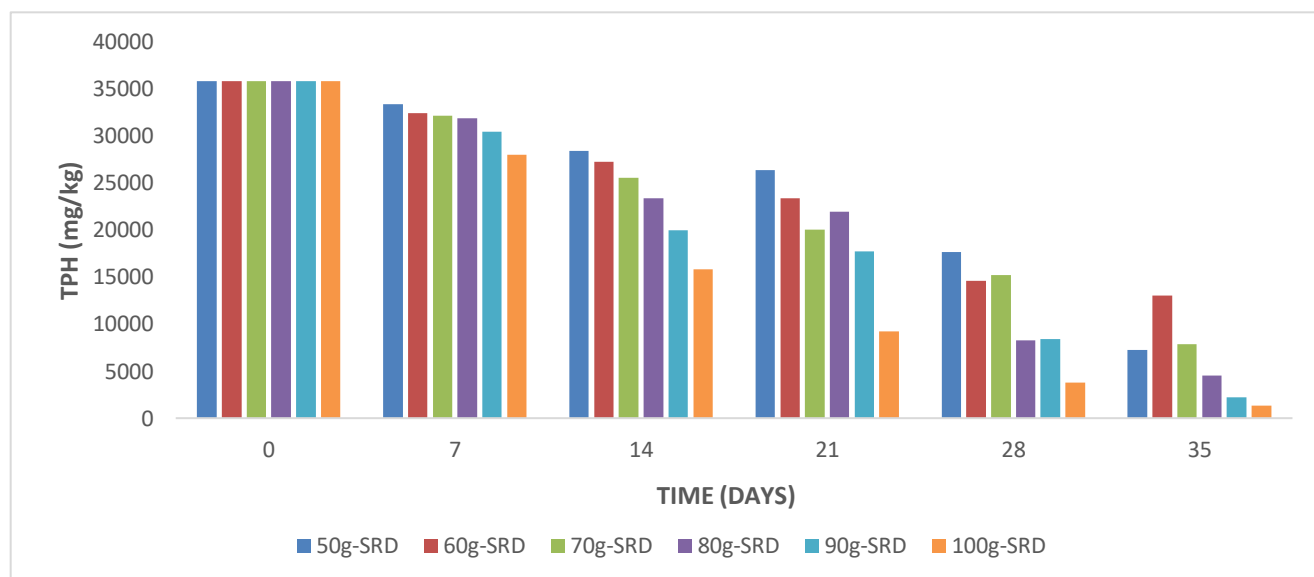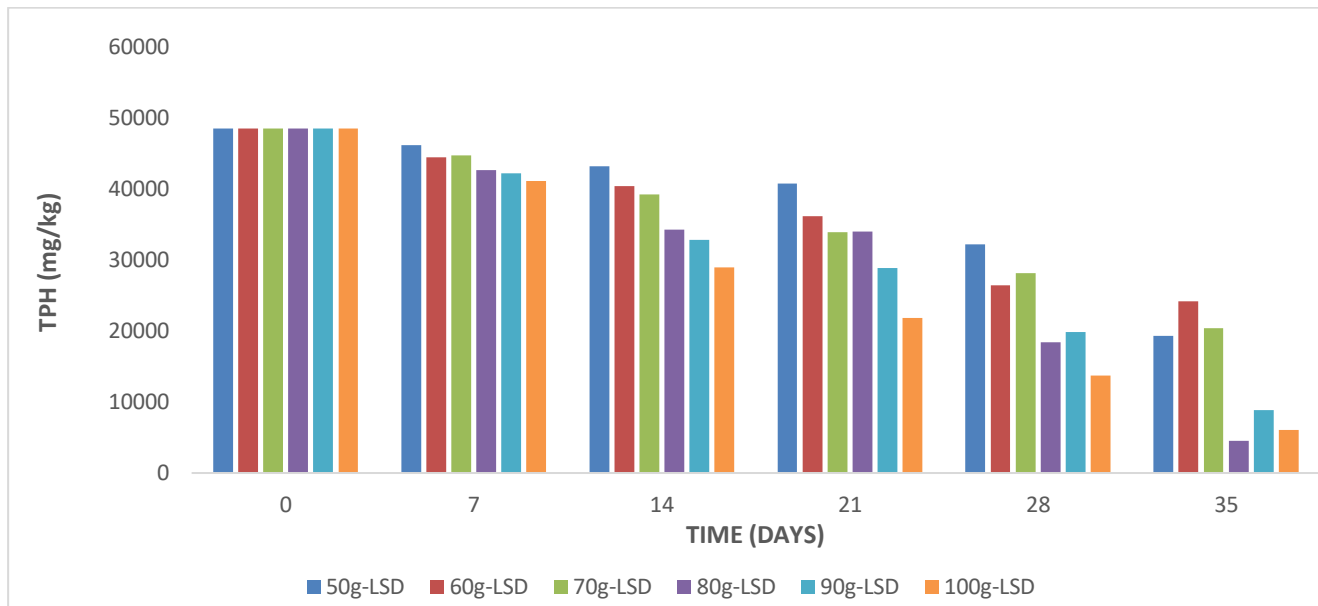

Fig. 2: TPH remediation in sandy soils using varying quantities of blended room-dried *A. indica* leaves

The soil and crude oil used in the experiment were subjected to physicochemical tests to ascertain their physical and chemical constituents. The results are shown in the Tables 2-7.

Models for the different quantities of *A. indica* leaves

The least square method was used to compute the model that best fits each of the plots. In the model, y represents TPH and x represents Time. The regression analysis of 50 g

A. indica leaves (TPH vs time) is shown in Eqs. 1-4, whereas Eqs. 5-8 showed the regression analysis of 60 g *A. indica* leaves (TPH vs time) and regression analysis of 70 g *A. indica* leaves (TPH vs time) are shown in Eqs. 9-12. Regression analysis of 80 g *A. indica* leaves (TPH vs time) is shown in Eqs. 13-16, while regression analysis of 90 g *A. indica* leaves (TPH vs time) is shown in Eqs. 17-20. 13-16 and for 100 g *A. indica* leaves (TPH vs time), it is shown in Eqs. 21-24.

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Fig. 3: TPH remediation in loamy soils using varying quantities of blended sun dried *A. indica leaves*
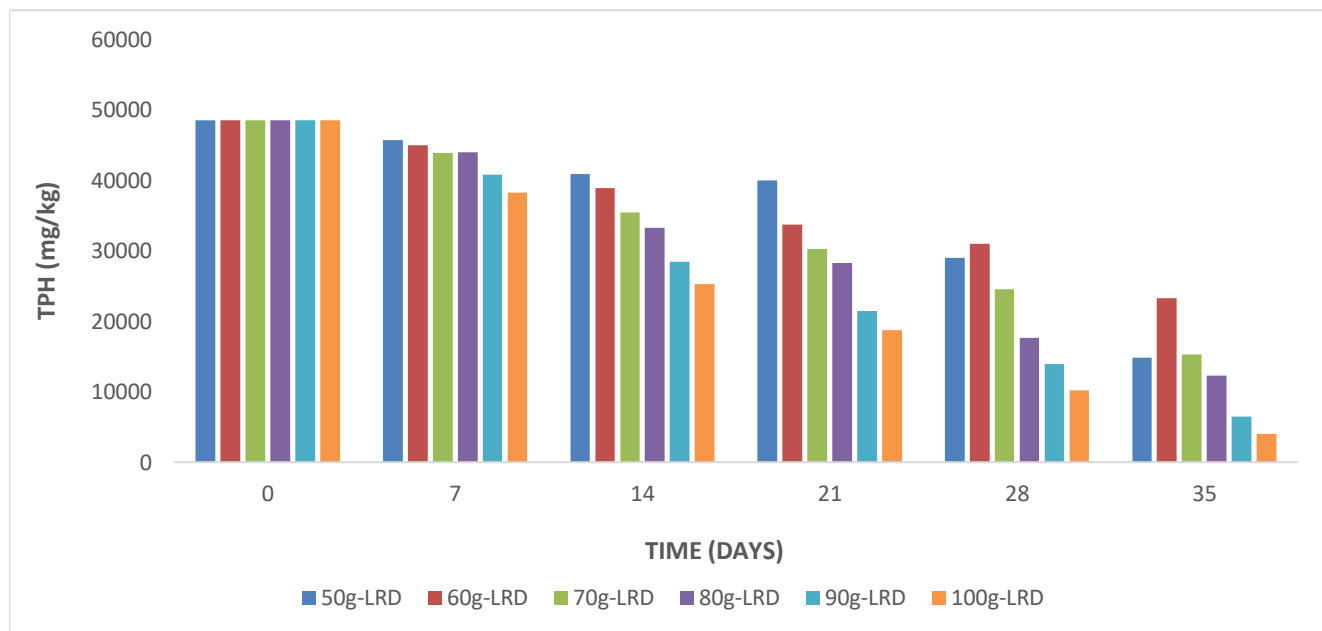
Fig. 4: TPH remediation in loamy soils using varying quantities of blended room-dried *A. indica* leaves

Having accuracies of 100%, these models can be used to predict the amount of TPH remediated at any other time. For instance, the TPH remediated at the fifth, tenth, fifteenth, twentieth and twenty-fifth day can be estimated by simply imputing any of the afore-stated days as a value of x in the models expressing either SSD, LSD, SRD or LRD. For 100 g SSD at the 5th, 10th, 15th, 20th, and 25 th day the regressions are shown in Eqs. 25-29, whereas, the regressions for 100 g LSD at the 5th, 10th, 15th, 20th, and 25th day are shown in Eqs. 30-34 and the SRD regressions for

100 g at the 5th, 10th, 15th, 20th, and 25th day are shown in Eqs. 35-39 and for 100 g LRD at the 5th, 10th, 15th, 20th, and 25th day, the regressions are shown in Eqs. 40-44.

The estimated values of TPH obtained from the regression models were seen to follow the trail of the experimental values of TPH. That is, the TPH estimated falls in line with the TPH obtained from experiment. For instance, TPH for SSD at the fifth day was estimated to be 32832.04mg/kg which is a good precedence for the experimental TPH of SSD at the seventh day observed to be

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29860.58mg/kg. Also, TPH estimated for LRD at the twentieth day is 19456.6mg/kg, a good precedence for the experimental TPH of LRD at the twenty-first day observed to be 18698.77mg/kg.

Sandy soils treated with sundried *A. indica* leaves (SSD)

Crude oil contaminated sandy soils treated with sundried *A. Indica* leaves were observed to contain the same amount of total petroleum hydrocarbon content (TPH) at the preliminary stage of the experiment as described in Figure 1. As shown in Figure 2, for a period of day 7 to day 14, it can be observed that TPH reduced with respect to quantity of *A. Indica* leaves used. This could be as a result of lot of factors of which most prominent is the steady exponential growth of hydrocarbon degraders (microbes) in each sample with respect to the quantity of *A. Indica* leaves used. Subsequently from day 21 to day 35, it can be observed that the reduction of TPH was erratic and did not completely follow the idea that the higher the quantity of the inoculant (*Azadirachta indica*), the greater the remediation. For instance, at day 35, it can be seen that 50 g of the inoculant caused more remediation than 60 g of inoculant.

Sandy soil treated with room-dried *A. indica* leaves (SRD)

This set of samples also followed the trend of SSD as it can be observed that at the beginning of the experiment, all the samples maintained the same range of TPH. From day 7 to day 14, the TPH is observed to also deplete with respect to the amount of *A. indica* leaves used. This is illustrated in Figure 2. For a period of day 21 to day 35, it can be seen that TPH depletion with respect to amount of inoculant used exhibited some irregularities among each group of data. That is, where some samples with lesser quantities of inoculants gave better remediation results than samples with higher quantities of inoculants.

Loamy soil treated with sundried *A. indica* leaves (LSD)

Crude oil contaminated Loamy soils treated with sundried *A. indica* leaves were observed to contain the same amount of total petroleum hydrocarbon content (TPH) at the preliminary stage of the experiment as described in Figure 3. From Figure 3, it can be observed that TPH reduced with respect to quantity of *A. indica* leaves used for a period of day 7 to day 14, . This could be as a result of lot of factors of which most prominent is the steady exponential growth of hydrocarbon degraders (microbes) in each sample with respect to the quantity of *A. indica* leaves used. Subsequently from day 21 to day 35, it can be observed that the reduction of TPH was erratic and did not completely follow the idea that the higher the quantity of the inoculant (*A. indica*), the greater the remediation. For instance, at day 35, it can be seen that 80 g of the inoculant caused more remediation than 100 g of inoculant.

Loamy soils treated with room-dried *A. indica* leaves (LRD)

This set of samples also followed the trend of SRD as it can be observed that at the beginning of the experiment, all the samples maintained the same range of TPH. From day 7 to day 14, the TPH is observed to also deplete with respect to the amount of *A. indica* leaves used. This is illustrated in Figure 4. For a period of day 21 to day 35, it can be seen that TPH depletion with respect to amount of inoculant used exhibited some irregularities among each group of the data. That is, where some samples with lesser quantities of inoculants gave better remediation results than samples with higher quantities of inoculants.

In the long run, it was observed that sandy soil treated with 100 g of blended room-dried *A. indica* leaves had the highest remediation as its TPH content dropped from 35818.69 mg/kg to 1349.109 mg/kg in 35 days. It was also observed that loamy soil treated with sundried *A. indica* leaves had the lowest remediation as its TPH dropped from 48508.92 mg/kg to 24199.65 mg/kg in 35 days. Based on findings, it can be concluded that *A. indica* leaves powder is efficient to remediate soil contaminated with crude oil, which is one major issue of the soil contamination and using remediation agents form natural resource is cost effective and ecofriendly (Deivakumari et al., 2020; Ferguson et al., 2020; Kang et al., 2020; Vasilyeva et al., 2020).

CONCLUSIONS

In tandem with the objectives of this research, the following conclusion was drawn; *A. indica* leaves is a good crude oil bioremedant. Blended Room dried *A. indica* leaves gives better crude oil remediation compared to the sundried. Crude oil remediation occurs faster in sandy soils than in loamy soils. Every 1kg of soil polluted with 100ml of crude oil requires 100g of blended room dried *A. indica* leaves to achieve significant remediation of its TPH in 35 days. Oxygenation by addition of water enhances the rate of bioremediation In the course of the experiment, some limitations were experienced and adequate innovative ideas were put together to counter these limitations. In Subsequent research regarding treating crude oil contaminated soils using *A. Indica* leaves*. Based on observation, the recommendations are as; larger quantity of *A. indica* leaves should be used. Comparisons should be made between Wet-blended *A. indica* leaves and room-dried *A. indica* leaves in crude oil remediation. The soil samples should be moisturized with water at intervals to enable the proliferation of micro-organisms in the soil. The soil samples should be stirred at intervals to enable the movement of micro-organisms within the soil and long term experiment should be carried out for a longer period to monitor the TPH properly.

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