International Journal of Latest Engineering Research and Applications (IJLERA) ISSN: 2455-7137

Volume – 08, Issue – 11, November 2023, PP – 96-103

Comparative Study of Nano-Particle of Magnesium Oxide and Cow Dung for Hydrocarbon Polluted Soil Remediation

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Abstract: Evaluating the performance of chemical (fenton nano-particle) and biological (cow dung) methodon bioremediation of hydrocarbon polluted soilwas investigated in this study. The cow dung and loamy soil sample were obtained within Agricultural farm and Fenton (nano-particle) was produced in chemical/petrochemical laboratory River State University. 300g of Loamy soil were weight and poured into 14 rectors, 100ml crude was added into 12 reactors containing treatment (5g, 10g, 15g, 20g, 25g and 30g). While we have 2 control sample (unpolluted and polluted). The sample were monitored for 7^{th} days. i.e. 1^{st} weeks – 6^{th} weeks in 45 days and where taken to the laboratory to analysis the following parameters TPH, Bacterial, pH and physiochemical parameters (density & moisture content).The biological method slightly outperformed better than the chemical method. At end of the analysis, TPH degradation in soil samples treated with 30g the percentage degradation of TPH in loamy soil was obtained biological method 97.49%, 98.12%, 98.25%, 98.78%, 99.11% and 99.66%, while chemical method was obtained as 91.35%, 93.62%, 97.05%, 98.36%, 98.97% and 99.09% respectively. The first order degradation rate constant, k_d obtained across the treatment options range from 0.0159–0.01243day^{-1} for biological method, while 0.00161 – 0.1601day^{-1} chemical method, the half-life analysis showed that chemical method may take the longest time for TPH to degrade half its initial concentration, and biological method is fastest in soil. Also, the first order rate kinetics performed better than Michaelis-Menten equation. Finally, the study showed that biological method can be utilised as alternative bio-stimulant for crude oil remediation. Comparison of the different treatment performed better in 42th day, but 30g weight sample performed best among the treatment options across the both sample. At the end of the analysis, for 30g biological method percentage reduction of the pollutant 99.66% and chemical method 99.09%. The result indicates that the treatment for bioremediation would perform better if biological method is applied.

Keywords: Biological, Bioremediation, Degradation, Rate constant, Media, and TPH.

1. Introduction

Petroleum production facilities generate large quantities of oil wastes from drilling, production and processing activities. According to [1], "all activities involved in hydrocarbon production and exploration normally have one impact or the other on the environment". The contamination of the environment by hydrocarbon process occurs through exploitation, transport, and leakage of crude oil storage facilities, which are released into the environment, thereby causing environmental pollution [2]. Crude oil pollution in the air occurs via evaporation of volatile components or gas flaring, while on aquatic and land environments it can be via spillage from oil facilities. A study reported that land environment was the most affected of pollution from petroleum industry, mostly from leakage of pipelines, storage tanks and other oil facilities [3]. Crude oil contains toxic compounds and radioactive elements that are serious health concerns, and also affects plant growth [4]. Crude oil pollution, especially on soil, has the ability to bind soil particles together because of low water solubility that could reduce soil nutrients [5]. Most organic and inorganic chemicals are hazardous to soil, and can cause low yield of agricultural produce.[6]. Various methods are used for the treatment of petroleum hydrocarbon polluted soil, but the choice of method depends on the cost effectiveness, ability to remove contaminants and availability.[7]. The development of a sustainable method for the removal of petroleum contaminants from the environment is essential. In soil contaminated by petroleum hydrocarbons, methods such as biological, physical and chemical technique can be used to remediate a polluted soil.[8]. These methods ranged from conventional excavation and removal of affected soil to landfills, capping of affected areas in the site, stabilization of soil with specific materials and cement, incineration and the use of organisms. The conventional methods appear to be less costly and expertise, but they do not solve the problem entirely. For instance, the method of excavating and dumping of polluted soil in landfills has become uncommon because of the risks associated with excavating and transporting the polluted soil.

Some of these techniques are also expensive and unrealistic for the removal of some specific contaminations [8]; [9].When there is crude oil pollution on soil, it spreads and seeps into soil, which may be faster initially and then slowly down the soil column as time progresses Naturally, soil has the capacity to purify itself, but it may take a very long period of time for contaminants to be removed to a satisfactory level.[10]. So, to facilitate the process of soil decontamination, a polluted soil can be modified through application of substance that interacts with the pollutant thereby, removing it from the soil in lesser time compared to natural attenuation.[11]. Though, there are techniques that can remove contaminants from the soil, but the removal of hydrocarbon from soil in recent times, uses microorganism, fertilizer, plant and animal wastes because of their effectiveness and environmental friendliness.[12].

2. Materials and Methods:

2.1 Materials and Equipment Used for The Experiment

Auto sampler vials, 150 ML vial inserts and crimp seals, Vial crimper and decrimper, 10ml unto sampler syringe, A won polar capillary column with a 5% phenyl- methyl polysiloxane stationary phase such as DB-5 or PH – 5, Laboratory fume hood, Analytical balance capable of weighing oiling, Glass ware, Disposable pipettes: Pasture, 150 mm long by 5mm (fisher scientific 13678-68 or equitant), Glass pipettes: 0-1-1-0, accurate to 1% or better, Volumetric flasks : glass 10 and l00 ml, Agilent 6890 Gas chromatographs with a flame lunation Defector, pH meter with glass electrode, Thermometer, Glass beaker (100ml), Glass rod, Nutrient agar, Acidified potato dextrose agar containing streptomycin, Mineral salt agar, Distilled water, Petri dishes.

2.1.1 Reagents Used for the Analysis

Acetone, Carbon disulphide, Petroleum ether (optima grade), Hydrogen gas, Air, Nitrogen, Dichloromethane, Methylene, Methanol, Cyclohexane, Standard buffer solution, Buffer solution of pH 6.86, Buffer solution of pH 9.2.

2.2 Methods

2.2.1 Production (Magnesium Oxide(Nano particle)

40g sodium hydroxide dissolve in 250ml distilled water (exothermic reaction, co-precipitate method). 26g of Magnesium (ii) chloride is dissolve in 160ml of distilled water endothermic reaction ‘wet chemical method” both were mixed together to give a light yellowish substance.

6g of iron chloride ($FeCl_3$) is dissolved in 25ml of distilled water 14g of sodium hydroxide little by little to give a dark colour precipitation. Both the nanoparticle (magnesia oxide and fenton) reagent were dried into power form local grams produced 69.48 (magnesium oxide) +74.86 (magnesium oxide) = 144.34 grams.

2.2.2 Experimental Procedures

300g of Loamy soil was weighed into 14 rectors sample, out of 14 sample, 13 sample containing 300g of loamy soil were polluted with 100ml crude, while the remaining 1 server as control unpolluted soil, The 13 polluted sample were stirred for uniform m mixture, 50ml of distill-water were added and stirred then left for 3 days. After 3 days polluted loamy soil sample, unpolluted loamy soil sample and crude oil use to pollute the soil where taken to the lab to analysis the following parameters TPH, Bacterial, pH, density, moisture content and particle size, 3 days later the Fenton nano-particles (5g, 10g, 15g, 20g, 25g and 30g) produced and Cow-dung (5g, 10g, 15g, 20g, 25g and 30g) was introduced into 6 separate reactors, 1 sample for polluted soil control 2, Each of the 14 samples were taken to the lab to analysis the (TPH, Bacterial & pH) for 1^{st} weeks – 6^{th} weeks in 45 days.

2.2.3 GC Methodology for TPH and PAH

(a) Soil Sample Extraction

10g of soil sample was added an amber glass bottle. Anhydrous sodium sulphate (Na, So_2) was also added into the glass bottle containing the soil sample. The sample was stirred. The addition on Na. So_2, was to remove moisture from the sample, 300ml of surrogate (1-chlorocctadecane) standard was added to the soil sample polluted, unpolluted, treatment (5g, 10g, 15g, 20g, 25g and 30g). 30ml of dichloromethane (DCM) was added to the sample as extracting solvent and the bottle containing soil sample was corked very tight and transferred to a mechanical shaker.

The sample was agitated between 5 to 6h at room temperature using a mechanical shake. After agitation, the sample was allowed to settle for 1h and then filtered through 110mm filter paper into a clean beaker. The filtrate was allowed to concentrate to 1ml by evaporation overnight into a fume cupboard.

(b) Sample Clean Up

Sample clean-up was performed using glass column. Column preparation was carried out by inserting glass cotton into the column. Silica gel was dissolved with DCM to form slurry, and the slurry was added into the Colum, Anhydrous Na_2SO_4 was added into the column followed by addition of pentane. After preparation of the column, the concentrated sample extract was mixed with cyclohexane in a beaker and transferred into prepared column The sample extract was eluted using pentane as solvent and eluted sample collected in a beaker below the column. The sample was eluted further by adding more pentane into the column. After elution the column was rinsed with DCM. The eluted was allowed to stand overnight at room temperature in a fume cupboard for evaporation to take place.

(c) Sample Separation and Detection

The separation and detection of compounds in soil and groundwater samples were carried out using Agilent 6890N Gas Chromatograph – Flame lionization Detector (GC.FID) instrument. 3pl of concentrated sample eluted from column was injected into GC vial. The blank DCM was injected into micro-syringe of GC to clean the syringe (3 times) before taking the sample was injected analysis. The micro-syringe was further rinsed with the sample. The sample was injected into the column for separation of compounds in the sample. After separation the compounds were passed through a flame ionization detector. FID detects the compounds in the sample. The amount of TPH and PAH was resolved at a particular chromatogram in mg/kg for soil sample.

(d) Electrical Conductivity

The electrical conductivity meter was used to measure the electrical conductivity (EC) of the samples. The same procedure stated for pH measurement was used in the determination of EC. However, the EC electrode was thoroughly washed after each reading to avoid cross-contamination and error.

(e) Total Organic Carbon

Total Organic Carbon (TOC) was determined using a method described by [13]. Thus, 1.0g of soil samples was weighed into 250ml beaker, while 10ml of potassium dichromate solution was pipette into beakers and swirled gently to completely wet the soil sample. Thereafter, 20ml of concentrated H_2SO_4 was added using automatic pipette, and gently swirled for one minute to obtain a uniform suspension, as well as for effective and more complete oxidation before allowed to settle for about 30 minutes on asbestos sheet. On settling, 100ml of distilled water was added followed by addition of 3-4 drops of 0.5 ml diphenylamine indictor. The solution was titrated with 0.5N ferrous sulphate solution until the colour changes from violet to blue and finally bright green colour. The process was repeated on distilled water (blank titration), but without soil to standardize the dichromate. The TOC was calculated according to the formula.

$$TOC = Blank - \frac{volume\,of\ soil\ sample\,titre \times 0.195}{weight\,of\ soil\ sample} \times 100\% \qquad (1)$$

(f) Total Nitrogen Content

Total nitrogen content was determined using APHA 4500-NO_3B method (APHA, 1998). Thus, 10g of grinded and sieved soil sample containing 10 mg of nitrogen in a dried 500ml Macro-Kjeldahl flask was weighed. It is swirled for about 2 minutes followed by the addition of 20ml of distilled water, and then, allowed to settle for 30 minutes. A tablet of 1g K_2SO_4-H_2O mixture (catalyst), 10g of K_2SO_4 and 30ml concentrated H_2SO_4 were added to prepared sample in the flask and heated cautiously on digestion stand. Upon the notice of water content and frothing, the heating was increased until a clear digest was obtained. The heating was regulated so that H_2SO_4 is about half way up the neck of the flask. After the heating process, the flask was allowed to cool, while 100ml of water was added slowly. The digest was carefully transferred into another clean 750ml Macro-Kjeldahl flask. All soil particles in the original digestion flask were retained due to the severe bumping soil can cause during the Kjeldahl distillation. Soil residue was washed with 50 ml distilled water four times and the aliquant transferred into same flask. Addition of 50ml H_3BO_3 indicator solution into a 500ml Erlenmeyer flask was followed, which was placed under the condenser of distillation apparatus. The 750ml Kjeldahl flask was also attached to the distillation apparatus. About 150ml of 10N NaOH was added into the distillation flask through the opening funnel, and the distillation was stopped after 150ml of the distillate was collected. The NH_4-N in the distillate was determined by titrating with 0.01N standard H_2SO_4 using 25ml burette graduated at 0.1ml intervals. The colour of the end point changed from green to pink. The percentage of nitrogen in the soil was calculated using equation (3.3).

$$N(\%) = \frac{(T-B)\times N \times 1400}{S} \times 100\% \quad (2)$$

Where: T = Sample Titration (ml), B = Blank Titration (ml), N = Normality of H_2SO_4 andS = Sample weight (mg).

(g) Phosphorous Content

Phosphorus content was determined according to APHA method $4500 - PO_4^{3-}$ (APHA, 1998). 1.0g of representative soil sample was weighed into clean extraction flask and 10ml of Bray P-1 extracting solution (0.025N HCl and 0.03N NH_4F) was added and vigorously agitated for 1 minute before being filtered. 5ml of the filtrate was pipette into 25ml volumetric flask and diluted to about 20ml of distilled water, and then, by 4ml of ascorbic acid solution (1.056g ascorbic acid in 200ml molybdate-tartrate solution), which were diluted. The diluted solution was allowed to settle for at least 30 minutes. The recording of data was done after a clear colour has been developed.

(h) Procedure for Total Bacterial Count (TBC) Analysis

Microbiological analysis enumeration of heterotrophic bacteria and fungi was carried out by pour plating technique. This was done by inoculating 0-1ml tenfold serrating diluted sample onto nutrients agar (bacterial), acidified streptomycin (1mg/100ml) (fungal) and mineral salt agar (MSA) (hydrocarbon degraders). The mineral salt media of Miu *et al* (1978) as modified by Okpokwasili and Amanchukwu (1988) contains the following composition in gram per liter of distilled water, N_aC_L 10g, Mg $S0_4$. $7H_2$.0, 9.42g, K_{CL} 0.29g, $HP0_4$ 1.2g, $KH2P0_4$ 0.83g, $NaN0_2$0.42g, Agar – Agar 16g, pH 7.2 and 2 nill at petrol/Diesel. The inoculated nutrient agar plates were incubated at 37^0C for 24 hours while the potato dextrose Agar plates were incubated at room temperature counted and expressed as colony firming units per gram (Cfu/ml).

2.3 Model Development

2.3.1 First Order Degradation Rate Kinetics

The first order kinetic model is one of the effective models used to study bioremediation process. Though, in literature, this model often expressed in its final state without showing the necessary steps as shown in equation (5). The biodegradation rate model is used to predict the TPH concentration in soils at any time under bioremediation process after the determination of the degradation rate constant. In chemical systems, the principle of mass balance is often applied to develop a mathematical expression describing a chemical system. Hence, the bio-kinetic model for TPH degradation rate in soil was developed based on the principle of mass balance. The principle of mass balance is stated in Equation (3).

$$\left\{\begin{array}{c}\text{Inflow of}\\ \text{mass into}\\ \text{system}\end{array}\right\} = \left\{\begin{array}{c}\text{Outflow of}\\ \text{mass from}\\ \text{system}\end{array}\right\} + \left\{\begin{array}{c}\text{Rate of}\\ \text{degradation}\\ \text{due to}\\ \text{reaction}\end{array}\right\} + \left\{\begin{array}{c}\text{Rate of}\\ \text{accumulation of}\\ \text{mass within}\\ \text{system}\end{array}\right\} \quad (3)$$

Now, substituting Equation (3.5) through (3.8) into Equation (3.4) gives

$$Q_o C_{TPH(o)} = QC_{TPH} - r_{TPH}V + \frac{d(C_{TPH}V)}{dt} \quad (4)$$

However, to obtain the instantaneous TPH concentration, exponential of both sides of equation (4) is taken to give:

$$C_{TPH(t)} = C_{TPH(o)}e^{-k_d t} \quad (5)$$

3. Results and Discussion

3.1 Physicochemical Properties of Soil before and after Pollution

The physicochemical properties of the soils before and after pollution are shown in Table 1.

Table 1: Physicochemical Properties of Soils before and after Pollution

Parameters	**Loamy Soil**			
	Before	**After**	**Biological(30g)**	**Chemical(30g)**

pH	6.5	4.1	6.3	5.9
EC (µS/cm)	352.43	843.61	432.13	567.34
TOC (%)	2.56	5.38	2.3	3.10
P (%)	1.46	0.93	1.8	1.5
N (%)	23.02	0.07	33.00	26.12
K (%)	32.84	1.42	43.61	30.23s
TBC (cfu/ml)	4.98×10^3	2.16×10^2	13.81×10^5	9.51×10^5

The physicochemical properties of loamy soils before and after being polluted by crude oil are shown in Table 1. The change in the physicochemical properties of the after pollution shows that crude oil has significant impact on soil. From the analysis, it was shown that pH, phosphorus (P), nitrogen (N), potassium (K) and Total Bateria Counts(TBC) in the soils were reduced after the crude oil pollution. On the other hand, the electrical conductivity (EC) and total organic carbon (TOC) in the soils increased after the pollution.

3.2 Total Heterotrophic Bacteria Count

The samples were analysed to ascertain the bacteria growth so as to determine the treatment option that has the most influence on TPH degradation under the Nano-particle Also, identification of hydrocarbon degrading bacteria and TPH analysis was conducted by the 7 days mark. The following were identified and isolated as hydrocarbon degrading bacteria in the analysis of bacteria isolates: Nano-particle (*Campylobacter sp*. and *Listeria monocytogenessp).* The growth analysis of TBC at the various days is shown in Figure 1.

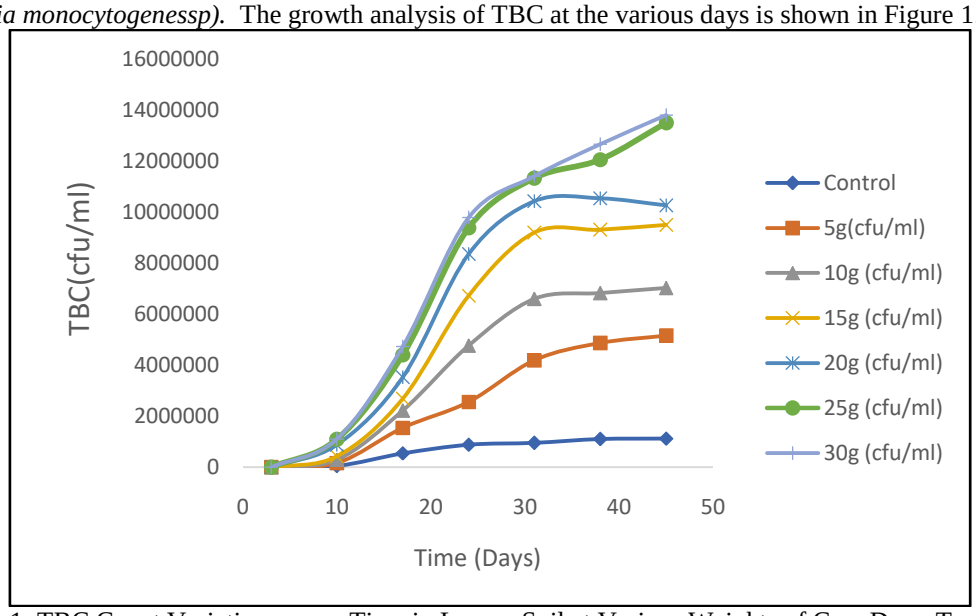

Figure 1: TBC Count Variation versus Time in Loamy Soil at Various Weights of Cow Dung Treatment

Figure 1 shows the growth rate of TBC in loamy soil amended with different weights of cow dung (treatment) with time. After pollution, TBC count reduced in the soils, but upon implementation of treatment increases the population of TBC in the soil as indicated by the profiles in Figure 1, TBC showed a rapid increase as treatment weight was increased.

3.3 TPH Degradation in Soils under the Influence of Treatment

This study showed that the weight of treatment applied in crude oil contaminated soils has effect on TPH degradation as time of bioremediation increases. Therefore, this section presents the results of the TPH recorded during the investigation periods. Thus, the degradation pattern of TPH in loamy soil under nanoparticle(treatment) compared with control samples as investigated with time at various weights of treatment is shown in Figure 2

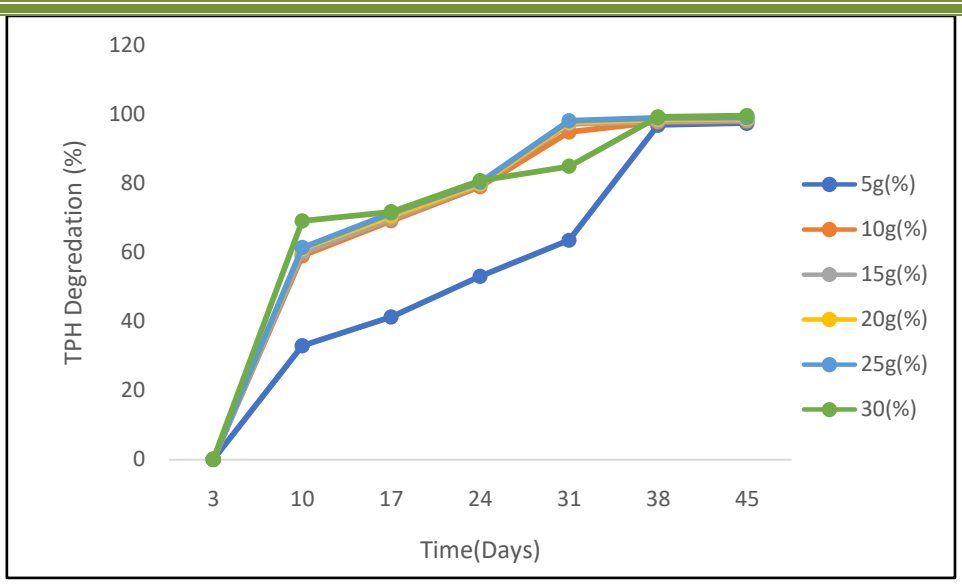

Figure 2: TPH Degradation in Loamy Soil versus Time at Various Weights of Nano-Particle Treatment

The degradation of TPH in loamy soil under treatment with nanoparticles is shown in Figure 2. The profiles of TPH percentage degradation in loamy soil indicate that increase in time also increases the percentage degradation. This implied that the concentration of TPH reduced with time. It was also observed from the chart that the TPH percentage degradation rate did not show substantial increase with treatment weight though 5g performed poorly in comparison with the others which mostly overlapped one another in terms of rate of degradation. Thus, after 45 days of the experimental analysis, the percentage degradation of TPH in loamy soil was obtained as 97.49%, 98.12%, 98.25%, 98.78%, 99.11% and 99.66% for 5g, 10g, 15g, 20g, 25g and 30g weights, respectively.

3.4 Comparison of Treatment Performance in the Soil

The performances of nanoparticles and cow dungas bio-stimulant for the degradation of TPH from crude oil polluted loamy soil was compared. Figure 4 compared all the treatment options with time at 30g.

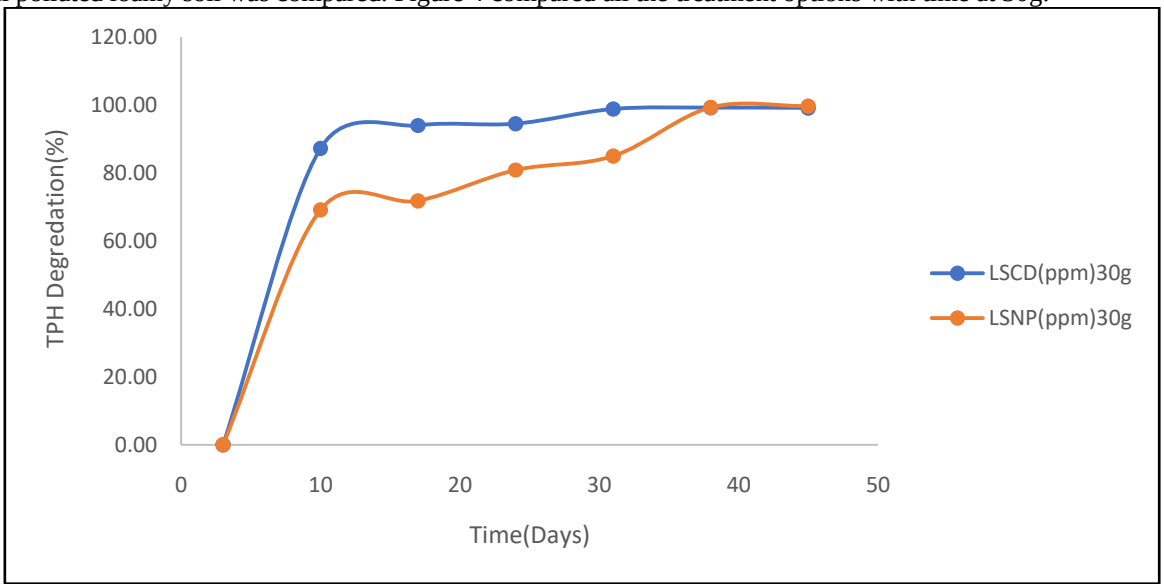

Figure 4: Comparison of TPH Removalin the Different Treatment at 30g.

Figure 4 shows the TPH percentage degradation Comparison of cow dung and nanoparticle treatment for the loamy soil, at 30g weight. As indicated in the profiles, the TPH percentage degradation in sample amended by cow dung sample was slightly higher that of sample with nanoparticle sample. The rate is faster with a steep slope in the cow dung samples as against the nanoparticles.

3.5.1 Evaluation of First Order Rate Constant and Half Life

The degradation rate constant in the first order rate kinetic model was determined by fitting regression equations for the different treatment options. From the determined rate constant, the time taken for the TPH concentration to reduce to half its initial concentration (half-life) was then evaluated. From the evaluated degradation rate constant, the estimated time at which the TPH concentration would degrade to half its initial concentration is given in Table 2 for the respective treatment option. Based on the evaluated time, it will take about 43 days for the TPH concentration to reduce to half its initial concentration if it were allowed to degrade naturally (control sample) in loamy soil, but the addition of 5g cow dung treatment caused the time to reduce to about 6 days to attain 50% degradation, which even reduced further to just about 21 days when the treatment weight increased to 30g. This implied that increase in treatment weight reduces the time at which the TPH degraded to half of its initial concentration. For soil amended by cow dung and NPK fertilizer. This reduction in half life was attributed to increase in degradation rate. This is evident in the value of the degradation rate constant, which was lowest in the control sample, but increased as the treatment weight was increased.

Table 2: Rate Constant and Model for Loamy Soil under Cow Dung Treatment

Weight (g)	k **(day^{-1})**	**Predictive Model**	**t$^{1/2}$ (days)**
Control	0.0159	$C_{TPH} = 81570.80e^{-0.0159t}$	43.591
5	0.0651	$C_{TPH} = 42066.21e^{-0.0651t}$	10.646
10	0.0744	$C_{TPH} = 41772.77e^{-0.0744t}$	9.316
15	0.0967	$C_{TPH} = 47667.26e^{-0.0967t}$	7.167
20	0.1091	$C_{TPH} = 48533.04e^{-0.1091t}$	6.352
25	0.121	$C_{TPH} = 51688.99e^{-0.121t}$	5.728
30	0.1243	$C_{TPH} = 49563.01e^{-0.1243t}$	5.576

4. Conclusion

The performance of Cow dung and Nano-particle treatment has been investigated for bioremediation of crude oil polluted loamy soil. The characterized loamy soil before and after pollution showed that the physicochemical properties of the soils changed immediately after polluted by crude oil, and this implied that crude oil has significant impact on soils. Thus pH, phosphorus, nitrogen, potassium and Total Bateria Counts in the soils were reduced after pollution, while electrical conductivity and total organic carbon were increased. Analysis of bacteria growth for the various treatment options shows that Total Bacteria Count (TBC) in the soil amended with different weights of *cow dung* (treatment) reduced immediately after pollution, but the TBC population increased significantly as time and treatment weight increased. The microbial growth rate attained a stationary state at some point in time, but the bacteria growth in control samples was very slow, indicating that *cow dung* stimulated the growth rate of bacteria in the soil.Thepercentage degradation of Total Petroleum Hydrocarbon (TPH) in loamy soils under cow dung and nanoparticle treatmentincreased with time and treatment weight. There was rapid increase in percentage degradation in the treated soil compared to the gradual increase recorded in the control samples for the various soils.The highest TPH degradation was recorded on the 45th day across the various treatment weights, implying that duration of remediation influenced the degradation efficiency. Also,Treatment improved the degradation rate of TPH in the soils. Comparison of the different treatment options in remediation of the polluted soils showed that the all the treatment options performed better on the 45th day, but the 30g weight samples performed much better than the other treatment options across the soil types. TPH percentage degradation across the treatment options was highest in Nano-particle treatment soil compared with Cow dung treatment soil. At the end of the analysis, for 30g Nano-particle treatment, the TPH degradation percentages were recorded as 97.48% to 99.66% for Nano-particle, while 91.35% to 99.10% were recorded for Cow dung treatment, respectively. Similarly, the Nano-particle samples slightly edged the Cow dung samples soils, indicating that treatment for bioremediation would perform better if Nano-particle as against Cow dung.

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