Investigating the effect of Using Cow Dung and Water Leaf on Bioremediation of Crude Oil Polluted Soil

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Abstract

This study investigated the use of fresh Talinum triangular (Water-leaves) and fresh cow dung for bioremediation of crude oil-polluted soils. Water leaves and cow dung were used as bio-stimulant for remediation of crude oil polluted soil. The physicochemical properties of the soils before and after pollution were analyzed. Also, the Total Heterotrophic Bacteria count (THB) and Total Petroleum Hydrocarbons (TPH) content were analyzed every 7 days for 35 days. The results obtained from the physicochemical analysis showed that there was an increase in the pH of the soils after contamination, while there were decreases in the soils' moisture content, total organic carbon, total nitrogen, and phosphorus contents, which differ remarkably from the control samples, implying that the water leaves and cow dung treatments after pollution were effective. Also, as time and treatment weights were increased, the percentage degradation of TPH in the soil increased. Thus, the 150g weight samples exhibited the best performance on the 35th day, consistently demonstrating superior efficacy across various soil types. Waterleaf treatment outperformed Cow dung treatment in terms of TPH percentage degradation. Specifically, 150g Waterleaf treatment resulted in degradation percentages ranging from 81.27% to 89.50%, while Cow dung treatment achieved percentage of 72.24% to 86.88%. Further examination of TPH degradation rate constants and half-lives for Waterleaf and Cow dung treatments revealed distinct patterns. Waterleaf treatment displayed higher degradation rate constants (ranging from 0.0043 to 0.057 day^-1 for Cow dung and 0.0043 to 0.0509day^-1 for Waterleaf), corroborating its superior TPH removal efficiency. Half-life analysis indicated that Cow dung required more time for TPH to degrade to half its initial concentration, while Waterleaf treatment exhibited faster degradation. Conversely, the variation of Michaelis-Menten constants showed no clear pattern. In terms of model performance, the first-order kinetic rate model demonstrated better congruence between experimental TPH concentrations and the Michaelis-Menten equation.

Keywords: *Soil, Hydrocarbon, Remediation, Cow Dung, Water-leaves and kinetic models.*

Introduction

The Environment was created to serve man and other living things with benefits. Energy related environmental problems, including oil spills, air pollution, flooding and deforestation have become a threat to world's biodiversity and delicate ecosystems. Oil spill occurs more frequently in developing countries such as Nigeria and have been the cause of severe environmental damage.

For example, spills in Rivers and other water bodies in the creeks have caused damages to swamp itself, hurt the local fishing communities, covered beaches with crude, and greatly polluted the coastal soils. Although most environmental pollution was not deliberate, it is the human quest for good living and advancement in technology that brought about the operations of petroleum industries, including exploring, production (extraction), storing, transporting, and refining of crude oil and the storing, distribution, and handling of products that are potential soil contaminates (oily sludge). Accidental spills of crude oil and petroleum products during the handling, storing, and transporting operations are the principal causes of the formation of oil sludge in large quantities (Ehirim et al., 2020).

Petroleum production facilities generate large quantities of oily wastes from drilling, production, and processing activities. 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 processing occurs through exploitation, transport, and leakage of crude oil storage facilities, which are released into the environment, thereby causing environmental pollution. Crude oil pollution in the air occurs via evaporation of volatile components or gas flaring, while in aquatic and land environments it can occur via spillage from oil facilities. A study reported that the land environment was the most affected by pollution from the petroleum industry, mostly from leakage of pipelines, storage tanks, and other oil facilities (Nnaji, 2017). Crude oil contains toxic compounds and radioactive elements that can cause challenges to the health and also affect plant growth. Crude oil pollution, especially on soil, can bind soil particles together because of low water solubility which could reduce soil nutrients. Most organic and inorganic chemicals are hazardous to soil and can cause low yield of agricultural produce (Kuppusamy et al., 2016).

Bioremediation is a radiation technique that utilizes microorganisms or bio-stimulants such as enzymes, spent biomass, and fertilizers to remove contaminants from a contaminated environment through a metabolic process. Bioremediation is economical, adaptable, efficient, and eco-friendly to the environment (Jeon & Madsen, 2013). Bioremediation can be implemented through bio-stimulation, bio-augmentation, and phytoremediation, among others. Bio-stimulation refers to the use of fertilizer, plant and animal wastes, or any other agro-based material as amendment agents, to stimulate the growth of microorganisms; while in bio-augmentation bacteria or fungi are directly applied to enhance the degradation rate of pollutants (Varjani & Upasani, 2017). In Phytoremediation, a plant is planted in polluted soil to take up the contaminant as it grows.

Soil pollution arising from increasing demand for petroleum and its products has become a common problem in recent years. Soils polluted with petroleum hydrocarbons differ from unpolluted soils and do not support adequate crop growth and development. Hence, the need to treat these soils, and also reduce the negative impacts of the crude oil on the environment. Therefore, the capability of *cow dung and water leaf* to degrade the TPH in crude oil-polluted loamy soil is investigated. The choice for the using of *cow dung and water leaf* as bio-stimulant is based on their nutritional quality implying that they possess the ability to stimulate hydrocarbon-degrading bacteria necessary for effective bioremediation.

Materials and Methods

A collection of loamy soil samples was procured from Ugwuaji Community within Enugu State. In parallel, samples of waterleaf and cow dung were acquired from the Agriculture farm at Rivers State University. The aforementioned specimens were meticulously stored within appropriate containers. Additionally, a representative crude oil sample was sourced from the Port-Harcourt Refinery, situated in Rivers State.

The assembled samples were subsequently transported to the Chemical/Petrochemical Laboratory within the premises of Rivers State University in Port Harcourt, for comprehensive analysis. The waterleaf, cow dung, and soil samples were subjected to specific preparatory procedures to facilitate subsequent processing stages.

Materials

The materials used in the experiment include 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, Laboratory fume hood, Analytical balance, Glass ware, Mineral salt agar, Distilled water, Petri dishes**,** Scientific Calculator, Cow dung, Nano particle, sieve merge.

Methods

A total of 4 kilograms of soil samples were utilized for the study. Cow dung and waterleaf specimens were procured from the agriculture farm at Rivers State University. Ten empty batch reactors were employed, loaded with 400 grams of soil samples. The samples were left in the reactors for three days. Subsequently, seven reactors were subjected to pollution by introducing 200 milliliters of crude oil into each reactor. The mixture was thoroughly blended to ensure homogeneity, after which the reactors were left undisturbed for an additional three-day period. Among the reactors, Reactor 1 was the control and remained devoid of crude oil and nutrients. Reactor 2, also a control, contained 2400 grams of slit loam soil within a plastic batch reactor. Before pollution and post-pollution, all samples were subjected to thorough analysis. To ensure uniform crude oil concentration within the soil samples, the polluted samples were meticulously mixed and left undisturbed for three days to facilitate settling. Following a three-day soil settlement period, soil treatment was initiated, involving the application of treatments ranging from 10 to 50 grams of cow dung and waterleaf.

The physicochemical attributes of loamy soils were assessed both before and after pollution. These attributes encompassed pH, particle size distribution, electrical conductivity, nitrogen content, total organic carbon content, and phosphorus content. Additionally, the study involved the analysis of Total Bacterial Count (TBC) and Total Petroleum Hydrocarbon at 7-day intervals during the treatment phase.

Determination of Total Petroleum Hydrocarbon

The analysis of the Total Petroleum Hydrocarbon was done using a Gas Chromatography — Flame Ionization Detector Model HP 5890 series II, U.S.A. This analysis was done in the Endpoint laboratory. Total Petroleum Hydrocarbon was obtained using a calibrated graph as a reference.

Soil pH Analysis

A quantity of 20 grams of air-dried soil with a particle size of 2mm was precisely weighed and placed into a beaker. Subsequently, 50 milliliters of distilled water were introduced into the beaker, and a thorough mixing was performed using a glass rod for approximately 5 minutes. The mixture was then left undisturbed for thirty minutes.

During this interval, the pH meter was activated for 15 minutes. The glass electrode within the pH meter underwent standardization using a pH 7 standard buffer solution. This calibration procedure also involved either pH 4 or pH 9.2 buffer solutions. The glass electrodes were then immersed into the beakers containing the soil-water suspension, all while ensuring continuous stirring. As the pH value was detected, the pH meter was switched to the pH reading mode. Following a 30-second stabilization period, the pH value was meticulously recorded to the nearest 0.1 unit.

After recording, the pH meter was placed in standby mode, and the electrodes were gently extracted from the soil suspension. They were subsequently cleansed using distilled water. To ensure accuracy, the electrodes were rinsed and carefully dried using filter paper before the next determination.

Standardization of the glass electrodes was performed after every ten determinations. During periods of inactivity, the electrodes were immersed in distilled water. The reference electrode was consistently maintained with saturated potassium chloride in contact with solid potassium chloride crystals.
The range pH is classified at

i. Less than 4.5 is extremely acidic
ii. 4.6 to 5.2 strongly acidic
iii. 5.3 to 6.0 moderately acidic
iv. 6.1 to 6.5 slightly acidic
v. 6.6 to 7.0 neutral
vi. 7.1 to 7.5 slightly alkaline
vii. 7.6 to 8.3 moderately alkaline
viii. 8.4 to 9.0 strongly alkaline
ix. Greater than 9.0 is extremely alkaline

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.

Total Organic Carbon

Total Organic Carbon (TOC) was determined using a method described by Umeda et al. (2017). Consequently, a mass of 1.0 grams of the soil samples was meticulously measured and placed within a 250ml beaker. the volume of 10ml from the potassium dichromate solution was carefully transferred into the beaker and then swirled gently to ensure complete saturation of the soil sample. 20 ml of the concentrated H_2SO_4 was introduced into the beaker with an automatic pipette. The mixture was then gently swirled for one minute, achieving a uniform suspension and an enhanced and thorough oxidation process. Following this, the mixture was allowed to settle for approximately 30 minutes on an asbestos sheet.

Once the settling had occurred, 100ml of distilled water was added to the mixture. This was promptly followed by 3 to 4 drops of a diphenylamine indicator solution measuring 0.5ml. The resulting solution was titrated using a 0.5N ferrous sulfate solution. Titration continued until the observable color transitioned from violet to blue, eventually reaching a vivid green hue.

This procedure was replicated using distilled water (a blank titration) but without soil. This blank titration served the purpose of standardizing the potassium dichromate solution. The TOC was calculated according to the formula.

$$TOC = Blank - \frac{volume of\ soil\ sample titre \times 0.195}{weight\ of\ soil\ sample} \times 100\% \qquad (2.1)$$

Total Nitrogen Content

The determination of total nitrogen content was conducted in accordance with the APHA 4500-NO3B method (APHA, 1998). To begin, a sample of soil, ground, and sieved, weighing 10g and containing approximately 10 mg of nitrogen, was measured and placed within a dried 500ML Macro-Kjeldahl flask. The contents were gently swirled for approximately 2 minutes. Following this, 20ml of distilled water were added to the flask, after which the mixture was allowed to settle for a duration of 30 minutes.

Into the prepared sample in the flask, a tablet of 1g K_2SO_4-H_2O mixture (acting as a catalyst), 10g of K_2SO_4, and 30 ml of concentrated H_2SO_4 were introduced. The flask was placed cautiously on a digestion stand and subjected to controlled heating. As signs of water content and frothing became apparent, the heat intensity was elevated until a clear digest was achieved.

The regulation of heating ensured that the level of H_2SO_4 in the flask remained approximately halfway up the neck. Once the heating process concluded, the flask was allowed to cool, and a gradual addition of 100ml of water followed.

The obtained digest was meticulously transferred into another clean 750ml Macro-Kjeldahl flask, while any sand particles present in the original digestion flask were retained. This precautionary step was taken to prevent severe bumping that sand particles might cause during the subsequent Kjeldahl distillation. The sand residue was washed using 50ml of distilled water in four iterations, and the collected aliquots were then added to the same flask.

Subsequently, 50ml of H_3BO_3 indicator solution was introduced into a 500ml Erlenmeyer flask. This flask was positioned beneath the condenser of the distillation apparatus. Simultaneously, the 750ml Kjeldahl flask was attached to the distillation apparatus. Through a funnel-like opening, approximately 150ml of 10N NaOH was carefully added to the distillation flask. The distillation process was halted once 150ml of distillate had been collected.

The ammonium nitrogen (NH_4-N) in the distillate was quantified through titration, employing a 0.01N standard H_2SO_4 solution. The titration process was carried out using a 25ml burette calibrated with intervals of 0.1ml. The color of the endpoint changed from green to pink. The percentage of nitrogen in the soil was calculated using equation (3.2).

$$N(\%) = \frac{(T - B) \times N \times 1400}{S} \times 100\% \quad (2.2)$$

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

Phosphorous Content

Phosphorus content was determined according to APHA method $4500\text{-}PO_4^{3-}$ (APHA, 1998). 1.0g of representative soil sample was weighed into a clean extraction flask and 10 ml 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 had been developed.

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 seriating diluted sample onto nutrients agar (bacterial), acidified streptomycin (1mg/100ml) (fungal), and mineral salt agar (MSA) (hydrocarbon degraders).

Total Petroleum Hydrocarbon (TPH) Analysis

The determination of Total Petroleum Hydrocarbons (TPH) was conducted using Gas Chromatography-Flame Ionization Detector (GC-FID). The protocol for TPH analysis is elucidated as follows:
Initially, the soil sample was carefully poured into a 1-liter separation funnel. Subsequently, 50 ml of methylene chloride was introduced into the sample bottle. The bottle was sealed and agitated for 30 seconds to ensure the inner surface was evenly coated. The solvent was then transferred from the sample bottle to the separation funnel. The funnel was shaken for 2 minutes, with intermittent venting to release excess pressure.

The organic layer was allowed to separate from the aqueous phase for a minimum of 10 minutes. The methylene chloride was extracted into a 250ml flask. A second volume of 60 ml of methylene chloride was added to the sample bottle. Rinsing of the separation funnel and the column was performed using 25ml of the solvent, directing the reinstate into the extract. This extraction process was repeated a second time, and the extracts from both repetitions were combined in an Erlenmeyer flask.

The third extraction followed the same procedure. The extracts obtained from all three extractions were filtered through a drying column comprising packed cotton wool, anhydrous sodium sulfate, and silica. The resultant extract was collected in a vial and subsequently concentrated. Concentration was achieved by boiling down the extract using nitrogen gas, ultimately reducing it to a volume of 1.0ml.

The concentrated extract was then mixed with 1.0 ml of the solvent. From this mixture, a volume of 1.0ml was injected into the gas chromatograph equipped with a flame ionization detector for TPH analysis.
The residual TPH percentage at any time was calculated using Equation (2.3).

$$TPH_R(\%) = \frac{TPH_i - TPH_f}{TPH_i} \times 100\% \quad (2.3)$$

Where: TPH_R is the residual TPH percentage with time, TPH_i is the initial concentration of TPH, and is the concentration of TPH measured with time.

3. RESULTS AND DISCUSSION

Investigations on the efficacy of cow dung and water leaves in bioremediation of crude oil-polluted soil have been carried out and the results are presented in this chapter.

3.1 Influence of Treatment on the Soil Physicochemical Properties

The behavior of the polluted soils after application of the cow dung and water leaves over the investigative period was studied through some of the soils' physicochemical properties, which were as shown in Figures 3.1 through 3.5.

3.1.1 influence of Treatment on the Soil pH

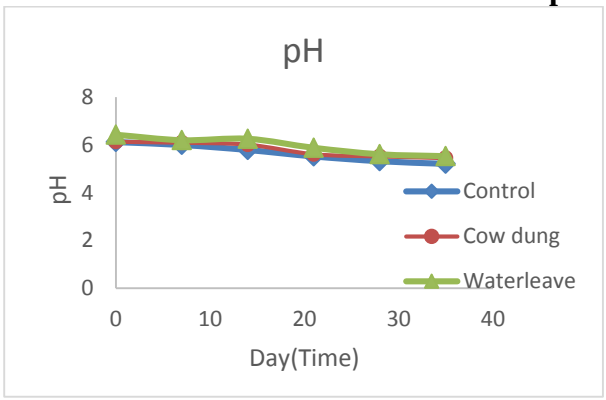


Figure 3:1: Variation of pH in Loamy Soil with Treatment

Figure 3.1 shows the variation of pH in crude oil-polluted loamy soil treated with cow dung and water leaves. There were changes from the initial conditions of the soil after pollution with crude oil. These physicochemical changes in the soils from their initial conditions have been attributed to the crude oil introduction. Thus, the pH of silt loam soil after pollution decreased from its initial condition of 5.01 to 5.4 in the control sample (CL), 5.23 in the sample treated with water leave particles (WL) and 5.11 in the sample treated with cow dung (CD) up to the seventh (7th) day of treatment, which then gradually increased to 5.4 in control sample, 6.53 in sample treated with water leave and 6.0 in sample treated with cow dung at the 35th day. Again, the increase in pH of soil samples with no treatment was low compared to the samples with treatment.

Influence of Treatment on the Soil Moisture Content

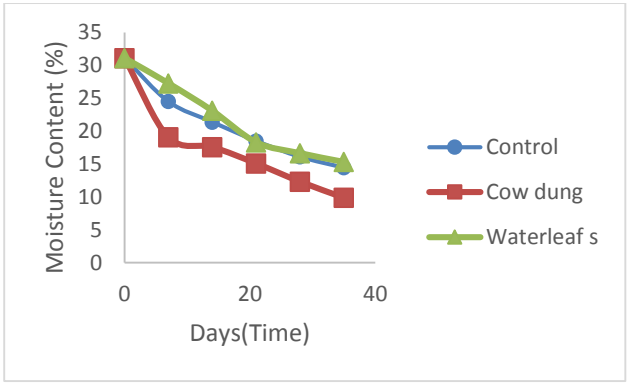


Figure 3.2: Variation of Moisture Content in Silt Loam Soil with Treatment

Figure 3.2 shows the variation in moisture content of crude oil-polluted loamy soil with treated and untreated samples. Unlike the soils' pH, moisture content decreases with time for both control and treated samples. Thus, moisture content decreases with time for both control and treated samples. Similarly, the moisture content in silt loam soil after pollution decreased from 31.12% to 14.45% in the control sample (CL), 15.34% in the sample treated with water leaves (WL) and 14.67% in the sample treated with cow dung (CD) at the end of the 35day treatment Again, the soil with no water leave treatment recorded moisture content than the other soil samples. The trends in moisture content obtained in this study are similar to those reported in a previous work (Umeda et al., 2017).

Influence of Treatment on the Soil Total Organic Carbon

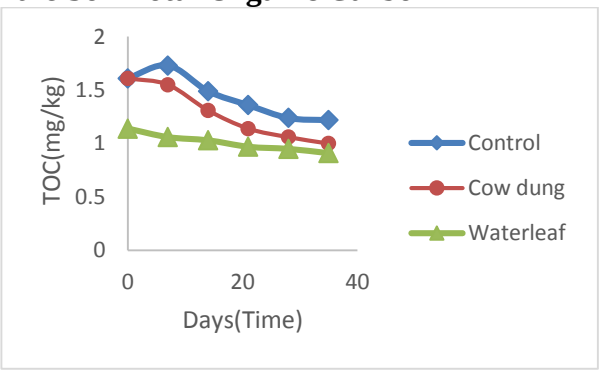


Figure 3.3: Variation of TOC in Silt Loam Soil with Treatment

Figure 3.3 shows the trends in TOC across the treatment samples. Like the soil's moisture content, the total organic carbon decreases. However, the TOC in the control samples were higher than samples with treatment. The TOC in silt loam soil before pollution was 1.61mg/kg, but at the end of the investigation (35 days), it decreased to 1.22mg/kg, 0.91mg/kg water leave and 1mglkg cow dung, treatment with water leave and treatment with cow dung respectively. Again, silt loam soil with no treatment recorded a higher concentration of TOC than the treated samples.

Influence of Treatment on the Soil Total Nitrogen

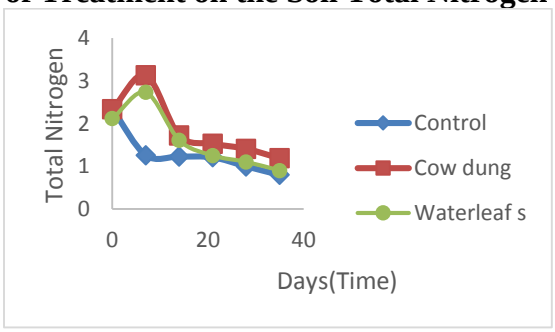


Figure 3.4: Variation of Total Nitrogen in Silt Loam Soil with Treatment

Variation in total nitrogen of treated and untreated crude oil polluted silt loam soil is shown in Figure 4.4. Again, total nitrogen in the treated soil initially increased within the first 7 days, and thereafter decreased gradually till the end of the investigation, on the contrary, there was no increase of total nitrogen at the first seen (7) days in the control sample. This is because addition of treatment improved the content of nitrogen in the polluted soil samples. The total nitrogen in silt loam soil before pollution was 2.34mg/kg, but after 35days of investigation, it decreased to 0.6mg/kg, while water leave treatment and cow dung treatment samples respectively (Table 4.4 of Appendix A), representing 80% and 90% reduction of total nitrogen in the respective soil samples. Total nitrogen content was most produced in the soil treated with cow dung, the control sample which has limited nitrogen production also does not contain the necessary nitrogen supplying nutrient to cause a speedy degradation of TPH in soil.

Influence of Treatment on the Soil Phosphorus Content

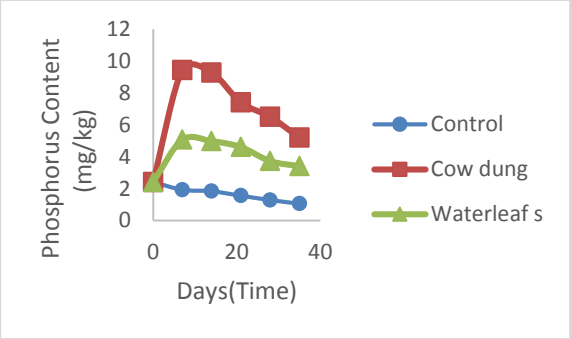


Figure 3.5: Variation of Phosphorus Content in Silt Loam Soil with 150g Treatment

Figure 4.5 shows the variation in phosphorus content of treated and untreated silt loam soil samples. The phosphorus content in the soil with water leaves and cow dung initially increased within the first 7 days before decreasing till the end of the investigation. As in total nitrogen, the initial increase in phosphorus content within the first 7 days was not observed in the control sample. However, from the analysis, phosphorus content in silt loam soil before pollution was 2.42mg/kg, but on the 35th day after pollution, it decreased to 1.06mg/kg, water leaves 5.2mg/kg and 3.41mg/kg cow dung treatment samples respectively.

Total Heterotrophic Bacteria Count

Analysis carried out on hydrocarbon bacteria was carried out on the soils before pollution to identify micro-organisms present. Also, as the experiment was in progress, the control and treatment samples were subjected to analysis to monitor the THB growth in the soils. the following micro-organisms were identified: Bacillus species, Pseudomonas species, and Proteus species. The Bacillus species with colonies in white color, have pointed edged center, and measure about 1-2cm. The Pseudomonas species appeared to be swarming in colonies, whose color can be described as green color with circular edges. Also, the Proteus species are flat with swarming colonies,

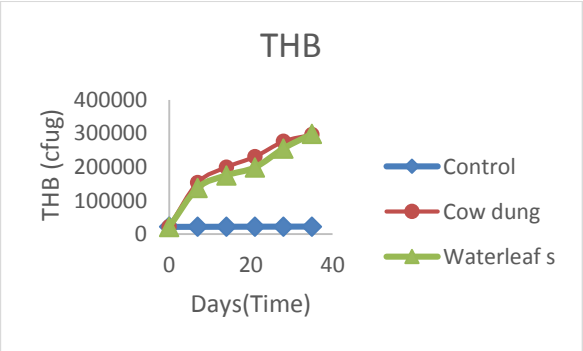


Figure 3.6: TBC Count Variation versus Time in Loamy Soil at Treatment of 150g Waterleaf and Cow Dung

Figure 3.6 depicts the Total Bacteria Count (TBC) dynamics within Loamy soil subjected to varying amendments of Waterleaf treatment weights over an extended period. Much like the preceding experiment involving Cow dung treatment, the introduction of the treatment led to a rapid elevation in the TBC populace within the soil, with a proportional amplification linked to treatment weight. Around the 35th day, the growth rates of microorganisms across all specimens started to approach a state of relative stability. This stabilization trend persisted in samples containing 50 to 150g even after the 35th day. As before, the untreated control sample of Loamy soil exhibited gradual bacterial proliferation.

The findings revealed that the Total Bacteria Counts within Loamy soil treated with Waterleafs exhibited an upward trajectory across the range of treatment variations. Specifically, the counts ranged from 21700 cfu/g for the control sample to 21813 cfu/g, water leaves 21700 cfu/g to 298754 cfu/g, and cow dung 298754 cfu/g, the samples treated with 150g of Waterleafs and cow dung, respectively. The graphical data aptly illustrate that the water leave-treated samples displayed higher TBC values compared to the soils treated with cow dung. A presentation of the Total Bacteria Count (TBC) recorded over time for Loamy soil under the both treatments.

TPH Degradation in Soils under the Influence of Treatment

This study underscores the influence of treatment weight applied in crude oil-contaminated soils on the degradation of Total Petroleum Hydrocarbons (TPH) as bioremediation progresses over time. Consequently, this section presents the outcomes of TPH degradation observed during the investigation periods. The degradation pattern of TPH in loamy soil treated with Waterleaf s is compared with control samples across various treatment weights and time intervals, as depicted in.

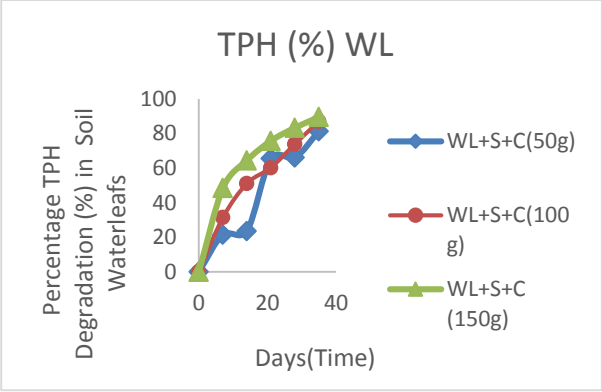


Figure 3.7: TPH Degradation in Loamy Soil versus Time at Various Weights of Waterleaf Treatment

Figure 3.7 portrays the degradation process of Total Petroleum Hydrocarbons (TPH) within loamy soil when subjected to Waterleaf treatment. The profiles of TPH percentage degradation within the loamy soil distinctly demonstrate that an extended time frame corresponds to an augmented percentage of degradation. This correlation directly implies a diminishing concentration of TPH over time. The graph also conveys that while the TPH percentage degradation rate did not display a substantial enhancement with higher treatment weights, the 50g, 100g, and 150g treatments exhibited similar levels of efficacy.

As a consequence of the comprehensive experimental study encompassing 35 days, the TPH percentage degradation within the loamy soil was quantified at 81.27%, 87.07%, and 89.50%, for treatment weights of 50g, 100g, and 150g, respectively. A comprehensive compilation of the TPH analysis results for loamy soil treated with Waterleafs. Notably, the most substantial TPH degradation was observed on the 35th day across varying treatment weights, highlighting the profound influence of the remediation duration on removal efficiency. The results obtained in this study are similar to the reported effect of treatment on crude oil-polluted soil. However, observed that a high concentration of crude oil in soil can negatively affect TPH degradation efficiency. The 5g sample recorded the poorest TPH degradation rate. This implied that the treatment contained nutrients that stimulated the hydrocarbon-degrading bacteria.

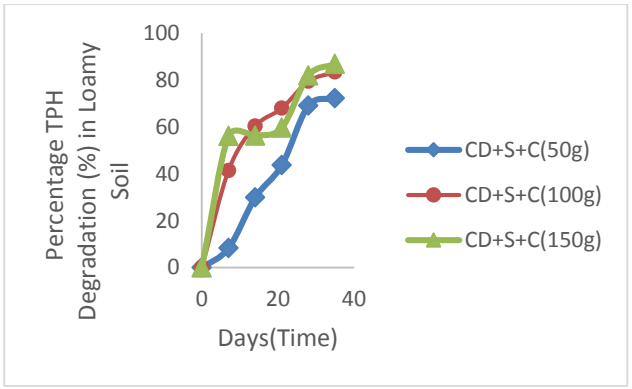
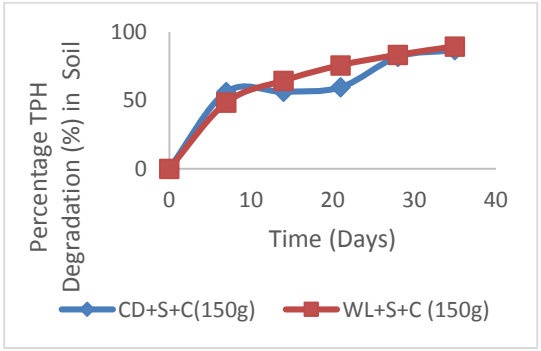


Figure 3.8: TPH Degradation versus Time in Loamy Soil at Various Weights of Cow Dung Treatment

Figure 3.8 portrays the profiles of TPH percentage degradation within Loamy soil subjected to Cow dung treatment. Analogous to the trends observed in Loamy soil treated with Waterleafs, the TPH degradation percentage within Loamy soil exhibited a proportional augmentation with an elongated duration of remediation. Furthermore, an incremental increase in the rate of TPH degradation was noticeable as the treatment weight was elevated. The control sample, similar to the Waterleaf treatment scenario, also demonstrated a reduction in TPH concentration over time, yet at a comparatively slower rate of degradation. The findings revealed that on the 35th day of the experimental study, the recorded TPH degradation percentages within Loamy soil across the spectrum of treatment options were as follows: 72.25%, 83.49%, and 86.89% for treatment weights of 50g, 100g and 150g, respectively. A comprehensive compilation of the TPH analysis results for Loamy soil treated with Cow dung.

Evaluation of Treatment Performance in the Soil

The effectiveness of water leaves and Cow dung as bio-stimulants for TPH degradation in crude oil-contaminated Loamy soil was compared. Figure 3.13 provides a comparison of all treatment options over time at a treatment weight of 150g. Meanwhile, Figure 3.5 presents a comparison of the outcomes obtained on the 35th day across various treatment weights for Cow dung and



waterleaf treatments in the soil samples.

Figure 3.9: Comparison of TPH Removal in the Different Treatment at 150g.

Figure 3.9 illustrates the juxtaposition of TPH percentage degradation between Cow dung treatment and Waterleaf treatment for loamy soil, both carried out using a treatment weight of 150g. As evident from the depicted profiles, the TPH percentage degradation in the Cow dung-treated sample slightly surpassed that of the sample treated with Waterleafs. Additionally, the degradation rate demonstrated a more rapid advancement with a steeper slope in the Cow dung-treated samples compared to the samples treated with Waterleafs.

Evaluation of TPH Degradation Rate Constants

The degradation of TPH in soil was further studied using the first-order kinetic model, which has been generally used for evaluation of crude oil degradation rate in soil.

Evaluation of First Order Rate Constant and Half-Life

The assessment of the degradation rate constant, employing the first-order rate kinetic model, entailed a juxtaposition between Equation (2.16) and the regression equations outlined in Figures 3.9 to 3.24, encompassing a range of treatment options. By utilizing the determined rate constant, the duration necessary for the TPH concentration to diminish to fifty percent of its initial concentration (commonly known as the half-life) was subsequently evaluated.

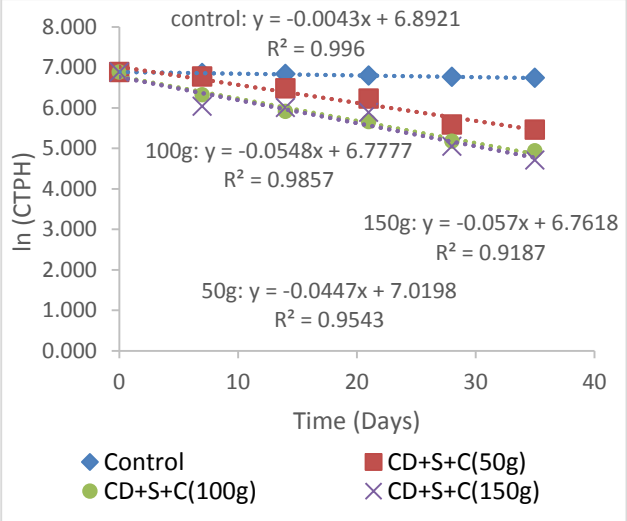


Figure 3.9: Plots for Evaluation of Rate Constant for Cow Dung Treatment in Loamy Soil

Figure 3.9 presents a series of distinctive linear regression equations that characterize various treatment scenarios applied to Loamy soil during Cow dung treatment. The degradation rate constant, referred to as Ks, was derived from these linear equations illustrated in the plot. This process involved a comparison between the provided equations in Figure 3.9 and Equation (2.16). Subsequently, the identified rate constant, together with the initial TPH concentration, was incorporated into Equation (2.17) to generate the final predictive model used for estimating residual TPH levels within the soil. Notably, the degradation rate constant exhibited variability in correspondence with treatment weight. A compilation of the obtained degradation rate constants can be found in Table 3.1. The first-order kinetic rate model, which integrates the degradation rate constants for the respective treatment options, is also documented in Table 3.1.

Utilizing the calculated degradation rate constants, the projected timeframe for the TPH concentration to decrease to half of its initial concentration is meticulously detailed in Table 3.1 for the respective treatment alternatives. Accordingly, considering the evaluated timeframes, the intrinsic degradation process (control sample) within the Loamy soil would demand approximately 161 days to achieve a 50% reduction in TPH concentration. However, the introduction of 50g of Cow dung treatment reduced this duration to about 15 days to attain 50% degradation. Furthermore, this period significantly diminished to 12 days as the treatment weight escalated to 100g, it held steady at 12 days even for 150g treatment. This observation underscores the notion that an increase in treatment weight leads to a notable reduction in the time required for TPH to degrade to half its initial concentration. This observation indicates that augmenting treatment weight leads to a reduction in the time required for TPH to degrade to half of its initial concentration.

Table 3.1: Rate Constant and Model for Loamy Soil under Cow dung Treatment

Weight (g)	k (day^{-1})	Predictive Model	$t^{1/2}$ (days)
Control	0.0043	$C_{TPH} = 984.47e^{-0.004t}$	161.186
50g	0.047	$C_{TPH} = 1118.6e^{-0.047t}$	14.7468
100g	0.0548	$C_{TPH} = 878.05e^{-0.055t}$	12.6478
150g	0.057	$C_{TPH} = 864.2e^{-0.057t}$	12.1596

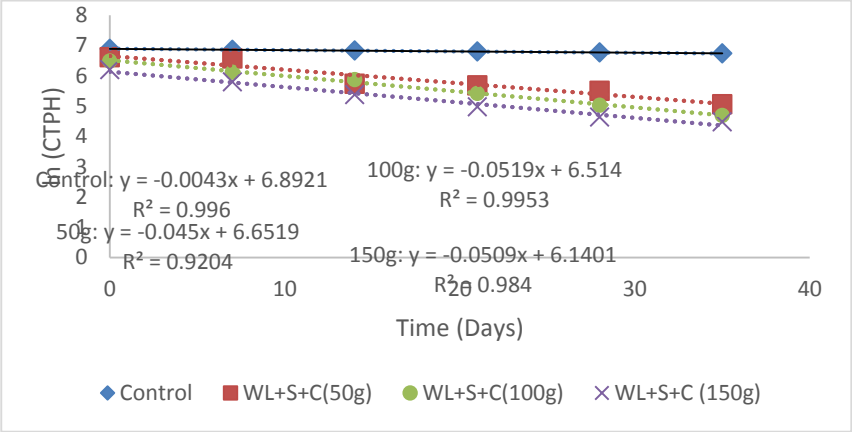


Figure 3.10: Plots for Evaluation of Rate Constant for Waterleaf Treatment in Loamy Soil

Figure 3.10 exhibits an array of unique linear regression equations signifying diverse treatment scenarios applied to Loamy soil during Waterleaf treatment. The determination of the degradation rate constant, designated as Ks, was derived from these linear equations featured on the plot. This determination entailed comparing the provided equations in Figure 3.10 with Equation (3.16). Subsequently, the established rate constant, coupled with the initial TPH concentration, was integrated into Equation (3.17) to yield the conclusive model utilized for projecting residual TPH levels within the Loamy soil. Noteworthy is the variability observed in the degradation rate constant, which correlates with the treatment weight. The tabulated degradation rate constants are available in Table 3.2. Furthermore, Table 3.2 provides the first-order kinetic rate model, encompassing the degradation rate constants unique to each treatment option.

Once again, by relying on the calculated degradation rate constants, the anticipated timeframe for the TPH concentration to decrease to half of its initial concentration within Loamy soil treated with Waterleafs is outlined in Table 3uhuvnnvvn.2. According to the analyzed timeframes, the investigation disclosed that without any amendment, approximately 161 days would be required for the TPH concentration to achieve 50% degradation in Loamy soil undergoing natural degradation (control sample). With the introduction of 50g of Waterleaf's treatment, this timeframe would be reduced to roughly 15 days to attain 50% degradation. This duration further diminishes to around 13 days when the treatment weight is escalated to 100g and holds steady for 150g treatment. Once more, the augmentation of treatment weight results in a shorter period needed for TPH to degrade to half of its initial concentration.

Notably, the pace of degradation, and consequently the degradation rate constant, exhibited its lowest value in the control sample under the Waterleaf treatment but showed an increase as the treatment weight was elevated. Intriguingly, the half-life in Loamy soil under both Cow dung and Waterleaf treatment was nearly identical in the control sample.

Table 4.2: Rate Constant and Model for Loamy Soil under Waterleaf Treatment

Weight (g)	k (day^{-1})	Predictive Model	$t^{1/2}$ (days)
Control	0.0043	$C_{TPH} = 984.47e^{-0.0043t}$	161.186
50g	0.045	$C_{TPH} = 774.25e^{-0.045t}$	15.4022
100g	0.0519	$C_{TPH} = 674.52e^{-0.0519t}$	13.3545
150g	0.0509	$C_{TPH} = 464.1e^{-0.0509t}$	13.6169

Conclusion

The study's main focus was on assessing the effectiveness of Cow dung and Waterleaf treatments for remediating crude oil-contaminated loamy soil. Water leaves and cow dung were used fresh and silt loams soil were used for this study. The physicochemical properties of the soils before and after pollution were analyzed. Also, the Total Heterotrophic Bacteria count (THB) and Total Petroleum Hydrocarbons (TPH) content were analyzed every 7 days for 35 days. The results obtained from the physicochemical analysis showed that there was an increase in the pH of the soils after contamination, while there were decreases in the soils' moisture content, total organic carbon, total nitrogen, and phosphorus contents, which differ remarkably from the control samples, implying that the water leaves and cow dung treatments after pollution were effective. Also, as time and treatment weights were increased, the percentage degradation of TPH in the soil increased. Thus, the 150g weight samples exhibited the best performance on the 35th day, consistently demonstrating superior efficacy across various soil types. Waterleaf treatment outperformed Cow dung treatment in terms of TPH percentage degradation. Specifically, 150g Waterleaf treatment resulted in degradation percentages ranging from 81.27% to 89.50%, while Cow dung treatment achieved percentage of 72.24% to 86.88%. Further examination of TPH degradation rate constants and half-lives for Waterleaf and Cow dung treatments revealed distinct patterns. Waterleaf treatment displayed higher degradation rate constants (ranging from 0.0043 to 0.057 day^-1 for Cow dung and 0.0043 to 0.0509day^-1 for Waterleaf), corroborating its superior TPH removal efficiency. Half-life analysis indicated that Cow dung required more time for TPH to degrade to half its initial concentration, while Waterleaf treatment exhibited faster degradation. Conversely, the variation of Michaelis-Menten constants showed no clear pattern. In terms of model performance, the first-order kinetic rate model demonstrated better congruence between experimental TPH concentrations and the Michaelis-Menten equation. Comparing diverse treatment options for soil remediation, the 150g weight samples exhibited the best performance on the 35th day, consistently demonstrating superior efficacy across various soil types. Waterleaf treatment outperformed Cow dung treatment in terms of TPH percentage degradation. Specifically, 150g Waterleaf treatment resulted in degradation percentages ranging from 81.27% to 89.50%, while Cow dung treatment achieved percentages of 72.24% to 86.88%.

Further examination of TPH degradation rate constants and half-lives for Waterleaf and Cow dung treatments revealed distinct patterns. Waterleaf treatment displayed higher degradation rate constants (ranging from 0.0043 to 0.057 day^-1 for Cow dung and 0.0043 to 0.0509 day^-1 for Waterleaf), corroborating its superior TPH removal efficiency. Half-life analysis indicated that Cow dung required more time for TPH to degrade to half its initial concentration, while Waterleaf treatment exhibited faster degradation. Conversely, the variation of Michaelis-Menten constants showed no clear pattern.
In terms of model performance, the first kinetic rate model demonstrated better congruence between experimental TPH concentrations and the Michaelis-Menten equation.

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