213 *Egypt.J.Chem.* **Vol. 63**, No.8. pp.2827-2836 (2020)

Egyptian Journal of Chemistry
http://ejchem.journals.ekb.eg/

Bioremediation of *In-Situ* Crude Oil Contaminated Soil Using Selected Organic Dung

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THE potential of cow, pig and poultry dung for the bioremediation of in-situ crude oil contaminated soil for a period of 3-15 days at different soil and dung ratios were the objectives of this investigation. Using simulated soil, 200 g were measured into polyethylene bags labelled A,B,C,D,E, F and G. Soil physicochemical parameters such as particle size distribution, pH, organic carbon, organic matter, nitrogen and phosphorus were analysed using standard methods. Total Petroleum Hydrocarbon (TPH) concentrations were analyzed using spectrophotometry method at 420 nm. The concentrations of TPH decreased progressively with an increase in dung and time in the order cow dung > pig dung > poultry dung except the control soil that showed slight TPH reduction. Results also show that biostimulant efficiency (BE) increases with increase in dung and reduces with time. Results also reveal that dung with high bioremediation constant recorded a corresponding short half-life. Results show that there is a significant difference ($P < 0.05$) between dung type and stimulation time. Similarly, there is no significant difference ($P > 0.05$) in amended soil and time for poultry dung except for cow and pig dung. This study has shown that cow dung is more effective in bioremediation of TPH in crude oil contaminated soil.

Keywords: TPH, Bioremediation, Biostimulant efficiency, Dung, Contaminated soil.

Introduction

Petroleum is a complex mixture containing several and different saturated and unsaturated hydrocarbons, and polar-organic compounds [1]. Water and land pollution by the presence of crude oil and associated products could occur through natural (oil seeps from the bottom of oceans which enters the marine environment) and anthropogenic (equipment breakdown – tankers, barges, pipeline, refineries, drilling rigs and storage tanks) sources [2]. Environment and human health challenges associated land pollution with crude oil has drawn global attention [3-4]. Land pollution with crude oil adversely affects the soil and aquatic ecosystem through adsorption to soil particles, provision of excess carbon that might be unavailable for microbial use [5]. Also, crude oil contamination of soil has been reported to be the most life threatening due to its multiplier effects of soil not providing its traditional ecosystem support [6-8]. Remediation is the removal, destruction or transformation of contaminants to less harmful substances [9]. Land polluted with crude oil need to be reclaimed through treatment (physicochemical, thermal or biological) processes. The selection and application of remediation methods depend on the physicochemical and biological characteristics of the contaminants, treatment option and agents [10]. The remediation of soil contaminated with crude oil with the traditional physicochemical and thermal methods are known to be associated with changing complex or simple chemical structures to more hazardous complexes or simple by-products [10]. The traditional method is also expensive, laborious and do not always completely remove contaminants from soil [9]. Inadequate mineral

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Received 10/10/2019; Accepted 12/2/2020
DOI: 10.21608/ejchem.2020.18048.2098

nutrients, especially nitrogen, and phosphorus, often limit the growth of hydrocarbon utilizing bacteria in water and soil [11]. Addition of nitrogen, phosphorus and carbon as source of nutrient has shown to accelerate biodegradation of total petroleum hydrocarbons (TPH) in soil polluted with crude oil by stimulating the growth of microorganisms responsible for biodegradation of organic pollutants [12-14].

In Nigeria, pollution of terrestrial and aquatic ecosystem through oil spill is prevalent due to oil exploration, exploitation and allied activities. The continuous deposition and accumulation may pose human and environment health challenges due to its hazardous nature. The traditional physicochemical and thermal remediation techniques for attenuation of soil polluted with crude oil are known to be expensive and produce by-products that may be harmful to environment and human health. An alternative approach to remediation of soil polluted with crude oil is bioremediation [11]. Utilization of organic waste in the bioremediation of oil contaminated soil has been suggested [15]. This will reduce the amount of organic waste sent to landfill, thus reduce the emission of landfill gases, its associated pollution effects and serve as a cheap source of organic additive for remediation purposes. Nevertheless, extensive research is necessary to identify suitable techniques in utilization of organic wastes in bioremediation of crude oil contaminated soil. Therefore, the objective of this study is to identify the strategies and potential of applying selected cow, pig and poultry dung in enhancing remediation of land polluted with crude oil**.**

Materials And Methods

Soil and organic waste collection

Soil samples devoid of hydrocarbon contamination were sampled from site by the Faculty of Science Delta State University, Abraka using a clean shovel at a sample depth of 0 - 30 cm. cow, pig and poultry dung were collected from farms in Abraka. Crude oil with specific gravity of 0.85 g/cm^3 was collected from Seplat oil station, Sapele, Nigeria.

Sample preparation stimulation and amendment

Soil samples were air dried for 4 days and sieved by passing through a 1.5 mm mesh to remove dirt and suspended particles. 960 g of the soil was simulated with 282 ml of crude oil artificially in a clean dry polyethene bag and thoroughly mixed to achieve homogeity. Using the stimulated soil, 200 g was measured into 6 (six) clean dry polyethene bag labeled A, B, C, D, E, F and G. An experimental polyethene bag containing 200 g of natural soil without amendment serves as control The simulated soil sample labeled A, B, C, D, E, F and G were amended with dung in different ratio of 0.00% 2.56%, 5.26%, 8.11%, 11.11% 14.28% and 17.64%. These mixtures were thoroughly mixed separately to get a composite sample and exposed to the natural environment to allow proper decomposition for three (3) weeks and monitored for TPH concentrations at 3-day intervals.

Soil Physicochemical Properties

The pH of the soil samples was measured by a pH meter using the method described by Emoyan *et al* [16]. Phosphorus was determined using the alkaline oxidation method as described by Dick and Tabatabai [17]. Particle size distribution was determined by the hydrometer method as described by Sheldrick and Wang [18]. Moisture content was evaluated by the gravimetric method as described by Black [19]. Total nitrogen was determined by the microkjeldahl digestion method [20]. Organic carbon determination was by the modified Walkley-Black method [21] .

Determination of Total Petroleum Hydrocarbon (TPH)

The residual petroleum hydrocarbon content of the soil samples (amended and unamended) during study period was determined gravimetrically by solvent extraction method [15, 22]. Thus, 10 g of simulated soil samples (triplicates) was taken and transferred into a 50-ml flask and the hydrocarbon content in oil polluted soil was extracted using 20 ml of n-hexane. The mixture was shaken vigorously on a magnetic stirrer for 30 min and allowed to stand for 10 min until the hexane extract completely separate the oil from the soil sample. The solution was then filtered using Whatman 14 filter paper and the filtrate diluted by taking 1 ml of the extract into 50 ml of hexane. The absorbance of this solution was measured using UV visible spectrophotometry at a wavelength of 420 nm (Spectronic 721 Model) using n-hexane as blank. The total hydrocarbons in the soil sample was estimated with reference to a standard curve derived from fresh spent engine oil of different concentration diluted with n-hexane. The concentration of TPH was monitored at 3-day intervals over 15 days.

Statistical Evaluation and Models

Primary data were subjected to one-way analysis of variance (ANOVA) for level of significant differences at $p < 0.05$ using software statistical package for social sciences (SPSS), version 17.0.

Biodegradation percentage

The percentage of biodegradation was calculated using the following formula:

$$\% \text{ Biodegradation} = \frac{TPH_i - TPH_f}{TPH} \times 100 \quad (1)$$

Where: TPHi and TPH_f are the initial and final TPH, respectively.

Biostimulant Efficiency and Net loss of total hydrocarbon content

The dung efficiency in the oil contaminated soil was estimated by determining the percentage loss (PL) and biostimulant efficiency (BE) based on each soil amendment. The % biostimulant efficiency was calculated using the modified equation stated by Agarry, [23].

$$\% BE = \frac{PLTPH_{as} - PLTPH_{us}}{PLTPH_{as}} \times 100 \quad (2)$$

Where: $PLTPH_{as}$ and $PLTPH_{us}$ are amended soil and un-amended soil respectively.

First order kinetics and half-life for biodegradation of TPH

This model is given as

$$In[TPH]_t = -kt + In[TPH]_o \quad (3)$$

Where $[TPH]_o$ is the initial TPH concentrations in soil (g/kg), $[TPH]_f$ is the final TPH content in soil at time *t*, (day) , *k* is the biodegradation rate constant (day^{-1}) and t is time (day).

The Biodegradation half-life ($t_{1/2}$) of Petroleum hydrocarbons was estimated by model as stated in Agarry et al [23].

$$\text{Half life} = (t_{1/2}) = \frac{In2}{k} \quad (4)$$

Where *k* is the biodegradation rate constant (day^{-1}) from Equation 3.

Results and Discussion

Physicochemical Analysis

The baseline concentrations of physicochemical characteristics of soil and dung before amendment in this study, Table 2, show that pH level of the dung and soil in this study range between 5.86 and 7.49. The observed values could be used to determine the biodegradation effects on TPH. In soil, microbial metabolism of substrate is restricted to bio-available fractions in soil-pore or surfaces of soil particles [24]. Greater fungal populations are active in soil acidic pH, *Pencillium* species predominate in soil acidic pH with lower *Aspergiluss* population [25-26]. Research has shown that soil enzymatic actions are high at soil pH of 7.18 – 7.53 [26]. This suggests that the observed soil and dung pH range of 5.86 and 7.49 could be responsible for the degradation of TPH in this study. Organic carbon and organic matter of un-amended soil and dung range from 9.22 to 11.71% and 3.32 to 12.43% respectively, Table 2.

Several studies have shown that organic compounds – TPH, PAHs, PCB, dioxine etc – with high values of Kow and low solubility would most likely be retained by soil surface and less susceptible to environmental processes [16]. The particle size distribution obtained indicates that the soil consists of 95.79%, sand fraction with clay and silt having 2.00% and 2.21% respectively, Table 2. Also, fine soil particles (< 0.002 nm) have larger surface area; hence fine soil distribution is a measure of adsorption of contaminants than large particles [27]. Similarly, the adsorption of organic contaminants such as TPH in soil mostly occurs in the clay and silt fractions [28]. In this study, the particle size distribution shows that the soil is a sandy soil with 95%, this could retard adsorption and favors non-sorption of TPH on active soil surfaces and hence are available for biodegradation.

TABLE 1. Experimental design.

Samples	% Amendment	Weight of Soil	Weight of Dung
(A)	0 % amendment contains	200 g	0 g
(B)	2.56% amendment contains	195 g	5 g
(C)	5.26% amendment contains	190 g	10 g
(D)	8.11% amendment contains	185 g	15 g
(E)	11.11 % amendment contains	180 g	20 g
(F)	14.28% amendment contains	175 g	25 g
(G)	17.64% amendment contains	170 g	30 g

Stimulated soil versus remediation effects

Bioremediation of crude oil polluted soil was done using cow, pig and poultry dung to simulate the soil indigenous microbial population at different time interval. The concentrations of TPH after different stimulation of soil polluted with crude oil with variable organic wastes at 0, 2.56, 5.26, 8.11, 11.11, 14.28 and 17.64%, and 0, 3, 6, 9, 12 and 15 days treatment options, Table 3. From the remediation period of 0 - 15 days, in treatment option A, results of cow, pig and poultry dung show that there is no TPH concentrations variation. At simulation B, TPH concentrations reduced between 0.44 and 0.14 (cow dung), 0.44 and 0.25 (pig dung) and 0.44 and 0.28 (poultry dung). Similarly, results in amendment C show that TPH concentrations reduced between 0.44 and 0.13 (cow dung), 0.44 and 0.20 (pig dung) and 0.44 and 0.25 (poultry dung). Also, results show that TPH concentrations reduced between 0.44 and 0.11 (cow dung), 0.44 and 0.15 (pig dung) and 0.44 and 0.24 (poultry dung) at stimulation D. At treatment E, results show that TPH concentrations reduced from 0.44 to 0.11 (cow dung), 0.44 to 0.13 (pig dung) and 0.44 to 0.22 (poultry dung). Similarly, in treatment F, results show that TPH concentrations reduced from 0.44 to 0.10 (cow dung), 0.44 to 0.12 (pig dung) and 0.44 to 0.23 (pig dung). Similarly, results show that TPH concentrations reduced from 0.44 to 0.10 (cow dung), 0.44 to 0.14 (pig dung) and 0.44 to 0.19 (poultry dung) at stimulation G.

TABLE 2. Physicochemical characteristics of organic wastes and soil used for bioremediation.

Parameter	Soil	Cow Dung	Pig Dung	Poultry Dung
pH	5.86	7.22	7.32	7.49
Nitrogen (%)	0.17	0.62	0.58	0.49
Phosphorous (mg/kg)	8.12	634.34	624.18	612.23
Potassium (mg/kg)	72.30	82.10	72.45	71.46
Organic Carbon (%)	11.71	9.22	11.24	12.26
Organic Matter (%)	3.32	12.43	10.54	9.62
Moisture	2.42	18.25	17.36	17.46
C:N	28.1			
Clay (%)	2.00			
Silt (%)	2.21			
Sand (%)	95.79			

TABLE 3. Concentration of TPH in crude oil polluted and dung amended soil with cow, pig and poultry dung (mgg^{-1}).

Amended Soil	Dung	Day					
		0	3	6	9	12	15
A	Cow Dung	0.44	0.43	0.36	0.29	0.26	0.24
	Pig Dung	0.44	0.43	0.36	0.29	0.26	0.24
	Poultry Dung	0.44	0.43	0.36	0.29	0.26	0.24
B	Cow Dung	0.44	0.24	0.22	0.19	0.15	0.14
	Pig Dung	0.44	0.35	0.33	0.32	0.30	0.25
	Poultry Dung	0.44	0.36	0.34	0.30	0.29	0.28
C	Cow Dung	0.44	0.23	0.20	0.17	0.14	0.13
	Pig Dung	0.44	0.34	0.25	0.23	0.22	0.20
	Poultry Dung	0.44	0.35	0.34	0.32	0.28	0.25
D	Cow Dung	0.44	0.23	0.19	0.16	0.13	0.11
	Pig Dung	0.44	0.34	0.33	0.23	0.20	0.15
	Poultry Dung	0.44	0.34	0.33	0.31	0.29	0.24
E	Cow Dung	0.44	0.22	0.19	0.15	0.12	0.11
	Pig Dung	0.44	0.22	0.20	0.17	0.15	0.13
	Poultry Dung	0.44	0.32	0.30	0.28	0.25	0.22
F	Cow Dung	0.44	0.22	0.17	0.15	0.11	0.10
	Pig Dung	0.44	0.21	0.18	0.16	0.13	0.12
	Poultry Dung	0.44	0.31	0.28	0.26	0.25	0.23
G	Cow Dung	0.44	0.20	0.17	0.15	0.12	0.10
	Pig Dung	0.44	0.20	0.17	0.16	0.16	0.14
	Poultry Dung	0.44	0.30	0.25	0.23	0.21	0.19

The percentage reduction of TPH after treatment of crude oil contaminated soil with cow dung, pig dung and poultry dung between 3 to 15 days treatment shows that the percentage biodegradation ranged from 36.4 to 77.3%, Figure 1. This indicates that the carbon fractions of the crude oil in the amended samples were attenuated. The TPH was poorly reduced as observed in treatment B compared to C, D, E, F and G. In this study, high biodegradation of TPH between 6 and 15 days in soil amended with organic waste compared to A (control) amendment was observed. In 15 days remediation period, 17.64% stimulated soil showed that cow dung recorded the highest percentage biodegradation (77.3%) followed by pig dung (68.2%) with poultry dung having the least reduction (52.3%) compared to the control (45.5%). Results in this study show that crude oil polluted soil amended with cow, pig and poultry dung exhibit variable TPH biodegradability compared to un-amended soil. The percentage reductions of TPH show that there is significant increase in biodegradation between 5.26 and 17.64% amendment and 6 to 15 days remediation period, Figure 1.

This shows that an increase in time of amendment enhances the degradation properties of the parent microorganisms in the crude oil contaminated soil, thus allowing microorganisms to degrade the organic contaminant. This observation could be related to the feeding habit, biomass content and chemical composition of the organic waste particularly nitrogen and phosphorus in the three dung used in stimulating the indigenous hydrocarbon utilizing bacteria [29]. Results show that cow dung recorded the highest concentration of N and P hence the highest TPH degradation compared to pig and poultry dung. This shows that nitrogen and phosphorus are important nutrient needed by hydrocarbon utilizing bacteria to effectively degrade hydrocarbons in soil matrix [22, 30]. Also, the effects of cow dung application in TPH reduction may be due to the increase in HUB microbe population in cow dung that utilized the crude oil for carbon and energy source to degrade crude oil in the amended soil [31]. According to Oladotun and Adekunle [32], organic manure like cow dung enhances the rate of biodegradation of the contaminants and some of the products of biodegradation are useful organic nutrient, which do not inhibit the activities of beneficial microorganism and earthworms. The observed percentage (77.3%) degradation of TPH in this study shows that the HUB may be *Micrococcus* sp., *Pseudomonas* sp., *Bacillus* sp., *Corynebacterium* sp., *Nocardia* sp., *Achromobacter* and *Klebsiella sp.* as observed by different authors[15,32-36]. Statistical analysis of treatment options A to G shows that there is a significant difference at $P < 0.05$ between treatment options and stimulation time. This is indicative of positive contribution of organic waste to the bioremediation of petroleum hydrocarbons in the amended soil. Similarly, results show that there is a significant difference at $P < 0.05$ between cow and pig dung except poultry dung. This study has revealed that the use of organic manure for the bioremediation of petroleum impacted soil have more microbial consortium which degrades TPH as a result of increased percentage of manure. This may be due to microbial activities of indigenous petroleum utilizing microbes that may be present in crude oil polluted soil [36]. This study has also shown that nutrient supplementation enhances biodegradation rate which is in agreement with the works of Agamuthu, Agarry et al 2013, Obiakalaije et al [11, 37-39].

Biostimulant efficiency and biodegradation rate

Research has shown that biostimulants have degradation influence on several metabolic processes such as ion uptake, nucleic acid synthesis, photosynthesis and respiration. The biostimulant efficiency (BE) results of various dung treatments across bioremediation period from this study range between 95.78Gxy3 and -33.43Dz15. It could be deduced from the treatment options (2.56%, 5.26%, 8.11%, 11.11%, 14.28%, and 17.64%) and bioremediation period (3,6,9 and 12 days), that the biostimulant efficiency in this study is in the peak order: 95.78Gxy3 > 70.36FxGxy6 > 48.25ExFxGx9 > 45.47Fx12 > 39.33Fx15. Conversely, the lower limit is in the order: 86.26Bz3 > 19.82BzCz6 > -24.91ByCz9 > -28.62By12 > -33.43Dz15, (Figure 2). Results show that biostimulant efficiency increased down the group with increase in amendment option and decreases across the period with increase in biostimulation period. Biostimulant activity is at maximum in 17.64% (95.78Gxy3) amendment and minimum in 8.11% (-33.43Dz15) amendment with cow dung and poultry dung exhibiting high and low biostimulant efficiency respectively. Results also show that biostimulant efficiency reduces over bioremediation period; this observation could be related to the utilization of contained HUB in degradation of TPH. Results also show that biostimulant efficiency is a function of treatment option and bioremediation period, and directly proportional to percentage biodegradation. The biodegradation efficiency in this study could have been enhanced by microorganisms, soil physicochemical properties, soil/dung ratio, enzymes, and beneficial trace elements such as: cobalt, aluminum, sodium, selenium and silicon present in the soil. A similar trend was observed in studies reported by Agamuthu, and Obiakalaije et al [11, 39].

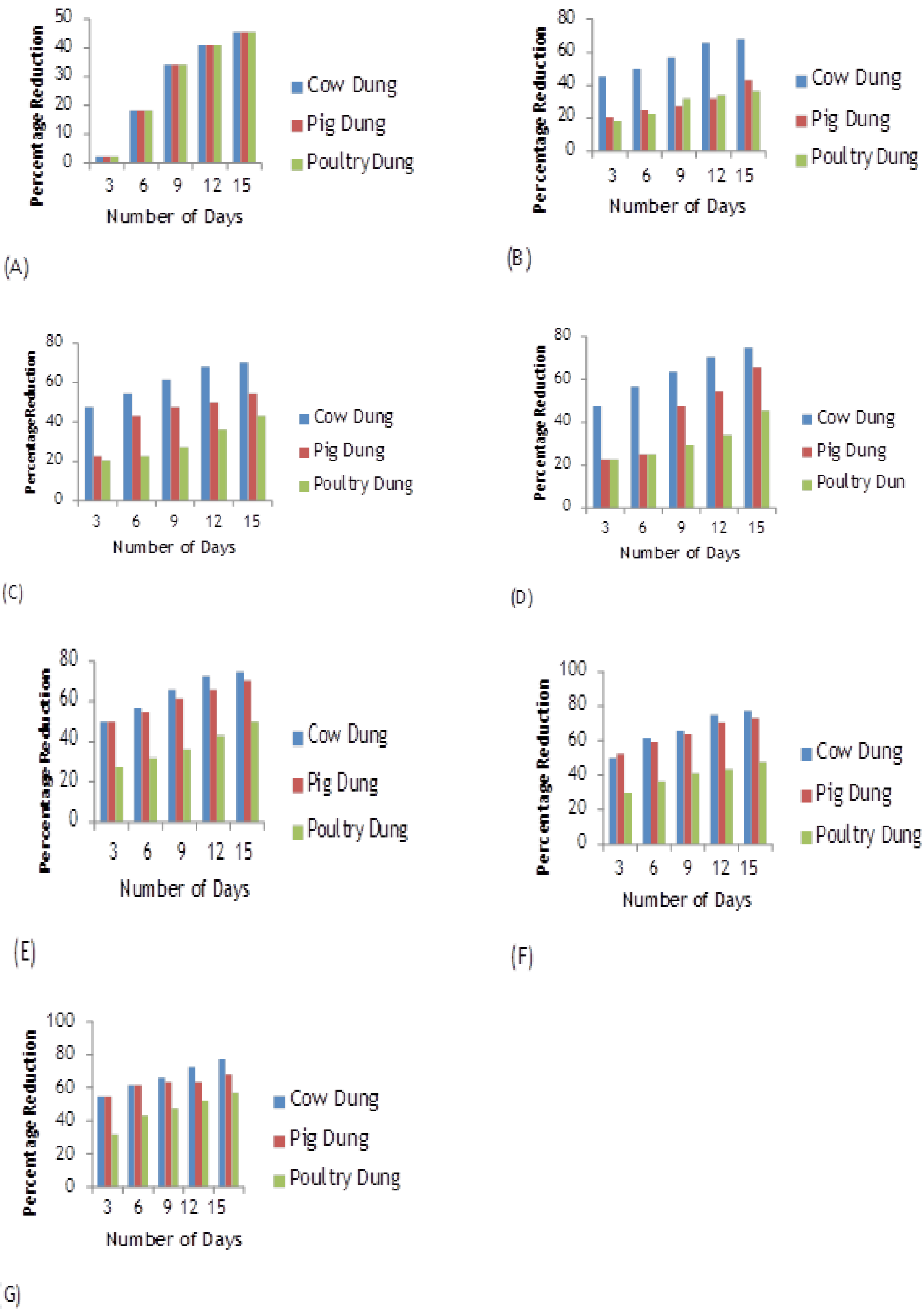

Fig. 1. Percentage reduction of TPH in cow, pig and poultry dung amended oil polluted soil at different remediation period.

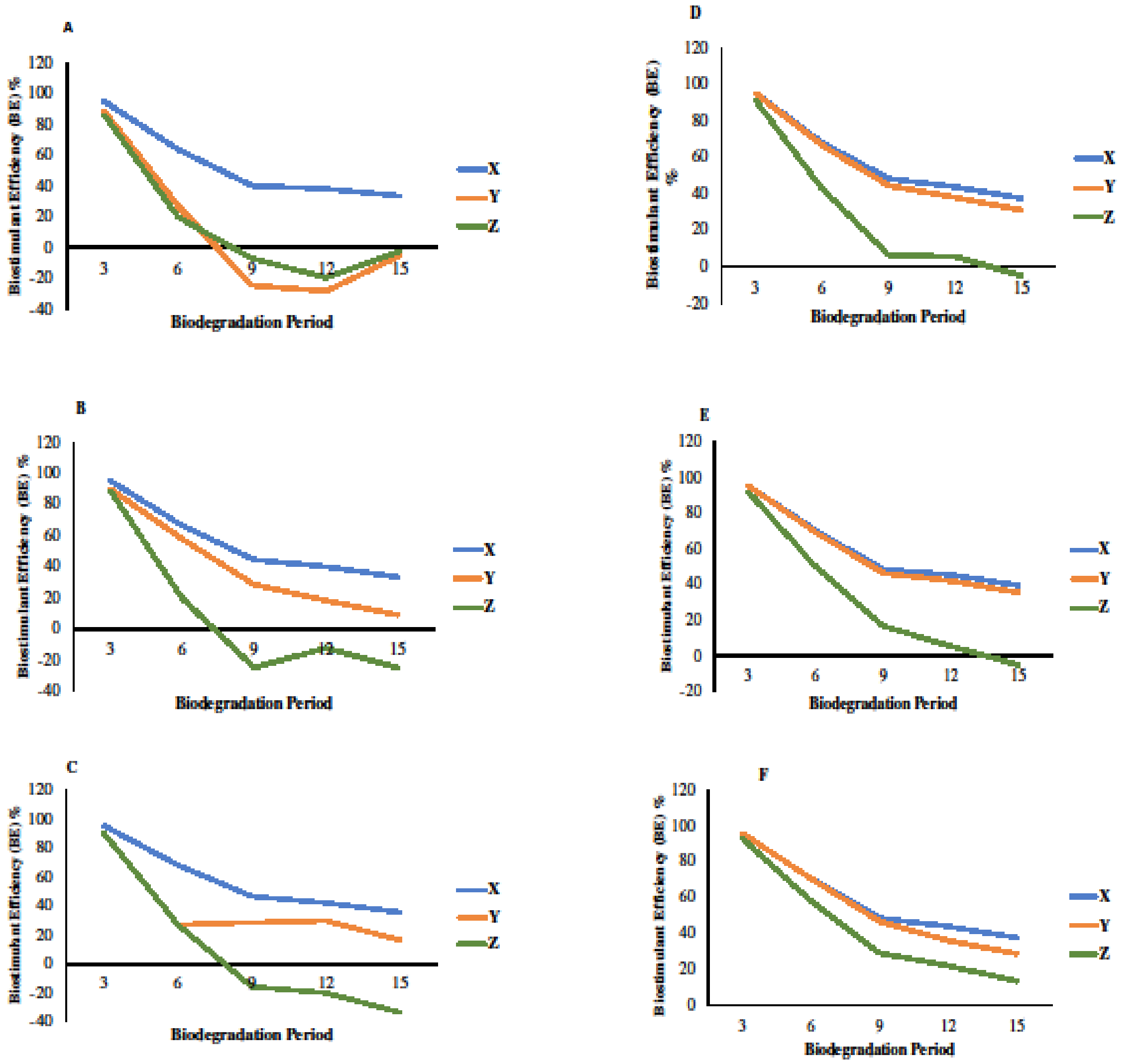

Fig. 2. Percentage reduction of TPH at different soil stimulation: A (0 %), B (2.56 %), C (5.26 %), D (8.11 %), E (11.11 %), F (14.28 %) and G (17.64 %).

Yeung et al, Abioye et al, Mohajeri et al Agarry et al , and Onuoha,, [15,36,38,40-41] had argued that the application of bioremediation kinetics in biodegradation studies gives information about bioremediation of soil contaminated with crude oil. This could be used to determine the concentration of contaminant that may likely be available at any time. Several studies have shown that first order kinetics could be effectively applied to explain the biodegradability of crude oil. Plotting the logarithm of TPH concentration versus time (day) presents appropriate information about the biodegradation rate. In this study, the result of biodegradation constant (K) and half-life ($t_{1/2}$) of TPH during the bioremediation process was calculated from the model, (equation 3 and 4). Furthermore, the bioremediation kinetics patterns also suggest a positive correlation coefficient R^2 for the reduction in TPH concentrations in all treatment options, Table 4. From this study, cow, pig and poultry dung recorded short half-life with a corresponding high biodegradation rate constant (k). Results shows that the upper and lower limits of biodegradation rate constant and half-life, in cow dung is in the order (0.0384 day^{-1}/18.1 days and 0.0284 day^{-1}24.4 days). Similarly, pig dung is in the following order (0.0967 day^{-1}/7.2 days and 0.0169 day^{-1}/41 days) and poultry dung (0.066 day^{-1}/10.5 days and 0.0353 day^{-1}/19.6 days). This is in agreement with other studies [23,38,42-45]. Results of biostimulant efficiency and k reveals that cow, pig and poultry dung were able to remediate crude oil contaminated soil in variable proportions.

TABLE 4. Biodegradation rate constant and half-life of crude oil polluted soil amended with cow, pig and poultry dung at remediation period of 3-15 days.

Amended Option	Dung	Biodegradation Rate Constant (K) day^{-1}	R^2	Half-life ($t^1/_2$) (day^{-1})
B	Cow Dung	0.0368	0.9796	18.806991
	Pig Dung	0.0591	0.9656	11.7230
	Poultry Dung	0.0599	0.8958	11.5735
C	Cow Dung	0.0338	0.9584	20.5121
	Pig Dung	0.0629	0.9659	11.0103
	Poultry Dung	0.0660	0.9825	10.4950
D	Cow Dung	0.0376	0.9635	18.4260
	Pig Dung	0.0967	0.9230	7.1640
	Poultry Dung	0.0568	0.9654	12.1975
E	Cow Dung	0.0384	0.9470	18.0510
	Pig Dung	0.0292	0.9093	23.7499
	Poultry Dung	0.0499	0.9965	13.8850
F	Cow Dung	0.0361	0.9313	19.2031
	Pig Dung	0.0276	0.9614	25.0760
	Poultry Dung	0.0353	0.8464	19.6290
G	Cow Dung	0.0284	0.9920	24.4050
	Pig Dung	0.0169	0.8864	41.0302
	Poultry Dung	0.0445	0.9121	15.5730

Conclusion and recommendations

The viability and potential of applying cow, pig and poultry dung in enhancing bioremediation of crude oil contaminated soil was investigated. This study has shown that acidic soil, sandy soil, soil with low organic carbon, and soil with high nitrogen and phosphorus content are favorable conditions for TPH degradation in crude oil contaminated soil. This study has established that cow dung is highly effective in degrading TPH in crude oil contaminated soil relative to pig and poultry dung, and the ratio of biodegradation depends on available soil nutrients. Results also show that biostimulant efficiency reduces over time of bioremediation period and is directly proportional to percentage biodegradation and a function of treatment option and bioremediation period. This study has further affirmed from the first order rate constant and half-life models, that dung-stimulated soil exhibit high efficiency in remediating crude oil contaminated soil. Results also show that there is no significant difference between treatment options and stimulation time, and between dung types except poultry dung. This study therefore recommends that further study be carried out at higher treatment options with increase in remediation time and pH variations, and identification of other organic waste with high HUB content may be carried out.

Acknowledgment

This research project was possible with full assistance of the laboratory technologist of the Department of Chemistry, Delta State University, Abraka. Special thanks are given to the cow, pig and poultry farmers of Abraka for the immeasurable assistance during the collection of samples.

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