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Research

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Kinetic Modelling of Insitu Treatment of Petroleum Hydrocarbon Contaminated Soil Using Bone Char and NPK Fertilizers

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Abstract

This study investigates the effectiveness of bone char (organic) and NPK (inorganic) fertilizers as stimulants in the degradability of petroleum hydrocarbon contaminants on soil. The Physicochemical properties of the hydrocarbon sludge were used to assess the effectiveness of this process over an 8-week period using 0.5 – 3.5kg mass of each fertilizer at different experiments. A first order kinetic model was used to estimate the rate of degradation of the total hydrocarbon content (THC) and total organic carbon (TOC) contaminants and the half-life of the remediation process. The microbial population within the period was also determined. The P-Value ($P < 0.05$) indicate that these fertilizers were effective in degrading these contaminants on the soil, because of the significant difference between the treated and the control soil samples. A direct relationship was observed between with the mass and performance of the fertilizers. With 3.5 kg mass of the fertilizers, rate constants of 0.018 day^{-1} and 0.019 day^{-1} were obtained for the removal of the THC and TOC contaminants, respectively using the bone char fertilizer, whereas NPK fertilizer gave rates of 0.03 day^{-1} and 0.023 day^{-1} respectively. The performance of the NPK fertilizer is attributable to its Nitrogen and Phosphorous content. The model adequately described the process and showed the effectiveness of both fertilizers in the remediation process.

Keywords: kinetic model, bioremediation, bone char, NPK, hydrocarbon, microbial growth

Introduction

Crude oil exploration and exploitation has given rise to the problem of environmental pollution in many parts of the world. The large scale production and oil exploration activities in the Niger Delta region of Nigeria, renowned for its huge deposit of crude oil and natural gas reserve, has given rise to oil spillage which has impacted negatively on the environment leading to air, water, and land pollution. The effects on terrestrial and aquatic lives are equally huge and devastating, and these consequences include surface layer poisoning, distortion of macro and micro ecology, as well as other environmental degradation and health impacts. The land desolation, soil and water body degradation associated with these spills leads to reduced agricultural production [1]. To this end, the need for remediation of these soils has become vital. Bioremediation has been shown to be an efficient and viable approach in the management of oil spilled soils. This technique involves the use of microorganisms such as bacteria, yeast, and fungi to degrade toxic and nontoxic substances. Hence, it involves the breakdown of these substances using living organisms [2, 3]. Various techniques of this approach exist and these include: bio-stimulation, bioaugmentation, bioventing, land farming, phytoremediation, intrinsic bioremediation and controlled natural attenuation, and could be applied in-situ or ex-situ depending on other factors of consideration [4 – 8].

This study which considered the bio-stimulation approach involved the enhancement of the hydrocarbon degradation process through microbial activities which were stimulated through the addition of nutrients. Wise *et al.* [2] and Chorom [9] reported that water, temperature, pH, oxygen content of the soil as well as availability of the contaminants to microorganisms could affect the degradation rate. The most widely reported application of this technique involves the use of NPK fertilizers. Increase in soil pH, mineral leaching, and other environmental effects are

among the adverse effects reported with the use of inorganic fertilizers, in addition to the huge cost consideration which remains a significant factor of consideration with these materials. On the other hand, organic fertilizers have been reported to fare better in these areas [10]. This study investigates the effectiveness of bone char (organic) and NPK (inorganic) fertilizers as stimulants in the remediation of an oil contaminated site with a view to determining the kinetics of the process.

Materials and Methods

Study Area

An experimental site located within the University of Port Harcourt (4.9069 °N, 6.9170°E), Rivers State-Nigeria was used for this study after due authorization by the University research and ethics committee. The area is a tropical rainforest region, which has an average daily minimum temperature of 23°C and a mean temperature of 31.5 °C. It has about seven (7) months of rainfall annually (April to October), an average annual rainfall of 2400 ml, and a mean relative humidity of 85% per month.

Sample Preparation

NPK Fertilizer

30g of NPK fertilizer was purchased from a commercial retail outlet in Port Harcourt metropolis, Rivers State, Nigeria.

Bone Char Fertilizer

Bone char sourced from an abattoir within the study area was subjected to pyrolysis treatment at the Chemical Engineering laboratory, University of Port Harcourt, to obtain an organic fertilizer.

Crude Oil sample

The crude oil sample used for this experiment was sourced from an oil spill site at Abua/Odual Local Government Area, Rivers State-Nigeria using a plastic container. 200 litres of the crude sample was collected from recovery tanks installed by the clean-up contractor. The sample was collected under the supervision and assessment of Department of Petroleum Resources (DPR) to ensure conformity and compliance with product and environment standards. Other chemicals used in this experiment were purchased from a retail outlet, Austino Laboratory Services Ltd, Choba, Port Harcourt (Nigeria) and were of analytical grade.

Soil Preparation and in-situ bioremediation procedure

The land portion, with no known history of crude oil contamination, was segmented into 7 cells of similar dimension demarcated from each other with a 10 cm wooden structure. Each cell, having a dimension of 1.5metres x 1.5metres and a depth of 30cm (contaminated soil depth), was polluted with 20 litres of the crude oil sample to form a sludge which was allowed to stand for 2 weeks under continuous monitoring. At the end of this period, the vegetations on the study site were observed to have died. Thereafter, different concentrations of the bone char and NPK fertilizers were administered on these cells (Table 1), and the soil tilled to expose its surface area for treatment and to enhance oxygen availability to bacteria and fungi for effective chemical reactions. Tilling of the soil was done in every 3 days whereas a daily irrigation of the site helped ensure that the optimal soil moisture condition of the site was maintained. Moreover, the experiment was conducted in an uncontrolled environment during the dry season. The effect of bioremediation on the contaminated soil was observed for a period of 8 weeks. Previous studies by Daka *et al.* [11] and Ayotamuno *et al.* [12] employed a similar approach under different experimental conditions.

The analytical soil samples were collected at depths of 6, 18, and 27cm from the cells at the experimental site using a spade. The soil samples were, thereafter, mixed into composite samples, labeled and sealed in small polyethylene bags for analysis.

Table 1: Soil treatment conditions

S/N	Site Code	Treatment		
		Crude oil sludge (l)	NPK (kg)	Bone Char (kg)
1	C	20	-	-
2	X ₁	20	0.5	-
3	X ₂	20	-	0.5
4	Y ₁	20	2	-
5	Y ₂	20	-	2
6	Z ₁	20	3.5	-
7	Z ₂	20	-	3.5

NB: C is the Control experiment

Characterization of Soil Sample

Physicochemical properties such as pH, Total Hydrocarbon content, organic carbon, extractable phosphorous and total nitrogen content were used as indices to evaluate the contamination levels of the soil. Samples collected from contaminated site were air dried and homogenized by passing them through a 2 mm mesh sieve. The physiochemical properties were determined as follows:

a. Total Hydrocarbon Content (THC)

10 g of the representative soil sample was shaken vigorously with 10 ml of toluene for 10 mins. The extract was analyzed with 420nm Bausch & Lomb spectronic-70 spectrophotometer (Bamko-surplus, Texas, United States). The concentration of the extract was obtained from the spectrophotometer and compared with a standard curve of another substance with known concentration. The total hydrocarbon content was evaluated with reference to the standard curve and multiplied by the appropriate dilution factor in line with the study of Ikiogha *et al.* [13].

b. Total Nitrogen (TN)

2 g of the soil sample was air dried, weighed and added into a 150ml beaker. 10 ml each of the following were added alongside: selenium, copper sulphate (CuSO_4), sodium sulphate (Na_2SO_4), and sulphuric acid (H_2SO_4). These reagents were of analytical grade. The mixture was agitated by gentle swirling and digested in a Foss Tecator digester (KjedahlTM Model 2300, Pittsburgh, USA) for 15 mins until an ash coloured solution was formed. The setup was heated for an hour and cooled for about same time. The digest was emptied into a 250ml capacity beaker and made up with distilled water. An auto-analyzer was used to determine the ammonium nitrogen of the aliquots. The percentage nitrogen content was thereafter evaluated with due consideration of the dilution factors.

c. pH

This was done using a corning pH meter model 7 (Corning Scientific, NY, USA) by dipping the electrode into a 1:25 sample and water suspension which had been stirred and allowed to equilibrate for about an hour.

d. Phosphorous Content

The phosphorus content was determined using the Bray and Kurtz method [14]. 2.85 g of sample was weighed into a clean and dry sample bottle. 20ml of Bray No.1 extractant was added (0.025N + 0.03N NH_4) and shaken for 60 sec. Thereafter, 10 ml filtrate was added into a 50ml capacity volumetric flask using a pipette. The mixture was diluted (made up) with about 20 ml of distilled water, after which 4 ml ascorbic acid solution was added. This setup was allowed to rest for 30 mins, during which a change in colour was observed, before being analyzed with a spectronic-20 spectrophotometer operating at 660nm wavelength. This analysis was carried out in accordance with the manufacturer's specification.

e. Total Organic Carbon (TOC)

This was evaluated using the wet combustion method by measuring 1g of the finely ground soil sample into 3 different 500 ml beakers with 10ml of potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) solution added alongside amidst continuous stirring using a magnetic stirrer. 20 ml sulphuric acid (H_2SO_4) was added to the content of the beakers using a graduated cylinder and, thereafter, the beaker was rotated and allowed to stand for 10 minutes while distilled water was equally added for dilution, by making up the solution to the 200 ml mark of the beaker. 25 ml of 10.5N ferrous ammonium sulphate ($\text{NH}_4\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$) and 0.4N potassium permanganate (KMnO_4) were also added under strong light.

f. Soil Texture

This was evaluated using the Bouyoucous hydrometer [15] in accordance with the method of Day [16].

Evaluation of Microbial Population

This process was carried out using the methods of Cowan and Steel [17] and Buchanan and Gibbons [18]. Prior to the experimental process, all the media and diluents were first sterilized in an autoclave operating at a temperature condition of 120°C for 15mins. The glass wares were dried in a hot air oven for 3 mins. The saline solution was prepared by weighing 0.85g NaCl into a clean 500ml beaker containing 100 ml of distilled water amidst continuous agitation. The solution was thereafter transferred into the various 9ml capacity test tubes used for the experiments for sterilization under a temperature condition of 120°C for 15mins. The solution was also used for the serial dilution of the soil samples. Cultivation and enumeration of the soil bacteria was done by adding 1g of the air-dried soil sample into 1ml of the sterile distilled water amidst vigorous agitation to obtain a homogeneous mixture. 1 ml of the 10^{-1} solution was added into a 9ml test tube containing the diluents and serially diluted stepwise to obtain a 10^{-3} dilution. A spatula was used to transfer 0.1ml aliquot of the solution into the sterilized nutrient agar plates in such a way that most of the surface area was exposed for reaction. The inoculated plates were incubated at a temperature of 37 °C for 24 hours, before the nutrient agar plates were examined for bacterial growth. The total viable heterotrophic bacteria were evaluated based on the number of the Colony Forming Units (CFU) using the colony counting technique to determine the number of cells capable of splitting [19]. The CFU was evaluated using the correlation:

$$\text{CFU/g} = \text{number of colonies} \times \text{dilution factor} / \text{volume of culture plate}$$

a. Identification and Isolation of soil bacteria: The bacteria culture was isolated by aseptically streaking colonies of different culture seen on the plate to a freshly prepared nutrient agar plate. The setup was incubated at 28 °C and allowed to stand for 24 hours to allow the bacteria grow. Based on the colony morphology and exhibition of zones of inhibition on the primary culture,

isolates were selected. Isolate purification was done using dilute nutrient broth and agar media and stocked at -80 °C. Characterization of these isolates was done using gram stain (methyl and violet test) to confirm the bacterial growth while the viable count was conducted manually. The isolates were observed under an inverted microscope at × 100 oil immersion following the method of Khan *et al.* [20] while the CFU was evaluated.

b. Isolation and identification of soil fungi: This was done using dilution plate method on potato dextrose agar. Isolates of soil fungi which were examined macroscopically and microscopically were identified by matching the characteristics of the fungus obtained from observations in accordance with the work of Umboh *et al.* [21].

Kinetics of the Process

With an assumption of a first order kinetic, the rate equation of the process for reactant of measured concentration (C) is given by:

$$\frac{\delta C}{\delta t} = -k_1 C \quad (1)$$

The half-life is given by:

$$t_{\frac{1}{2}} = \frac{\ln 2}{k_1} = \frac{0.6933}{k_1} \quad (2)$$

$$\ln C_t = -k_1 t + \ln C_0 \quad (3)$$

$$C_t = C_0 \exp(-k_1 t) \quad (4)$$

$$\frac{C_0 - C_t}{C_0} = FE \quad (5)$$

Where

FE = Fractional Efficiency

$$C_t = C_0(1 - FE) \quad (6)$$

$$t = -\frac{1}{k_1} \ln(1 - FE) \quad (7)$$

$$\ln(1 - FE) = -k_1 t \quad (8)$$

Equation (8) describes a straight line graph of the form $y = mx$

Where:

$$y = \ln(1 - FE)$$

$$m = -k_1$$

C_0 = initial concentration

C_t = Concentration at time t

t = elapsed time

Degradation

The hydrocarbon degradation was evaluated through the concentration of the Total Carbon

Content using the correlation:

$$D = \frac{TC_0 - TC_i}{TC_0} \times 100 \quad (9)$$

Where:

TC_0 = Initial Total Carbon Concentration

TC_i = Residual Total Carbon Concentration

Bio-Stimulation Efficiency

The bio-stimulation efficiency was used to estimate all the treatability options provided by the optimization rates of the bio-stimulants and was obtained using Equation 10. This was done to ascertain the effect of the biotic and abiotic factors in the bioremediation process.

$$BE = \frac{C_t - C_u}{C_t} \quad (10)$$

Where:

BE = Bio-stimulation efficiency

C_t = percentage of crude oil removed from the treated soil.

C_u = percentage of crude oil removed from the untreated soil (control)

Statistical Analysis

T-test was used to analyze the obtained THC data to evaluate the bioremediation process on the contaminated soil. The choice of the test instrument was based on the sample size. The treated soil samples were compared with that of the control site and the following conditional statements were considered:

- i. If $P > 0.05$ accept null hypothesis (H_o) (no appreciable effect on the process) and reject alternative hypothesis (H_i) (significant effect on the process).
- ii. If $P < 0.05$ reject null hypothesis (H_o) (no appreciable effect on the process) and we accept alternative hypothesis (H_i) (significant effect on the process).

The Pearson Correlation coefficient and t-stat values were also obtained. Microsoft excel and SPSS version 17 were used for this process.

Results and Discussion

Soil Properties

The particle size analysis of the soil texture within the depth of interest (30cm) prior to treatment showed it to comprise of the following: Soil – 75%, clay – 15% and silt – 10%. The soil texture, which is a classification of the soil based on its physical characteristics, was used to assess the soil sample to ascertain the air and water retention capability, rate of water movement in and out of the soil, as well as the ease of working with the soil sample. The texture triangle (Figure 1) indicate the soil texture to comprise of both sand and loamy sand and corroborates the work of Odokuma and Dickson [22] for the top soil of tropical rainforest.

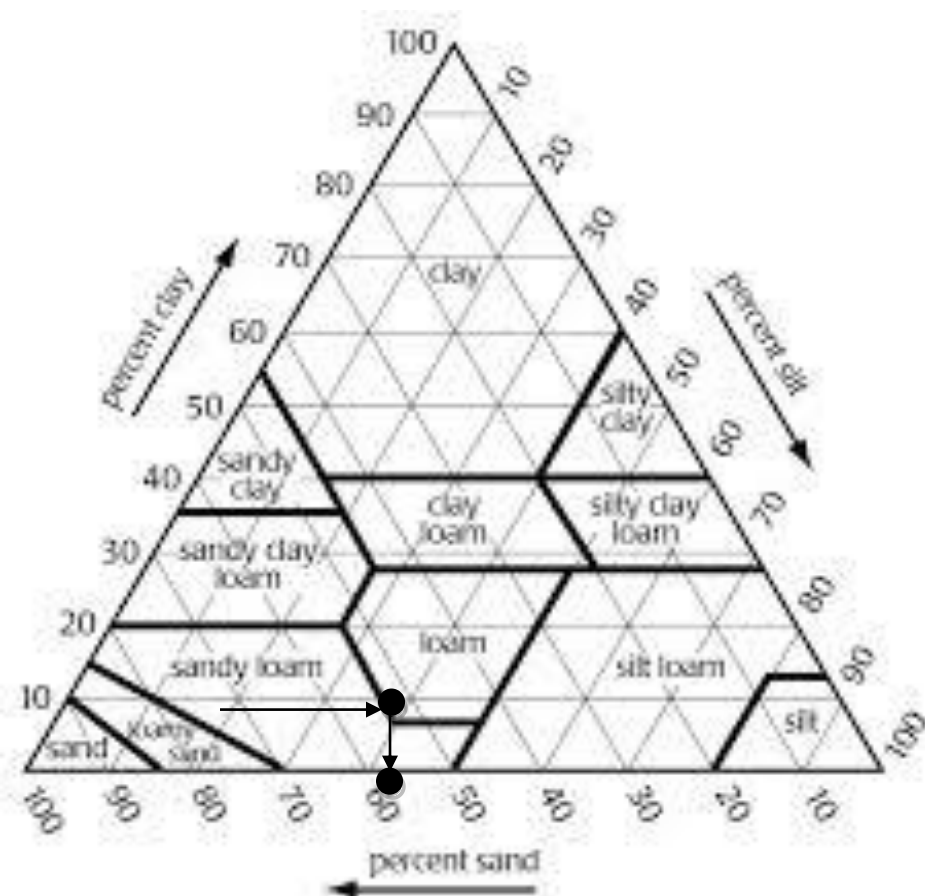


Figure 1: Soil Texture Triangle

247 The physicochemical and microbial properties of the soil samples (Table 2) are presented:

248 Table 2: Physicochemical and microbial properties of soil samples

Description	Property	Soil sample	
		Uncontaminated	Contaminated
Physicochemical Properties	Soil Porosity (%)	85	52
	THC (mg/kg)	136.74	198949.47
	Total Nitrogen (%)	0.85	0.27
	pH	5.86	6.74
	Phosphorous Content(mg/kg)	18.24	6.41
	Total Organic Carbon (%)	3.28	8.52
	Nitrate (mg/kg)	0.85	0.27
	Phosphate (mg/kg)	55.92	84.28
	Sodium (mg/kg)	10.28	4.17
	Potassium (mg/kg)	163.74	42.79
	Calcium (mg/kg)	1305.82	153.80
	Sulphate (mg/kg)	5.86	13.85
	Sodium Adsorption Ratio	2.05	11.47
	Moisture Content (%)	8.46	6.24
	Oil and Grease (mg/l)	305.86	14720.73
	CEC (meq/100g)	13.94	74
	Electrical Conductivity (µs/cm)	30	79
Microbial properties	Total Bacteria Count (cfu/ml)	3.46×10^6	1.31×10^2
	Hydrocarbon Utilizing Bacteria (cfu/ml)	2.23×10^2	1.64×10^2
	Hydrocarbon Utilizing Fungi (sfu/ml)	1.05×10^2	1.01×10^2

249

250 The physicochemical properties and microbial properties of the sandy loam soil were adversely

251 affected by the contamination of the petroleum hydrocarbon. Both the trace metal contents and

252 the microbial population of the soil were greatly depleted. The pH conditions in this study were

253 optimum and favourable for microbial growth and higher than the minimum growth pH reported

by Parker *et al.* [23]. Slight changes in the pH could alter the ionization of amino-acid functional groups and interfere with the hydrogen bonding leading to their denaturation thereby rendering them ineffective for use in the biodegradation of the hydrocarbon [24]. This study corroborates previous findings on the effect of petroleum hydrocarbon contamination on soil moisture [25]. Increase in the electrical conductivity and cation exchange capacity (CEC) indicates the dissociation of ion charges into the soil due to crude oil contamination. These ions are attracted to the water molecules in the soil which carry polar charges. The ionic solutions become electrolytes capable of conducting electricity.

Fertilizer Properties

The physiochemical properties of the bone char and NPK fertilizers (Table 3) are presented. The constituents of these fertilizers and their physical properties are critical to their performance in the degradation of the hydrocarbon contaminant.

Table 3: Physicochemical Properties of Bone Char

S/N	Property	Amount	
		Bone Char	NPK
1	pH	7.18	7.01
2	Moisture Content (%)	1.86	1.0
3	Density (g/ml)	3.06	1.26
4	Porosity (%)	5.80	15.0
5	Calcium ion (mg/kg)	3740.36	0.0
6	Total Nitrogen (%)	3.05	15.0
7	Potassium (%)	98.20	15.0
8	Phosphorous (%)	0	15.0

The effect of the structural-mechanical properties fertilizer granules have been reported [26] and these are fundamental in the exposure of the reactant surface area necessary for these reactions to occur as well as for the interaction of the microbes with the substrate. Various authors have shown the effect of these fertilizers on growth characteristics and soil nutrients [27 – 30] and bioremediation [31 – 35]. This study corroborates these findings. Moreover, the use of organic compounds such as formaldehyde and biuret, in the production of NPK fertilizer, would facilitate the microbial growth and the degradation process.

Effects of In-Situ Treatment of the Contaminated Soil

The effect of both grades of fertilizers (organic and inorganic) on the experimental site was evaluated through an analysis of the physicochemical properties of the soil samples (Table 2) and the Total Hydrocarbon Content (Figure 2) of the soil.

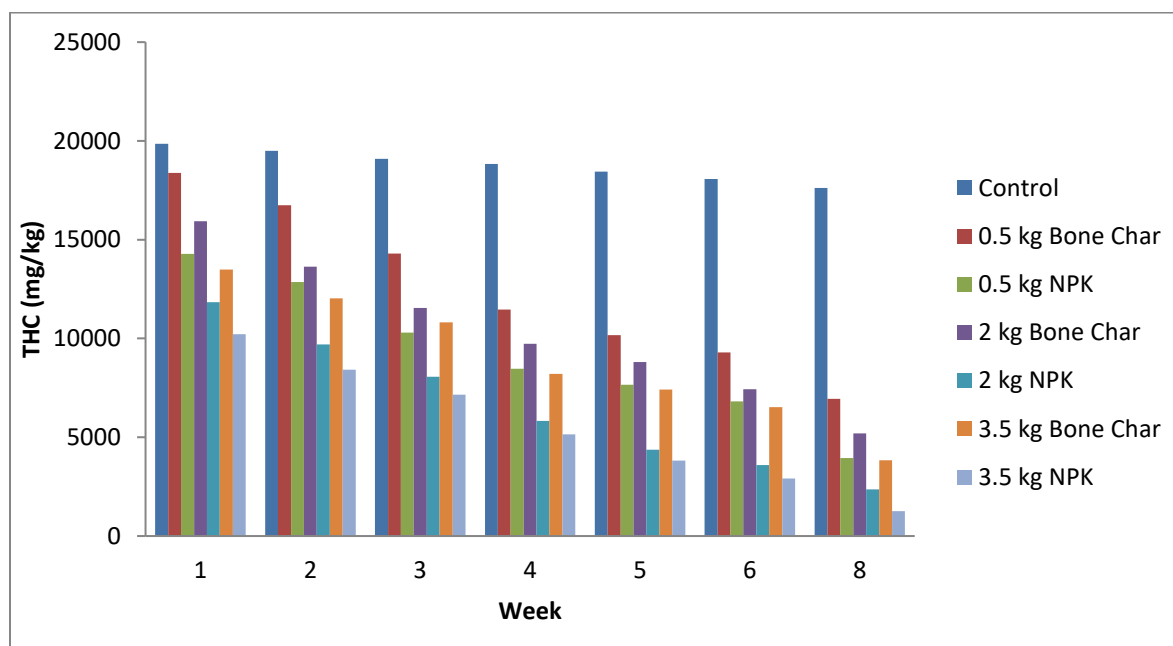


Figure 2: Bioremediation treatment on Total Hydrocarbon Content

Both grades of fertilizer drastically reduced the total hydrocarbon content of the contaminated soil over time. This indicates that bioremediation was effective in reducing the Total

Hydrocarbon Content from the contaminated soil. There is a link between the mass of fertilizer and performance in the bioremediation process for both grades of fertilizers. 3.5 kg mass of both fertilizers had the highest reduction of the THC and TOC contaminants; efficiency for all treated samples on the Total Hydrocarbon Content was above 60% (Figure 7a). Comparatively, the NPK grade gave a better performance; however, its synthetic nature could give rise to other adverse effects on the environment and potential increase in heavy metal concentration [31]. The effectiveness of the bone char grade of fertilizer could be linked with its ion content leading to the degradation of the hydrocarbon contaminant [36]. A similar pattern was observed for the TOC (Figure 3 and 7b) within this period which confirms the bioremediation effects of these grades of fertilizers.

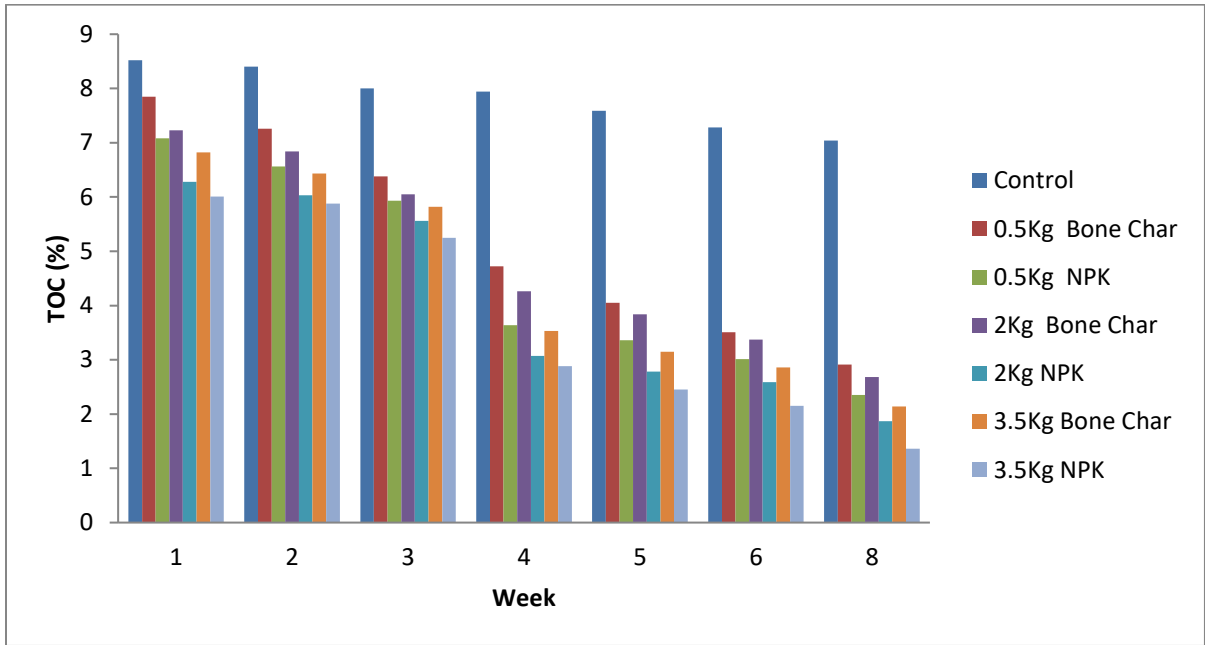


Figure 3: Effect of bioremediation treatment on Total Organic Carbon

The bacillus and pseudomonas species were predominant on the contaminated soil. Addition of these fertilizers resulted in a significant increment in the heterotrophic bacteria and heterotrophic

fungi. Total Bacterial Count (TBC) was used to obtain a quantitative idea of the microorganisms present in the soil. TBC is indicative of the number of colony forming units of the soil sample and formed the basis to analyze the degradation rate of the crude oil in the polluted soil. Prolonged period of the bioremediation treatment with both grades of fertilizers favoured the production of the microbes (Figure 4). The NPK fertilizer grade gave higher bacterial count than the Bone Char grade whereas higher mass of both fertilizers favoured the Total Bacterial Count and consequently their performance in the remediation process. Chorom *et al.* [9] reported that excessive application of fertilizers makes survival conditions of these microbes critical.

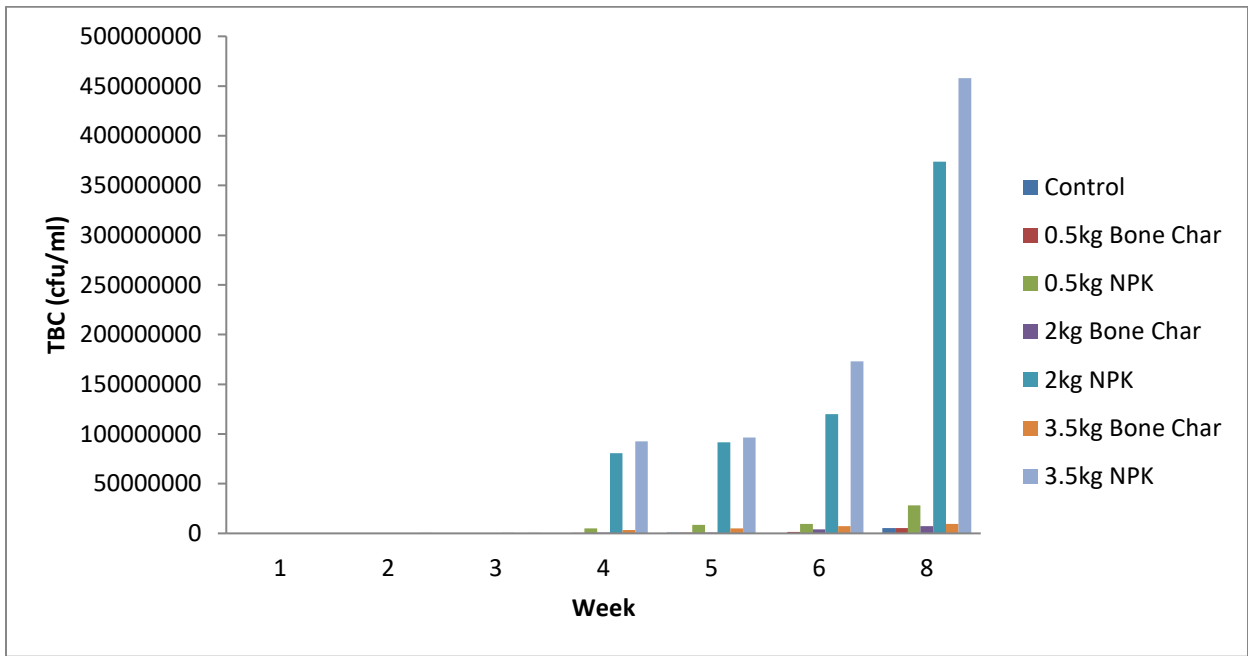


Figure 5: Effect of bioremediation treatment on Total Bacteria Count

Hydrocarbon Utilizing Bacteria (HUB) degrades aliphatic alkanes through various metabolic activities. They utilize the linear hydrocarbon chains in petroleum as their main energy source under aerobic conditions. Additionally, when exposed with other sufficient limiting nutrients like Nitrogen and Phosphorous, they produce surfactant glycolipids which help reduce water tension and the uptake of hydrocarbon eg alpha-proteobacteria *Alcanivorax*, *Alcanivorax borkumensis*

312 etc. Performance of the both grades of fertilizer in the isolation of the HUB was shown to be
313 time and dose-dependent (Figure 5). The higher performance of the NPK fertilizer in the
314 remediation process could be attributed to its higher Nitrogen and Phosphorous content which
315 makes it possible to isolate more of the desirable microbe population to produce the surfactant
316 glycol lipids that reduce surface tension thereby making it possible to degrade the contaminant
317 hydrocarbon . The bio-stimulation efficiency (Figure 8) further corroborates this.

318 Similarly, hydrocarbon utilizing fungi has proven to be very effective in the removal of a wide
319 array of toxins or contaminants from the environment. This is achieved through the degradation
320 of the hydrocarbon components to non-toxic substances. The growth rate of the HUF (Figure 6)
321 followed a similar pattern as that of the HUB, and the NPK fertilizer favoured the production of
322 the HUF as it consistently gave a higher count of the HUF than the bone char within the period
323 of study. Performance of the both grades of fertilizer in the isolation of the HUF has been shown
324 to be time and quantity-dependent. Kumar *et al.* [37] and Chikere *et al.* [38] reported the species
325 of this capable of degrading petroleum hydrocarbons in contaminated soils to include:
326 *Penicillium* sp., *Candida* sp., *A. Niger*, *Rhizopus* sp., *Mucor* sp., *Talaromyces* sp., *Saccharomyces*
327 sp., *Cladosporium* sp., *Fusarium* sp., *Rhodotorula* sp. and *Aspergillus flavus*,

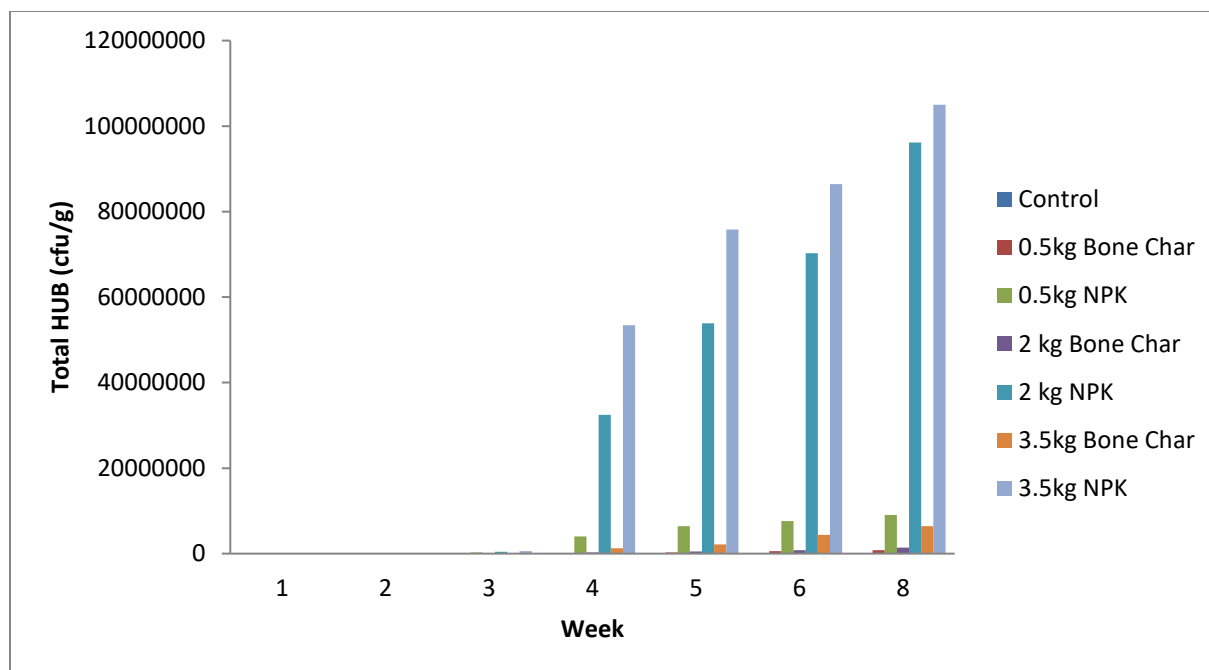


Figure 5: Heterotrophic Utilizing Bacteria

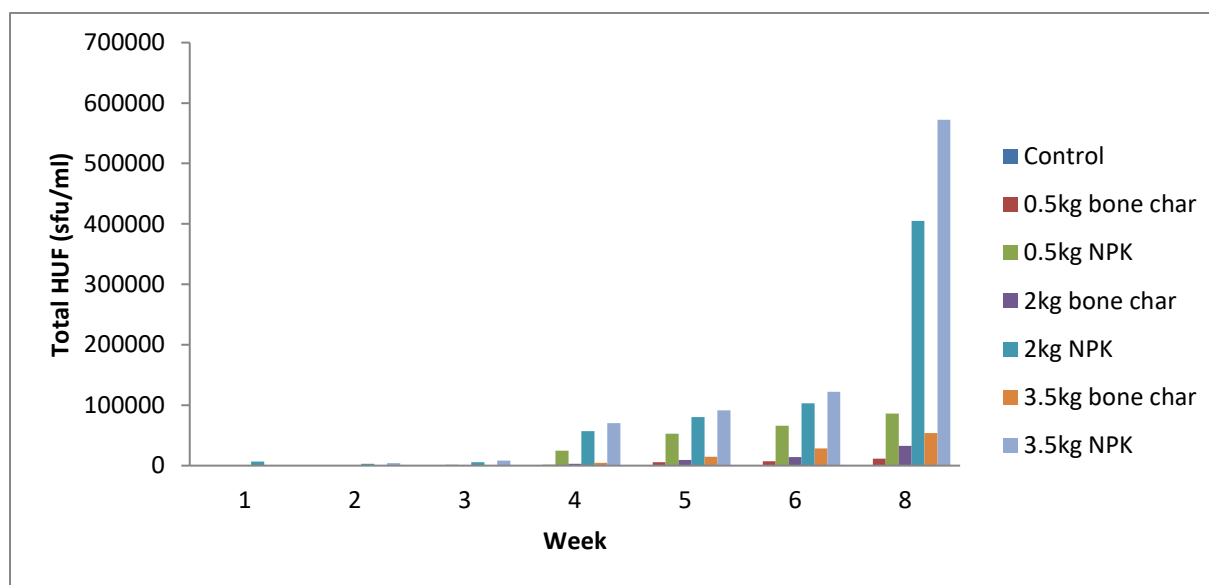


Figure 6: Heterotrophic Utilizing Fungi

The steady increase in the growth rate of these microorganisms with time entails a rapid reproduction of the crude oil degrading mechanisms which were responsible for the attenuation of the soil contaminants and this corroborates the works of Baheri and Meysami [39].

Statistical Analysis

The results of the statistical analysis are shown:

Table 4: Statistical Analysis

Soil Sample	Statistical Parameters					
	THC			TOC		
	R ²	t-Stat	P-Value	R ²	t-Stat	P-Value
0.5kg Bone Char	0.9865	4.92811	0.00264	0.96638	4.87701	0.00277
0.5 kg NPK	0.9878	9.04522	0.0001	0.93465	6.14734	0.00085
2kg Bone Char	0.9919	7.70261	0.00025	0.95774	6.07293	0.00091
2kg NPK	0.9826	12.0418	2E-05	0.91986	7.36838	0.00032
3.5kg Bone Char	0.9885	10.0233	5.7E-05	0.93606	6.47146	0.00065
3.5kg NPK	0.9929	14.476	6.8E-06	0.93571	7.56739	0.00028

The R², t-stat and P-values were used to study the effectiveness of the bioremediation treatment in the removal of the THC and TOC contents. The analysis was done in comparison with the control sample based on the P-value for significance level (P<0.05). Appreciable significant differences were observed for all the samples. Hence we reject the null hypothesis (H_0) and accept the alternative hypothesis (H_i). There was significant effect of the remediation treatment process on the contaminated soil which gave rise to an appreciable reduction of Total Hydrocarbon (THC) and Total Organic Carbon (TOC) contents.

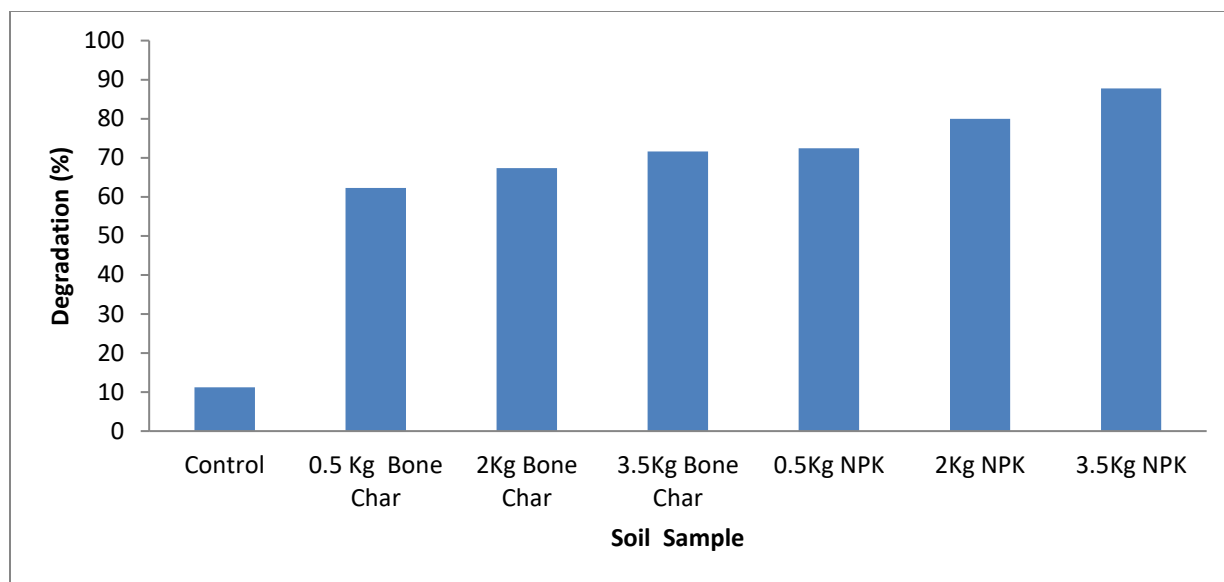


Figure 7a: Degradation of THC

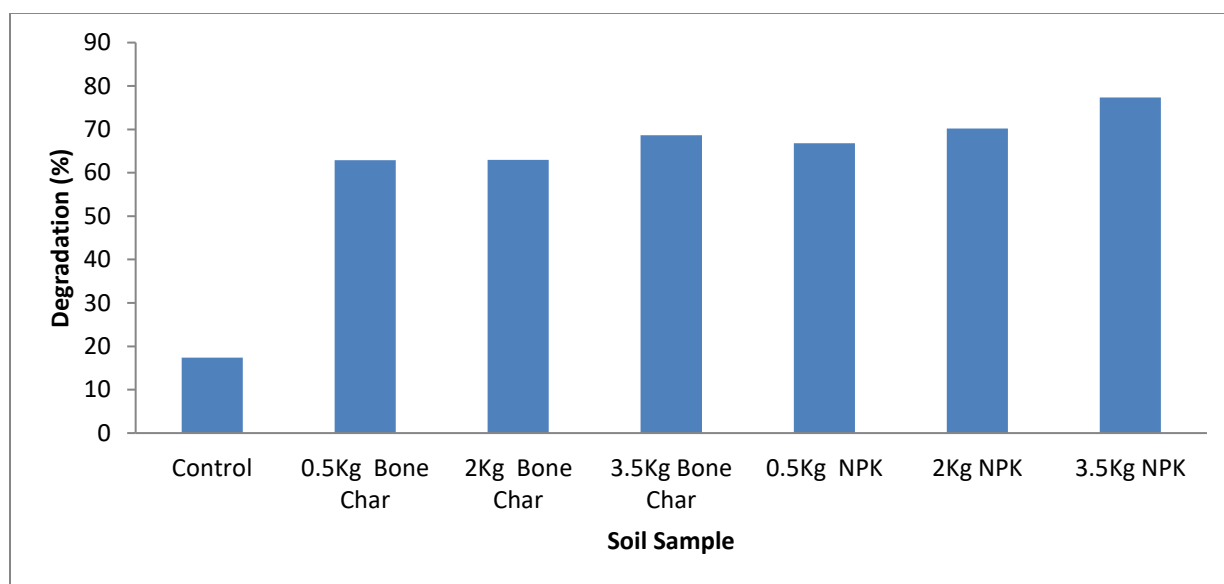


Figure 7b: Degradation of TOC

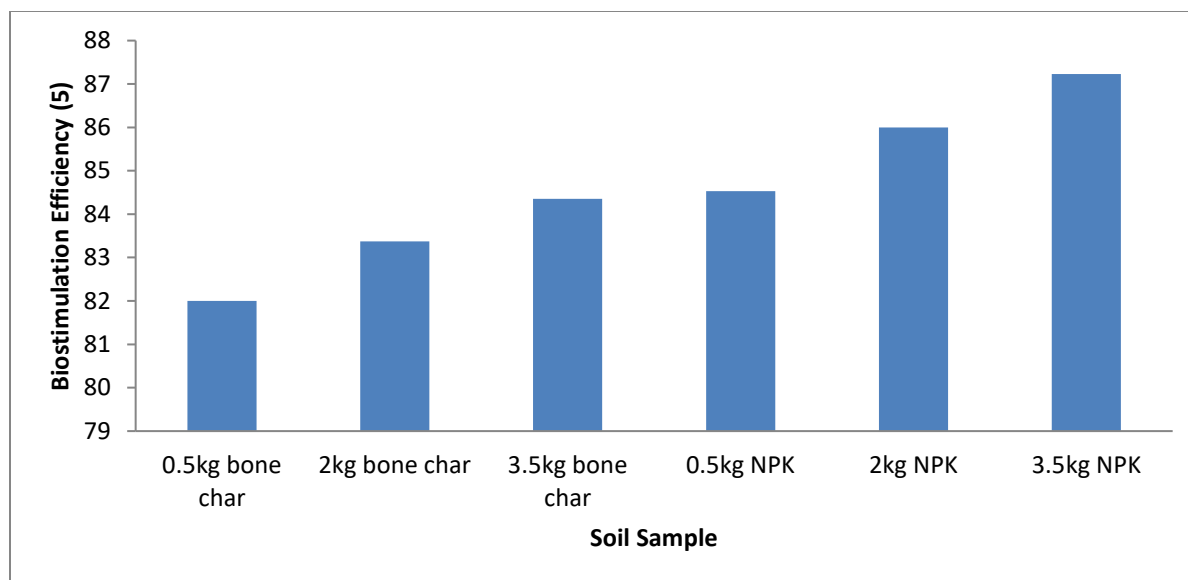


Figure 8: Biostimulation Efficiency

Kinetics of the Process

The first order kinetic model, which proposes that rate of degradation of substrate is directly proportional to its concentration, was used to model the degradation level of total hydrocarbon carbon (THC) and total organic carbon (TOC) content in the contaminated soil. High K value implies high degradation rate (Table 5), while the fractional efficiency was plotted against time for all samples (Figures 9 - 14).

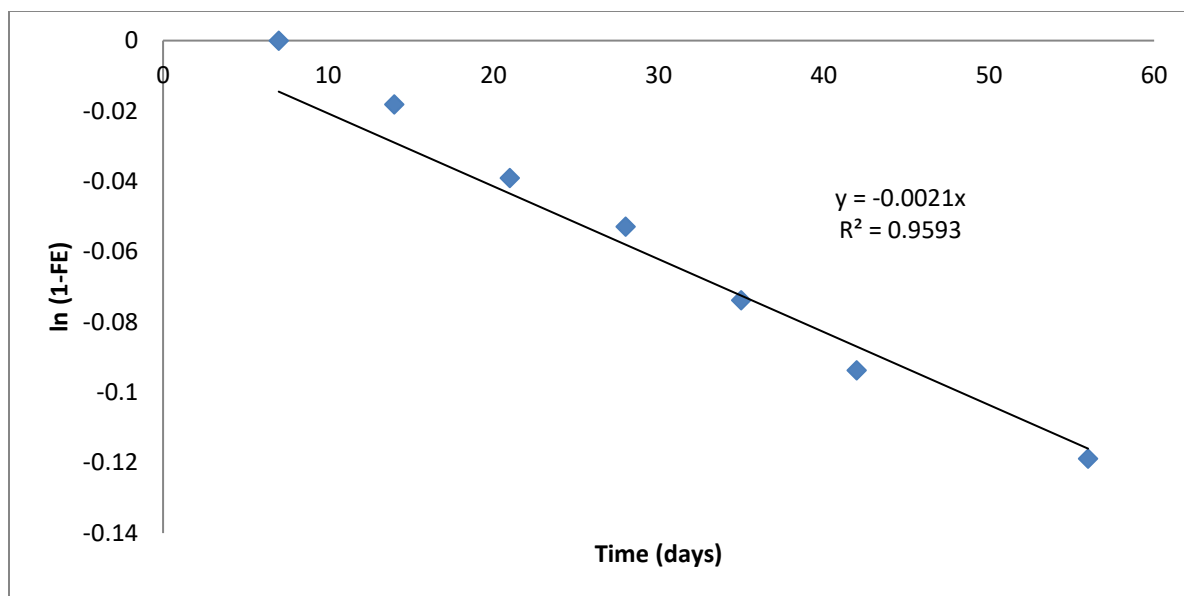


Fig 9: First Order Kinetic Pattern of THC reduction for the Control Sample

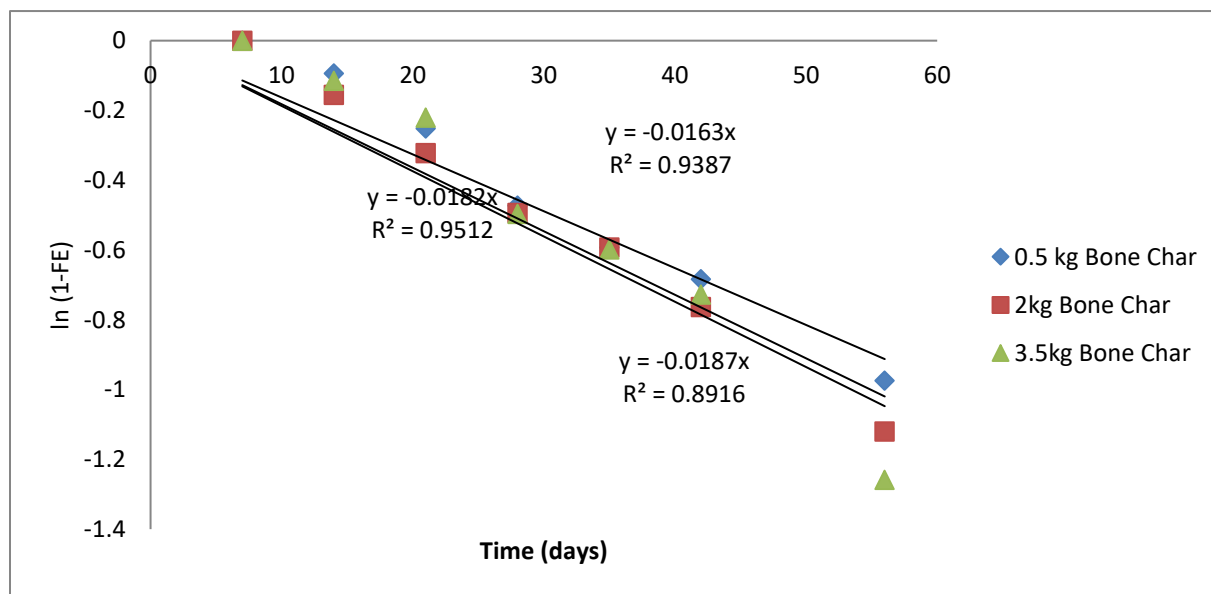


Fig 10: First Order Kinetic Pattern of THC reduction for Bone Char Treated Soil Samples

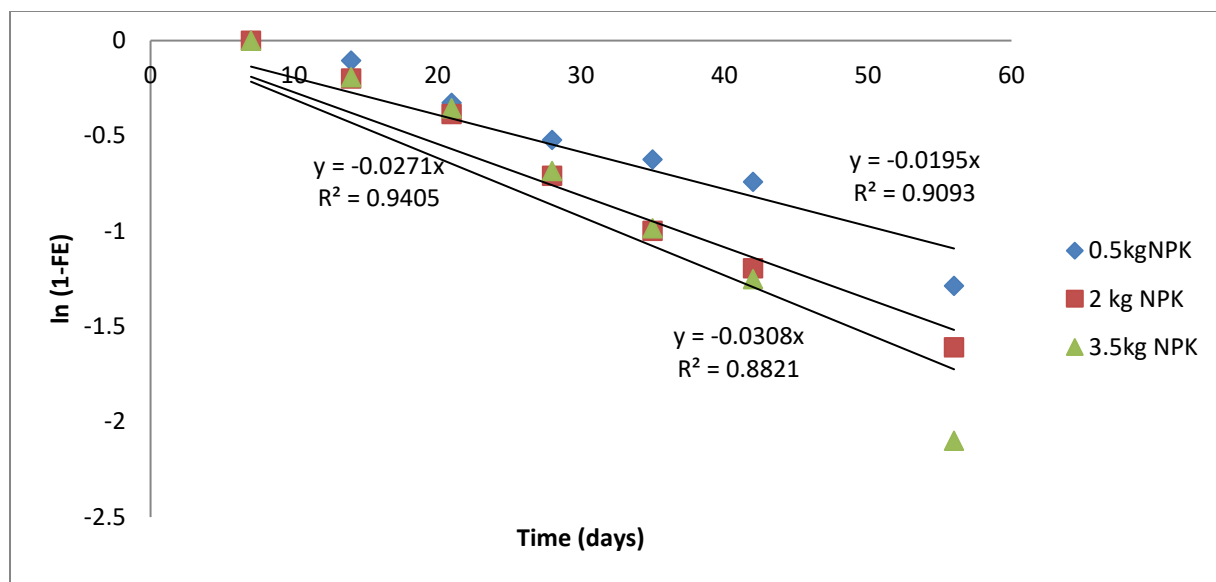


Fig 11: First Order Kinetic Pattern of THC reduction for NPK Treated Soil Samples

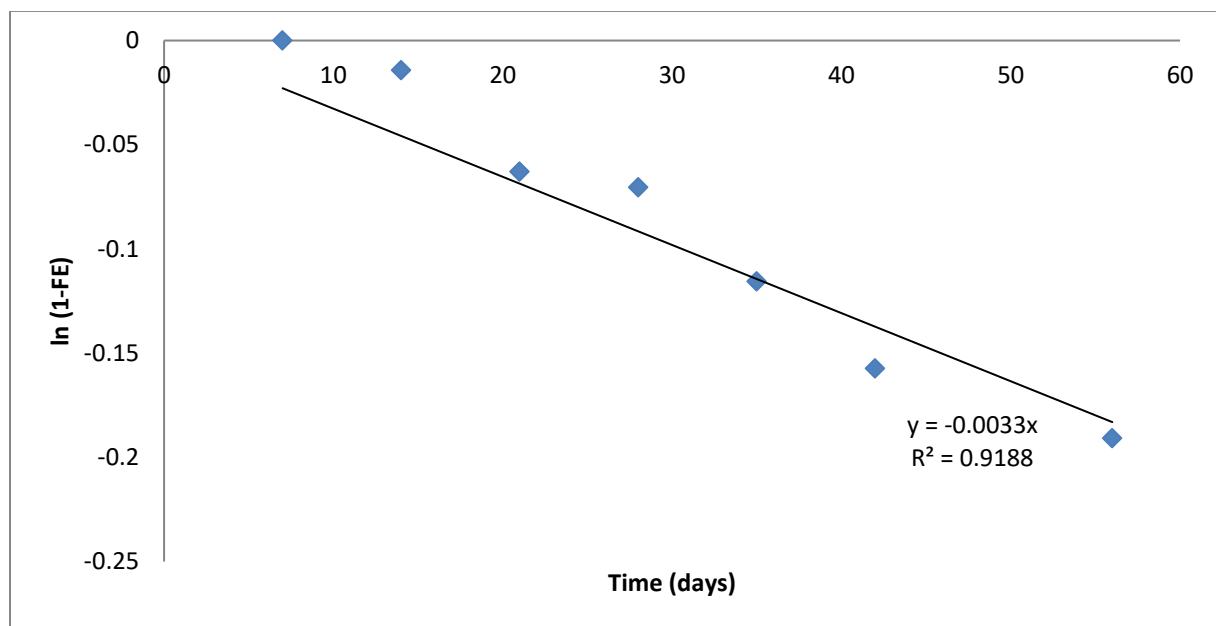


Fig 12: First Order Kinetic Pattern of TOC reduction for the Control Sample

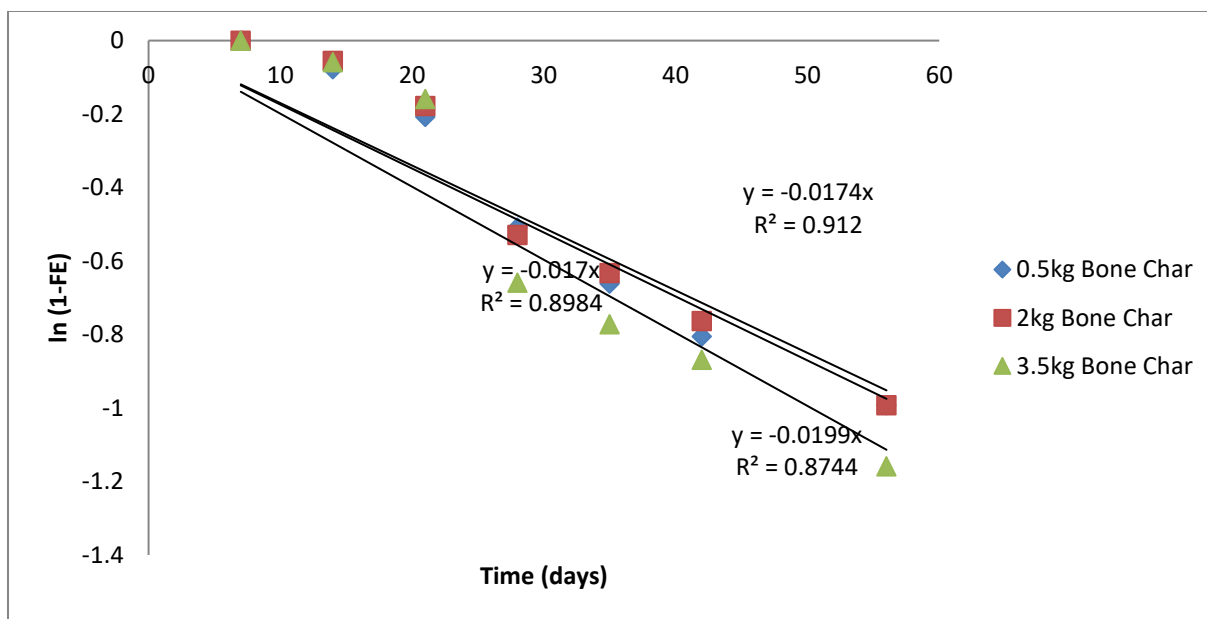


Fig 13: First Order Kinetic Pattern of TOC reduction for Bone Char Treated Soil Samples

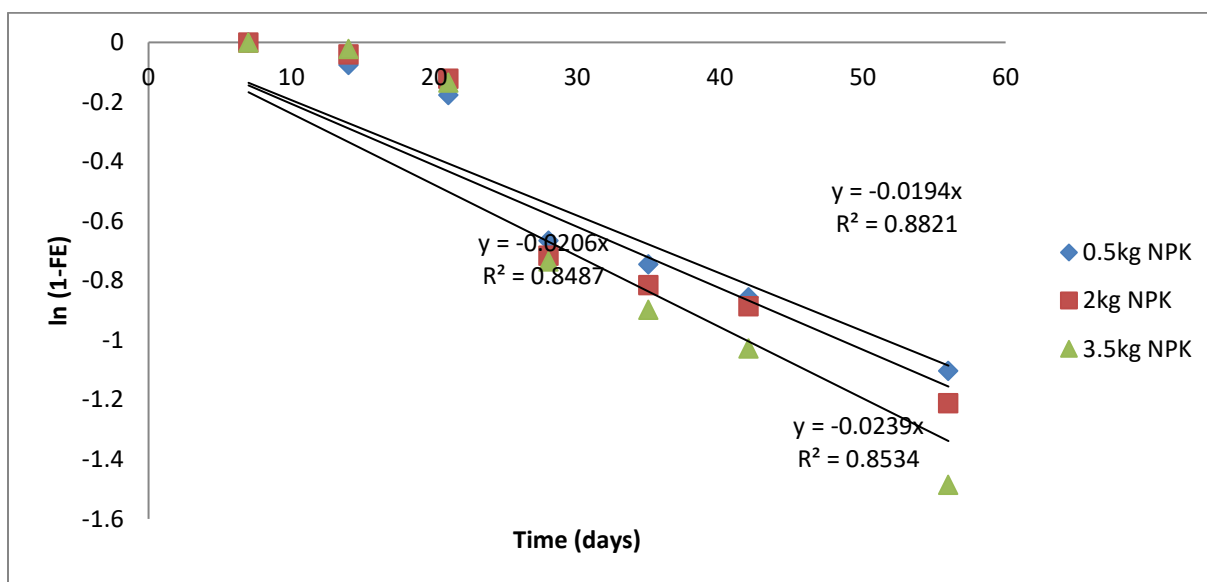


Fig 14: First Order Kinetic Pattern of TOC reduction for NPK Treated Soil Samples

375 Table5: Summary of the Kinetic Model Parameters for THC and TOC reduction

Treatment Condition	Model Parameters					
	THC			TOC		
	R ²	K (day ⁻¹)	Half life	R ²	K (day ⁻¹)	Half life
Control	0.959	0.002	346.65	0.918	0.003	231.1
0.5 Kg Bone Char	0.938	0.016	43.33125	0.912	0.017	40.78235
2.0Kg Bone Char	0.951	0.018	38.51667	0.898	0.017	40.78235
3.5Kg Bone Char	0.891	0.018	38.51667	0.874	0.019	36.48947
0.5Kg NPK	0.909	0.019	36.48947	0.882	0.019	36.48947
2.0Kg NPK	0.94	0.027	25.67778	0.848	0.02	34.665
3.5Kg NPK	0.882	0.03	23.11	0.853	0.023	30.14348

376

377 Positive correlations were observed for the degradation of THC and TOC in the soil with respect

378 to time. In both cases, 3.5kg NPK treatment had the highest degradation rate constant (0.03 and

379 0.023/day) and consequently half life of 23 and 30 days respectively. This is further proof of the

380 effectiveness of this pretreatment method in the bioremediation process. The high values of the

381 R² statistic across most of the treatment conditions indicate the sufficiency of the model in

382 predicting these processes. It also shows that the models adequately describe the variability of

383 the removal of the THC and TOC contaminants to the extent of their correlation. Physical

384 inspection of the site indicates tremendous effect of the remediation process on the contaminated

385 soil (Figure 15).

386 The general trend in the THC degradation profile was observed to follow similar trend reported

387 by Wami and Ogoni [40] Latinwo and Agarry [41] and Ofoegbu *et al.* [42]. The kinetics of the

remediation process also corroborated the physical evidence of the process (Figure 15). The kinetic studies of the first order constant obtained in this study, using NPK as biostimulant for the remediation of the soil, is well within the range of 0.0022 – 0.0335 day⁻¹ corroborates the range of 0.007 – 0.042 day⁻¹ and 0.003 - 0.024 day⁻¹ reported by Ofoegbu, *et al.* [42] and Latinwo and Agarry [41], respectively. Results from this work also show the significant effect and contribution of bone char as a bio-stimulant in the remediation of the hydrocarbon contaminated site. These effects were comparable to those of the NPK grade which indicate their suitability as a viable alternative. More so, the first order kinetics is comparable to that obtained for the NPK grade. Although the higher performance of the NPK grade fertilizer has been attributed to its Nitrogen and Phosphorous content, leaching of the soil nutrients and loss of soil texture are some of the side effects associated with its use and on the toxicity of aquatic life [43, 44]. In both cases, the bio-stimulation efficiency was above 70% for both THC and TOC in crude oil contaminated soil and the degradation was above 60% which is a strong indication of the effectiveness of the process. The R² value obtained from the kinetic models indicates the reliability of the models for future predictions and the attenuation of the samples. Based on the indices considered in this study, it can be deduced that enrichment of the bone char fertilizer can make it a credible alternative for NPK (inorganic fertilizer).



Delineated portion contaminated with crude oil

Crude oil contaminated soil with dead plants
(2weeks after contamination)

Remediation treatment with Bone Char/NPK

14 days after remediation treatment

30 days after remediation with growths

45 days after remediation

60 days after remediation

Figure 15: Pictorial views of experimental site at various stages of treatment

Conclusion

This work has successfully shown that the efficacy of the bone char (organic) and NPK (inorganic) fertilizers in the remediation of hydrocarbon contaminated soil. Vital information on the the kinetics of the process, degradation, and biostimulation efficiency have been provided. Although the inorganic fertilizer improved the contaminated soil, the organic fertilizer has been shown to provide a viable alternative. The amount of fertilizer had a direct effect on soil improvement and microbial growth. There treatment had a significant effects was significant effect on the contaminated soil which gave rise to an appreciable reduction of Total Hydrocarbon (THC) and Total Organic Carbon (TOC) contents. Enrichment of the organic fertilizer will boost its capacity for improving the recovery of oil contaminated soil of oil spilled sites across this region.

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437 **-Authors Contributions:** OOO: Data curation, Bench work and draft manuscript preparation,
438 data analysis and modelling; IJO: conceptualization, supervision and bench work

439 **-Competing Interests:** Authors confirm that there was no conflict of interest

440 **-Availability of data and materials:** All data have been provided

441

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Figures

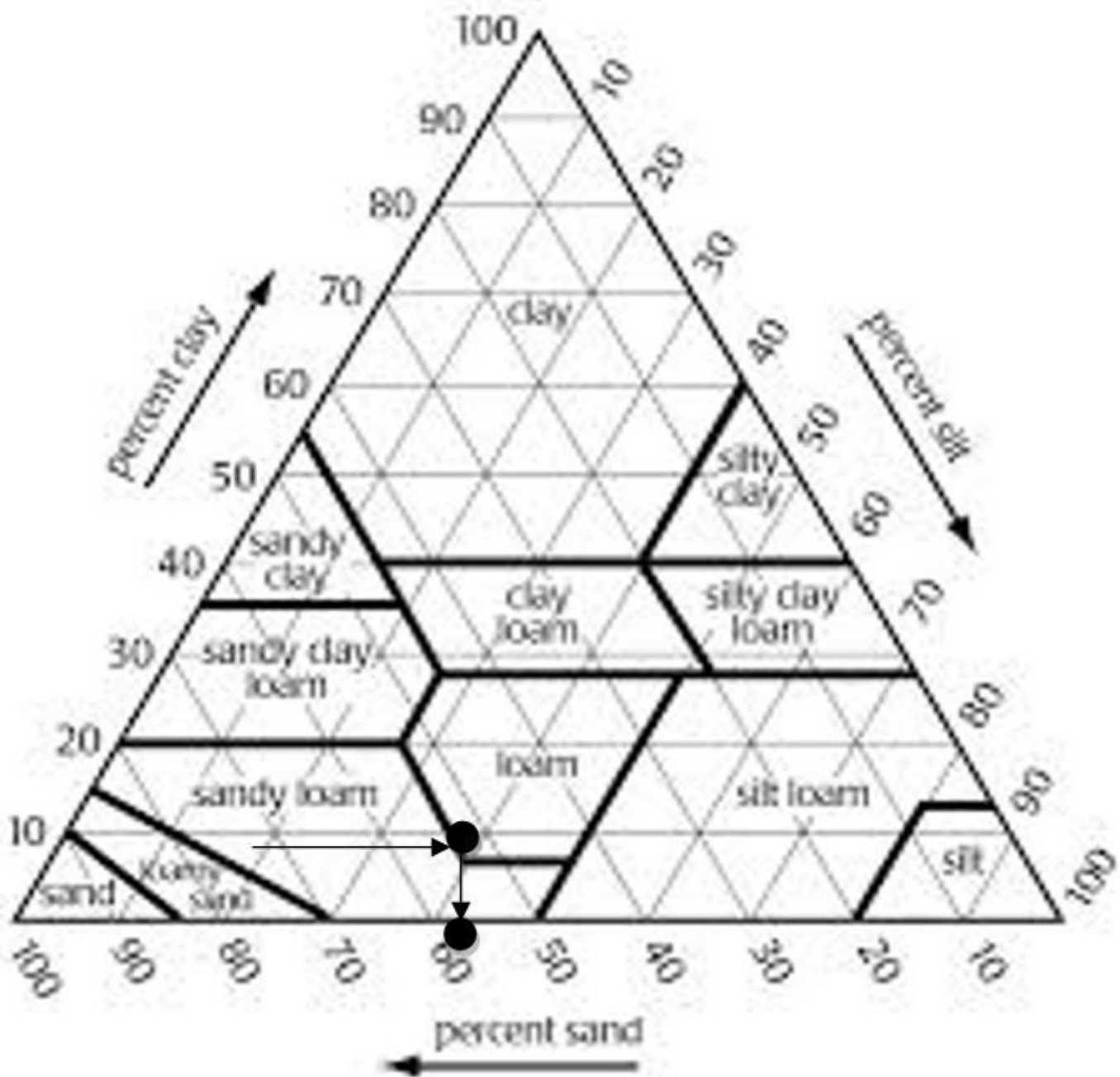


Figure 1

Soil Texture Triangle

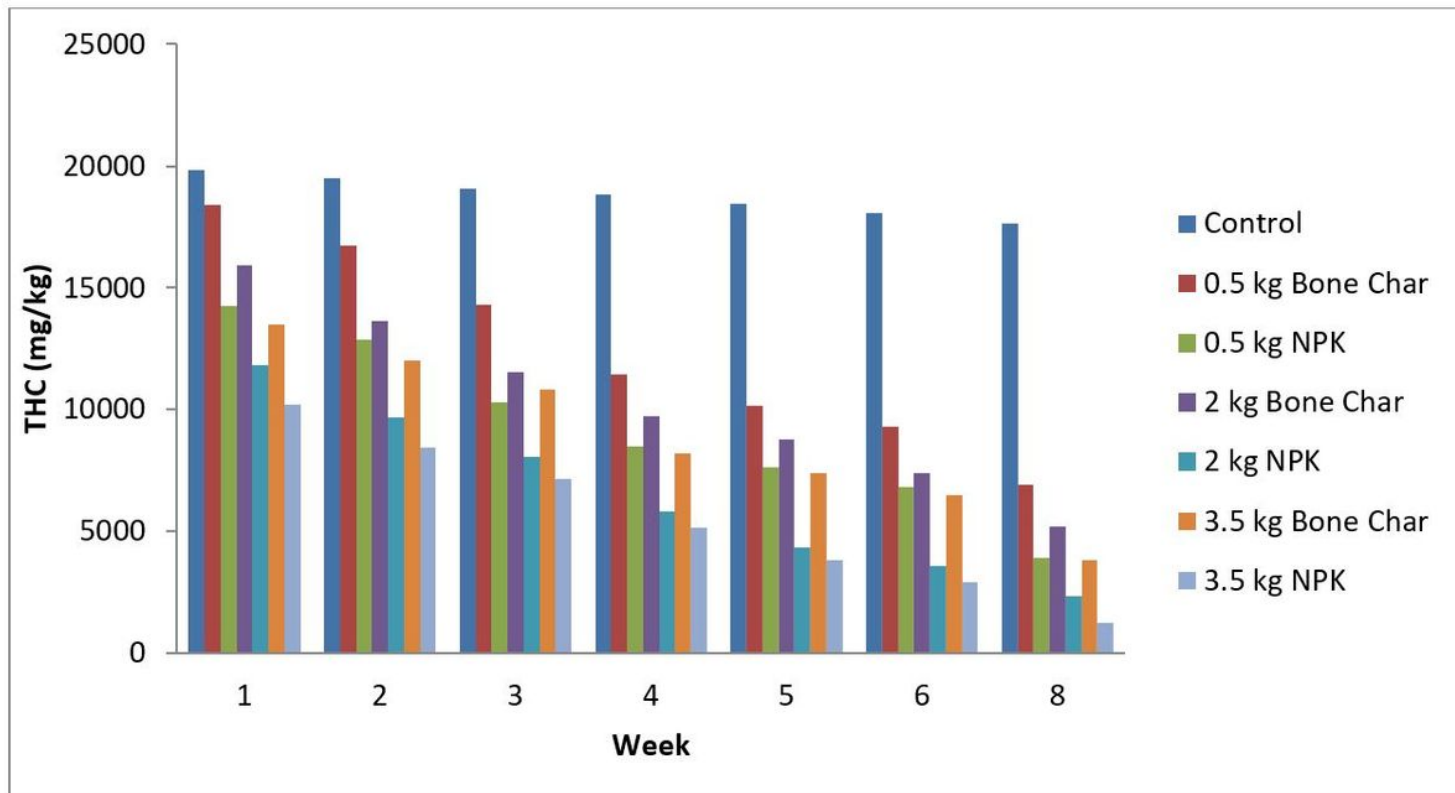


Figure 2

Bioremediation treatment on Total Hydrocarbon Content

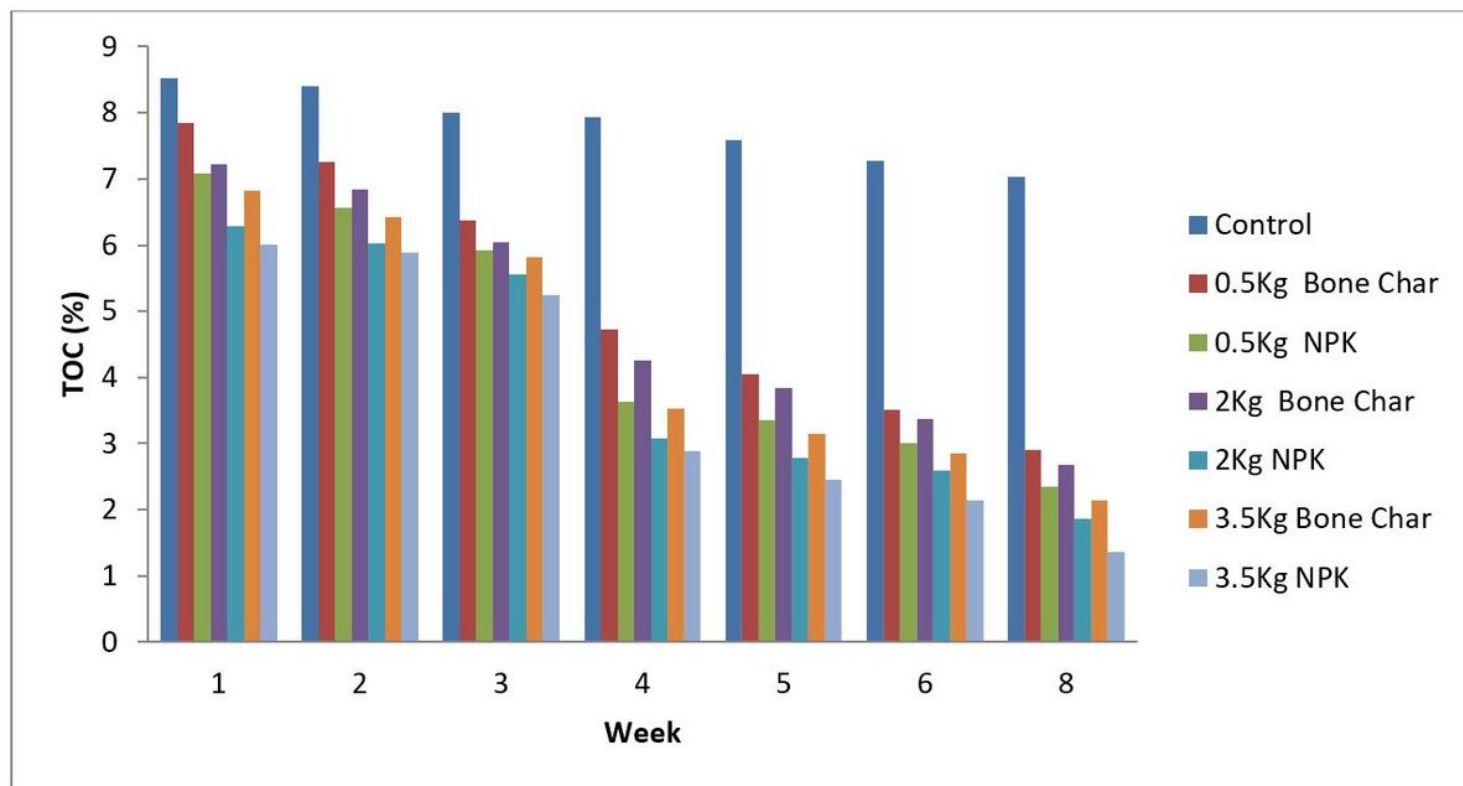


Figure 3

Effect of bioremediation treatment on Total Organic Carbon

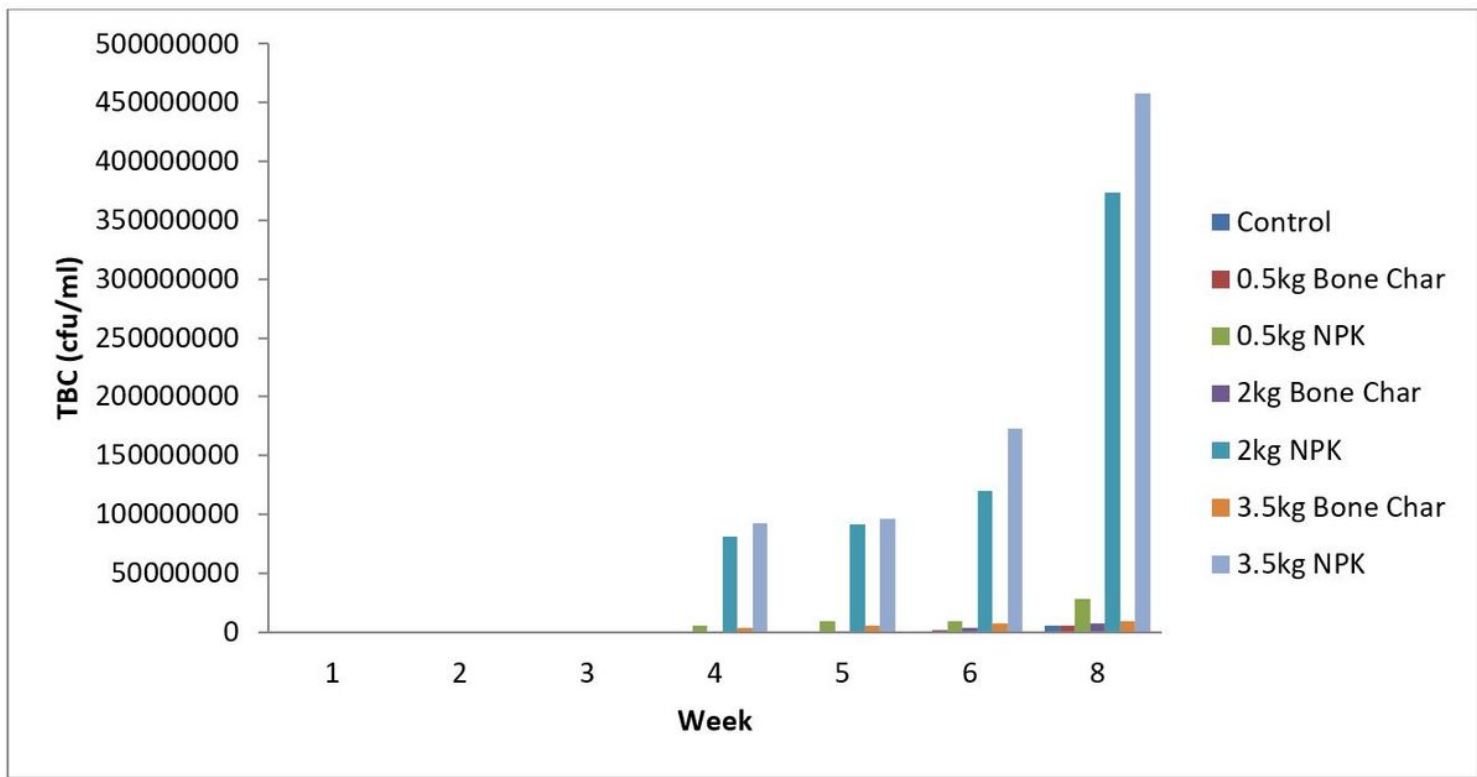


Figure 4

Effect of bioremediation treatment on Total Bacteria Count

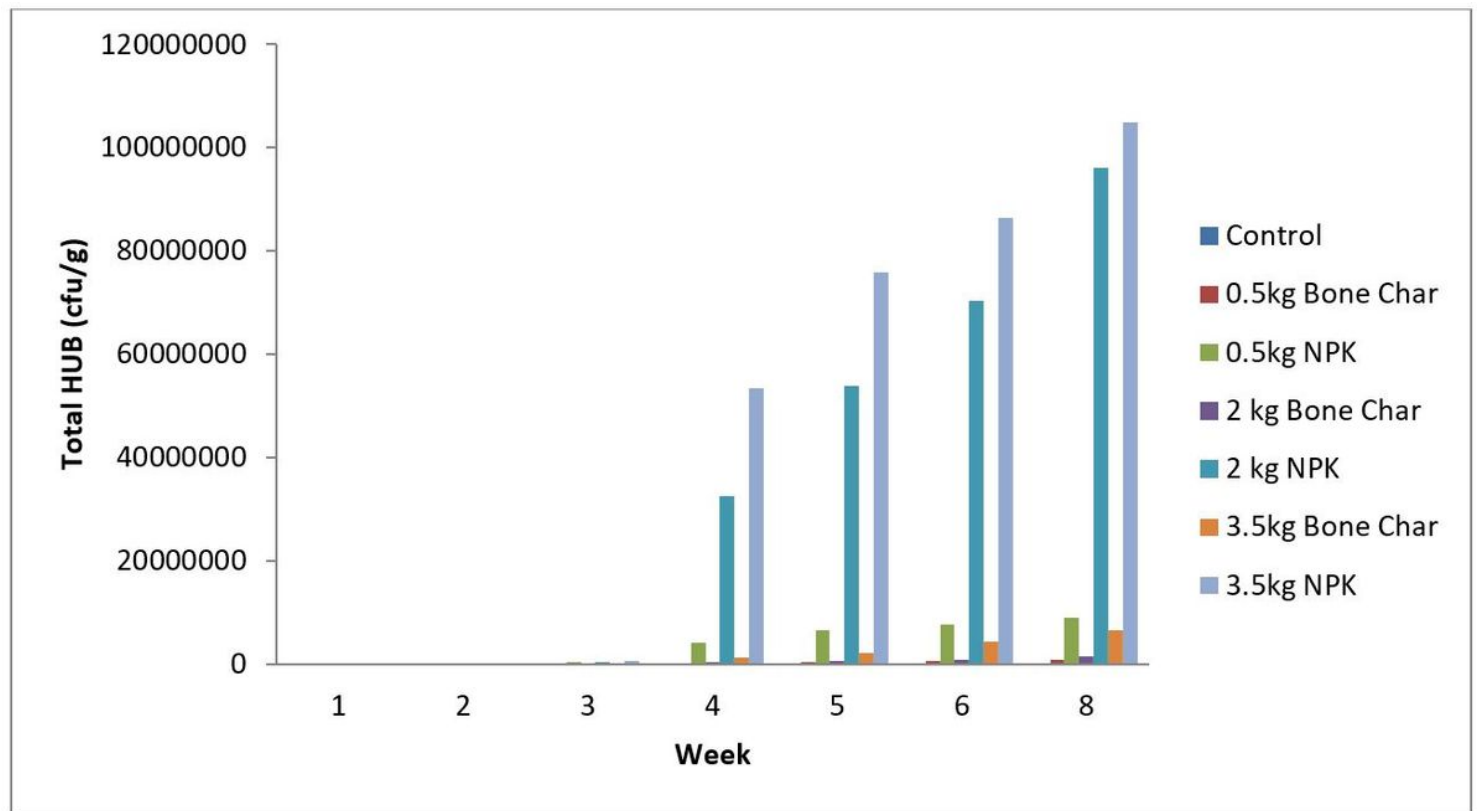


Figure 5

Heterotrophic Utilizing Bacteria

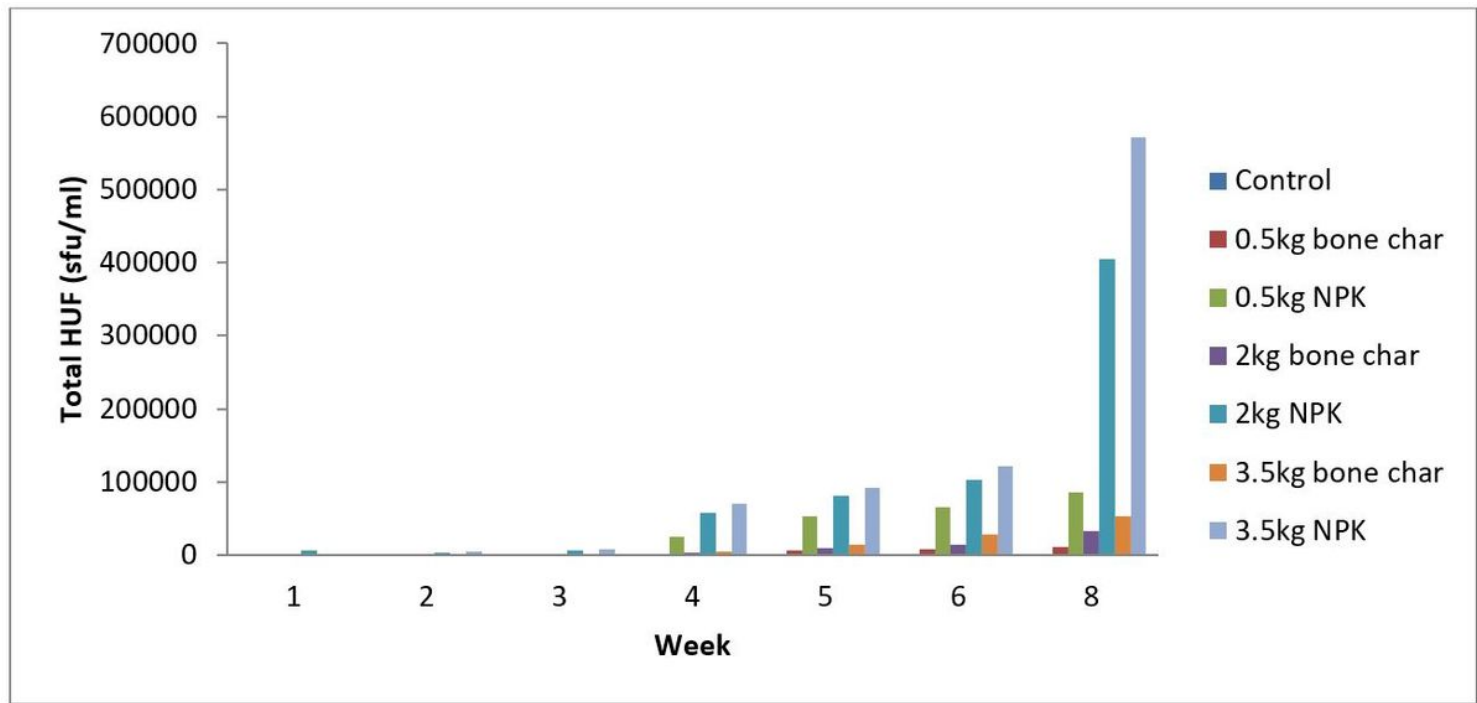


Figure 6

Heterotrophic Utilizing Fungi

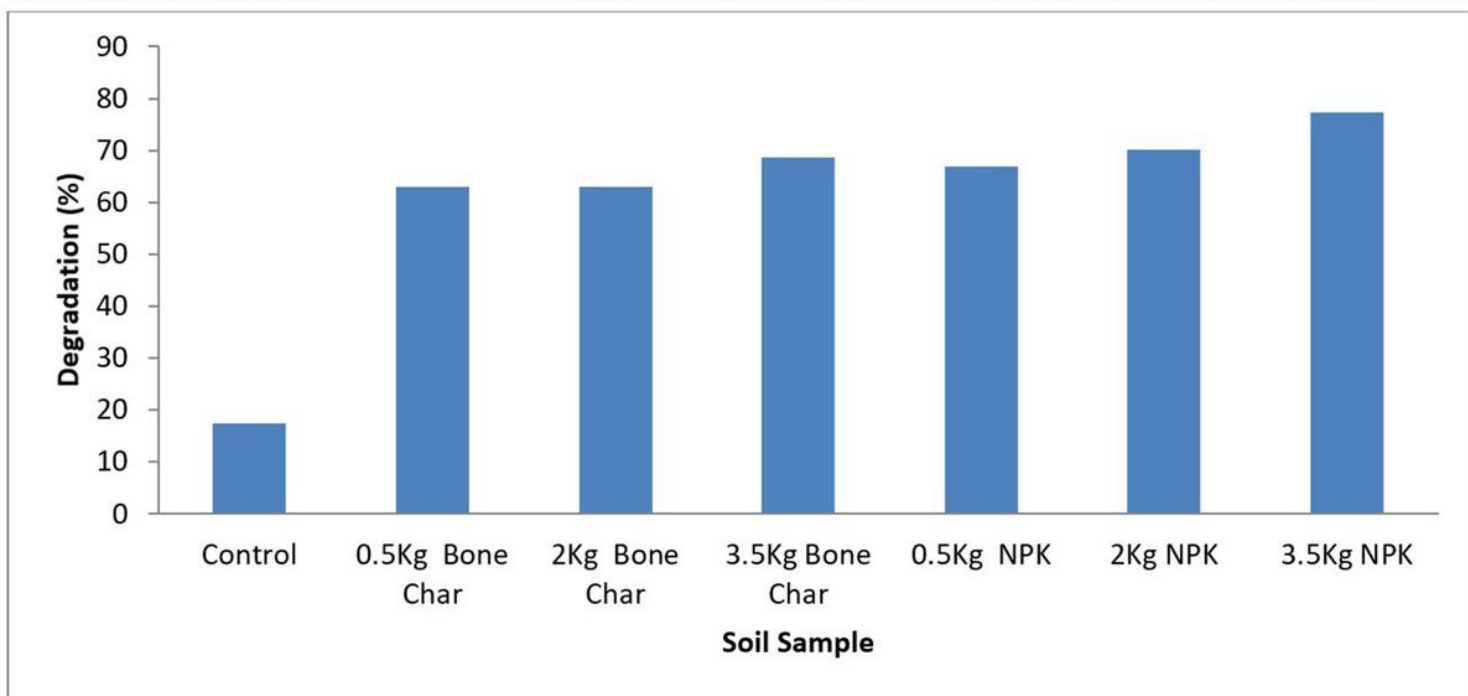
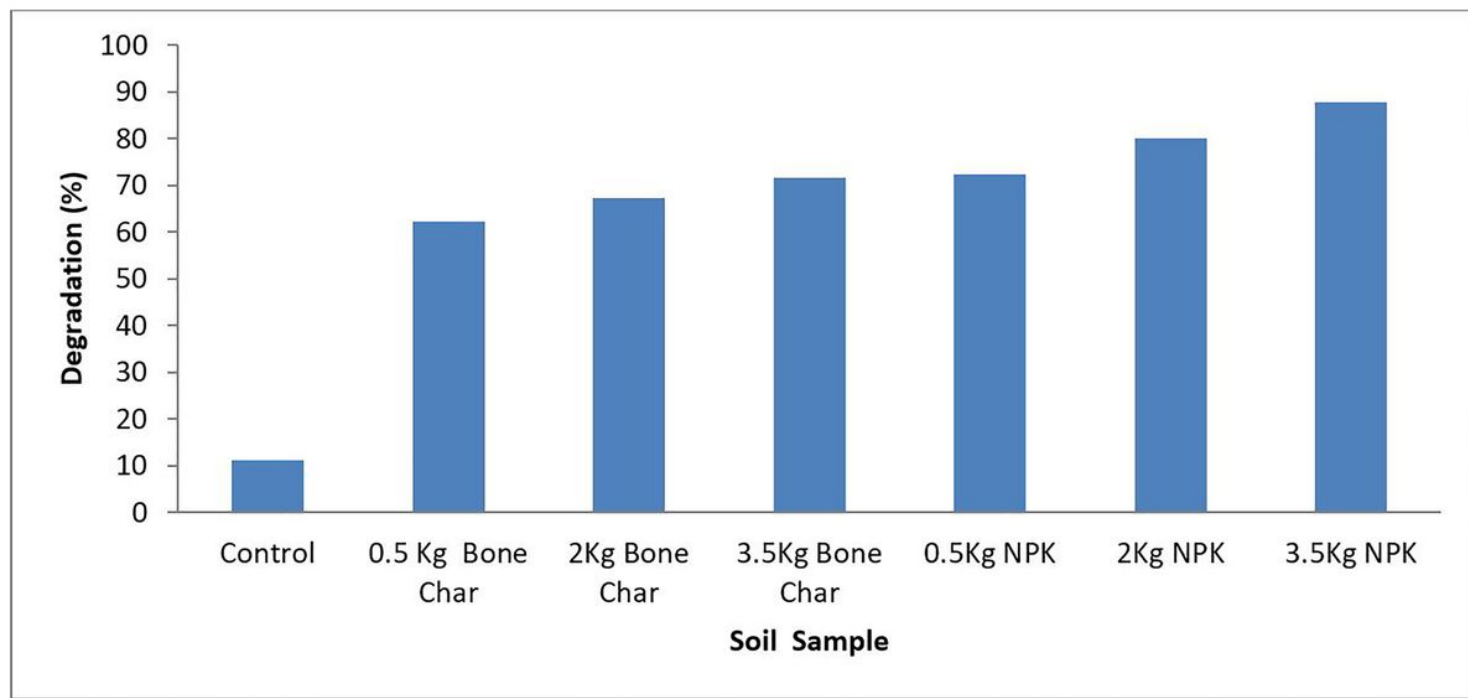


Figure 7

a: Degradation of THC. b: Degradation of TOC.

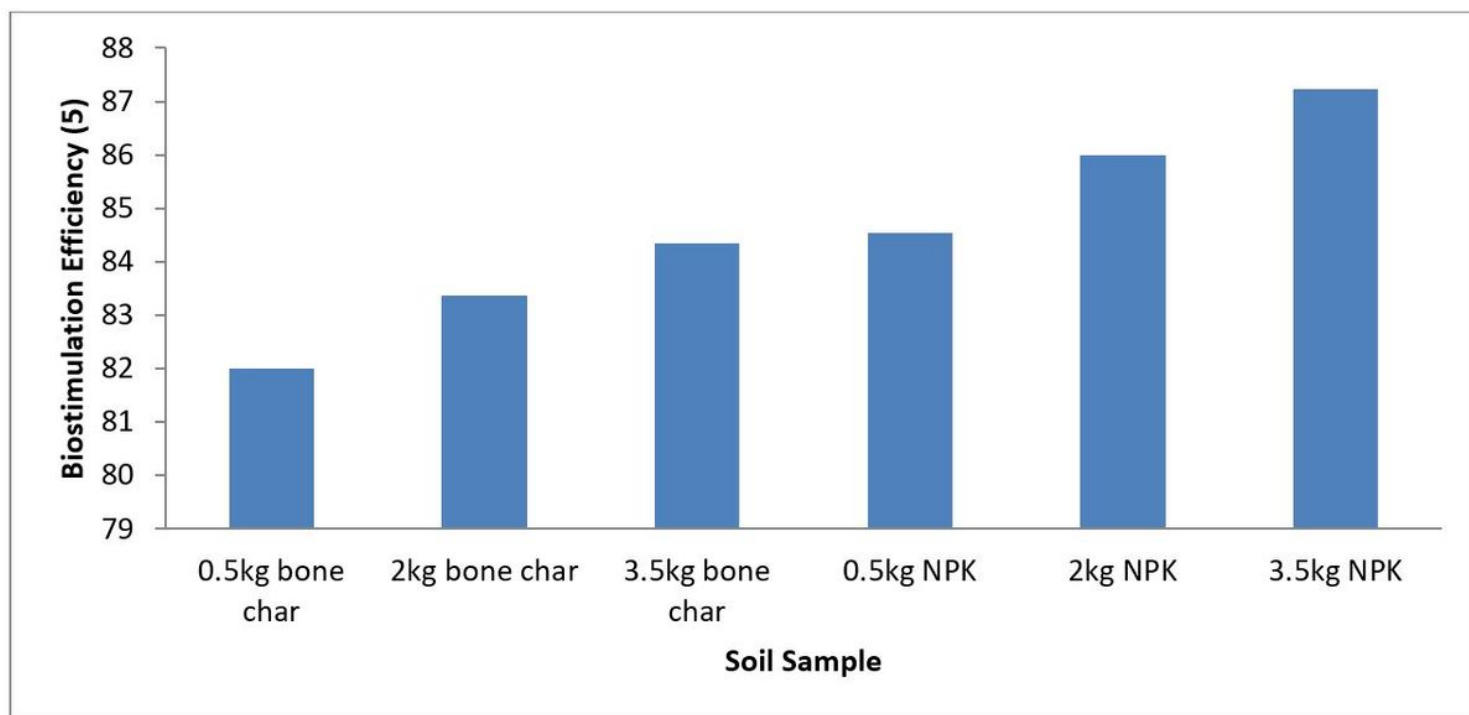


Figure 8

Biostimulation Efficiency

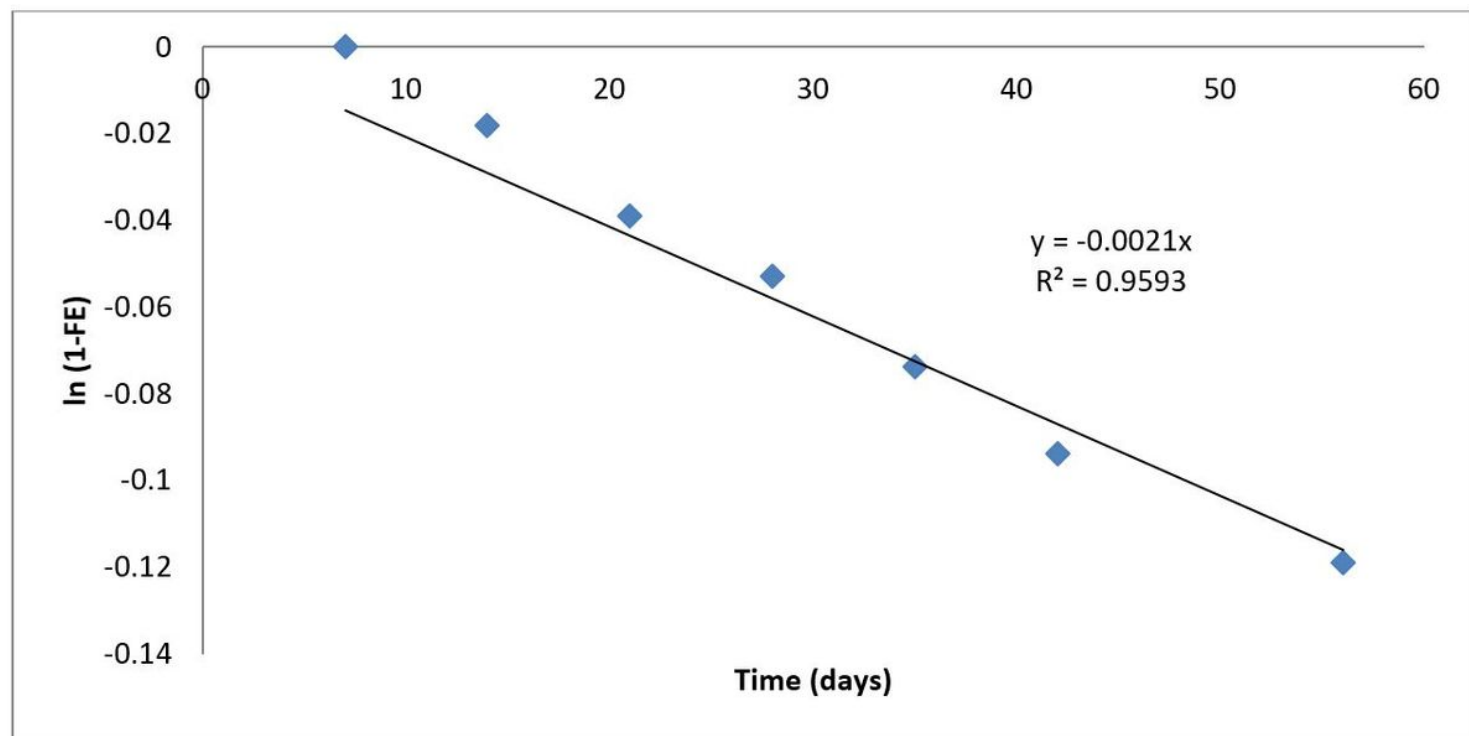


Figure 9

First Order Kinetic Pattern of THC reduction for the Control Sample

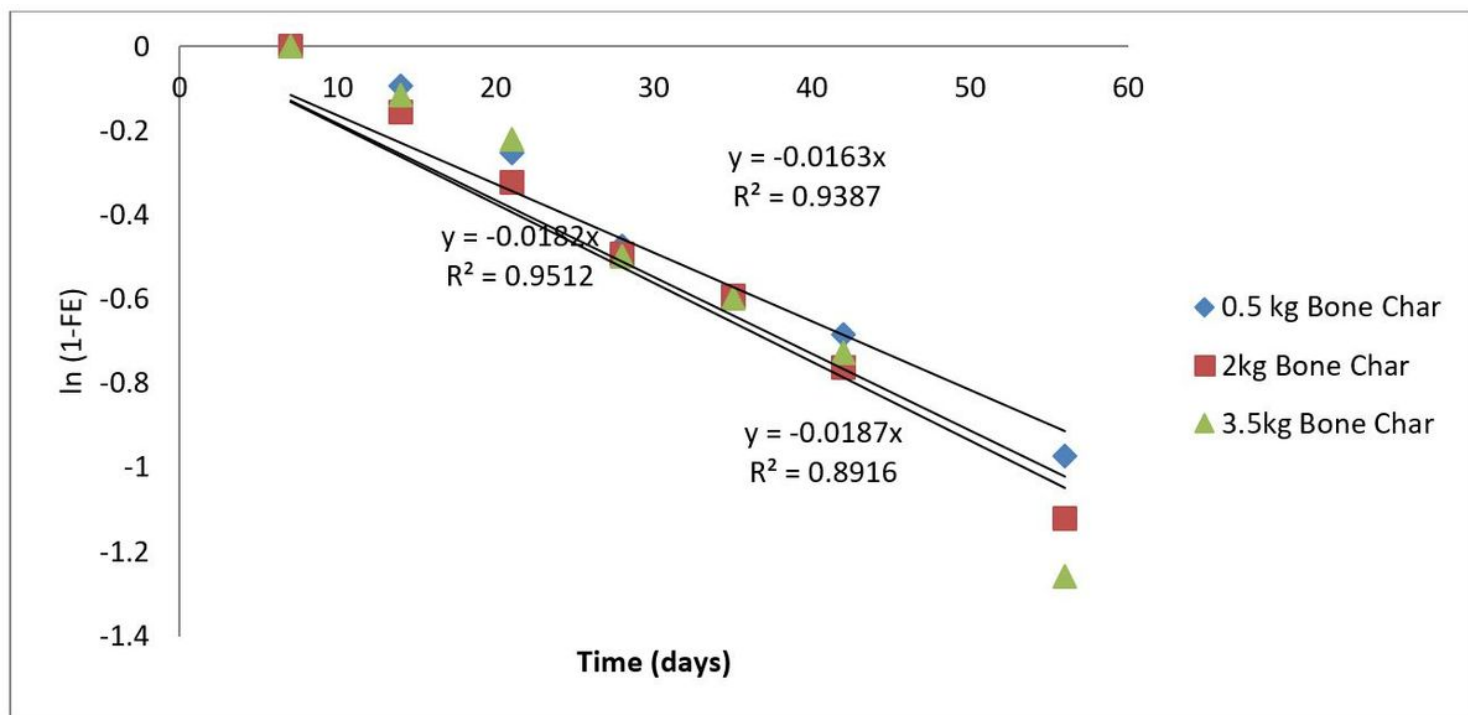


Figure 10

First Order Kinetic Pattern of THC reduction for Bone Char Treated Soil Samples

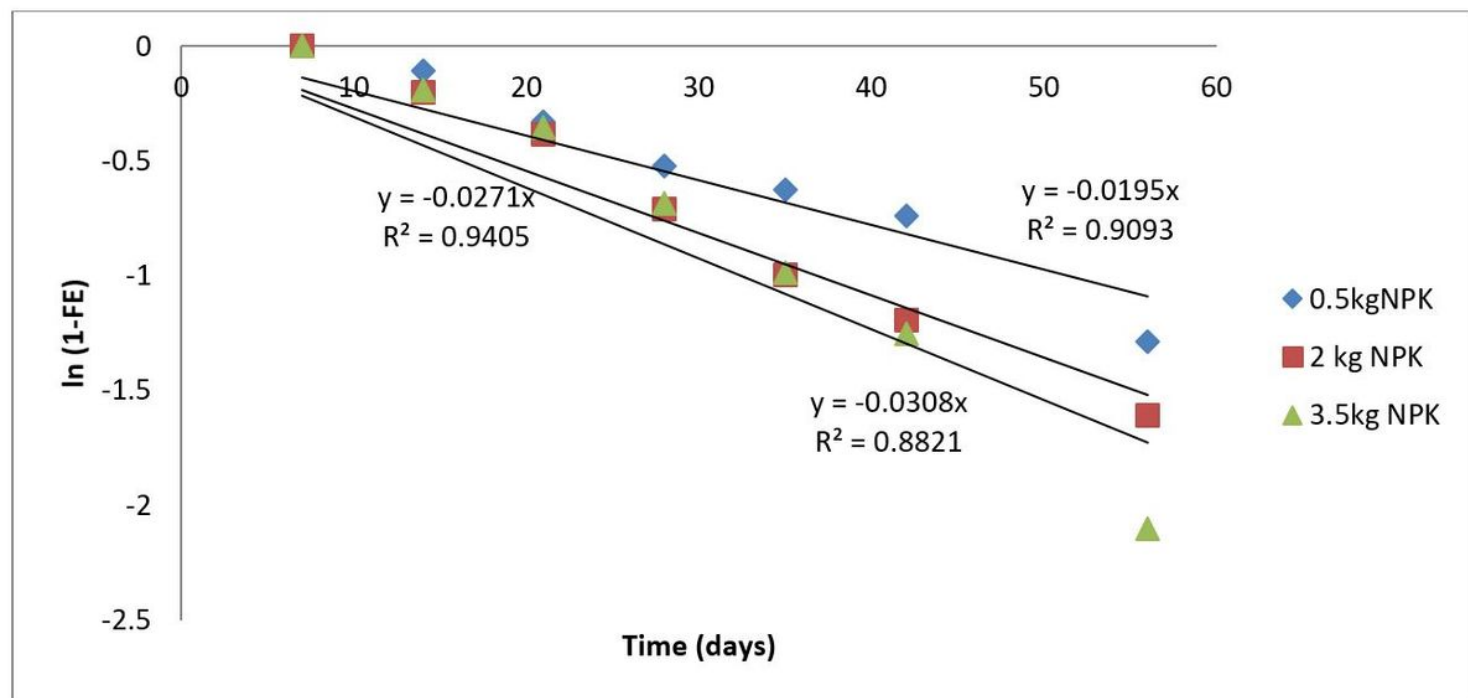


Figure 11

First Order Kinetic Pattern of THC reduction for NPK Treated Soil Samples

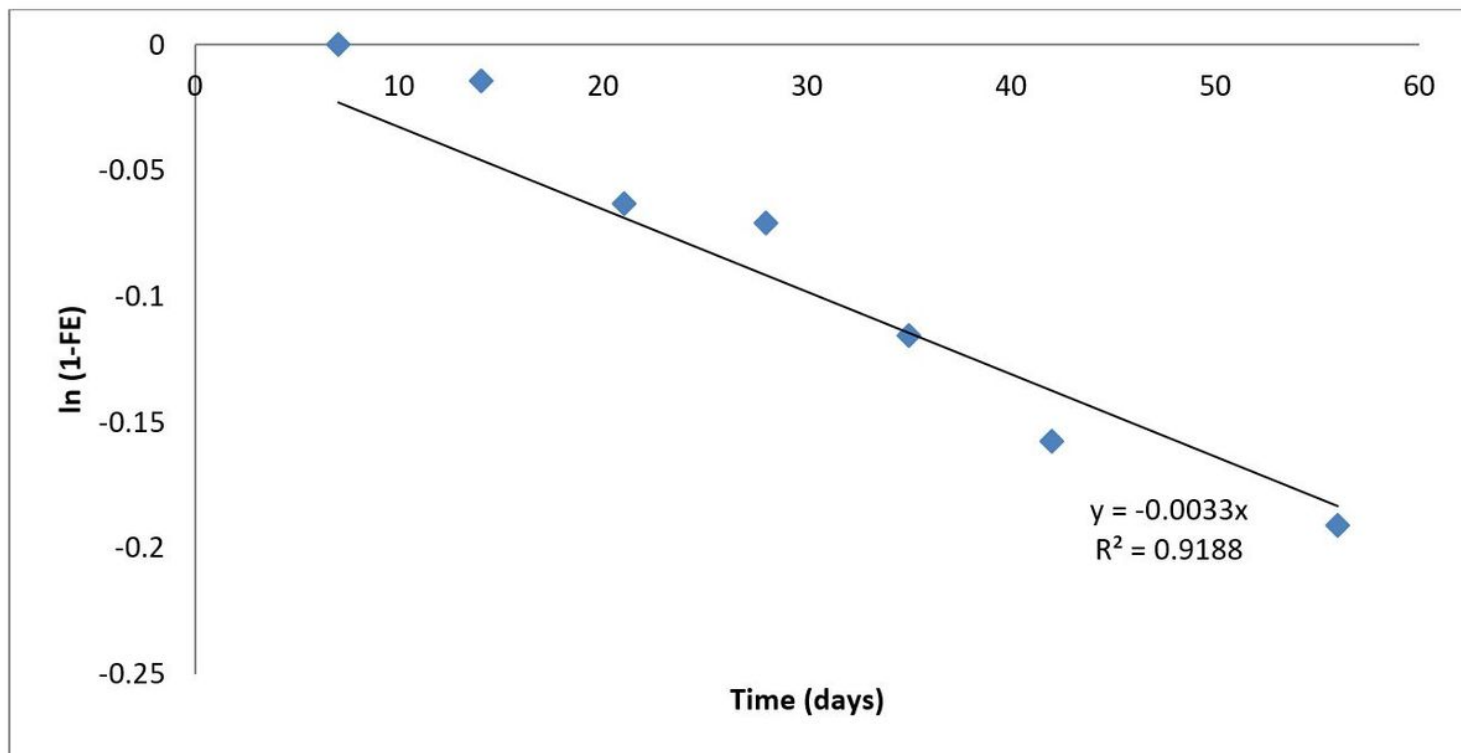


Figure 12

First Order Kinetic Pattern of TOC reduction for the Control Sample

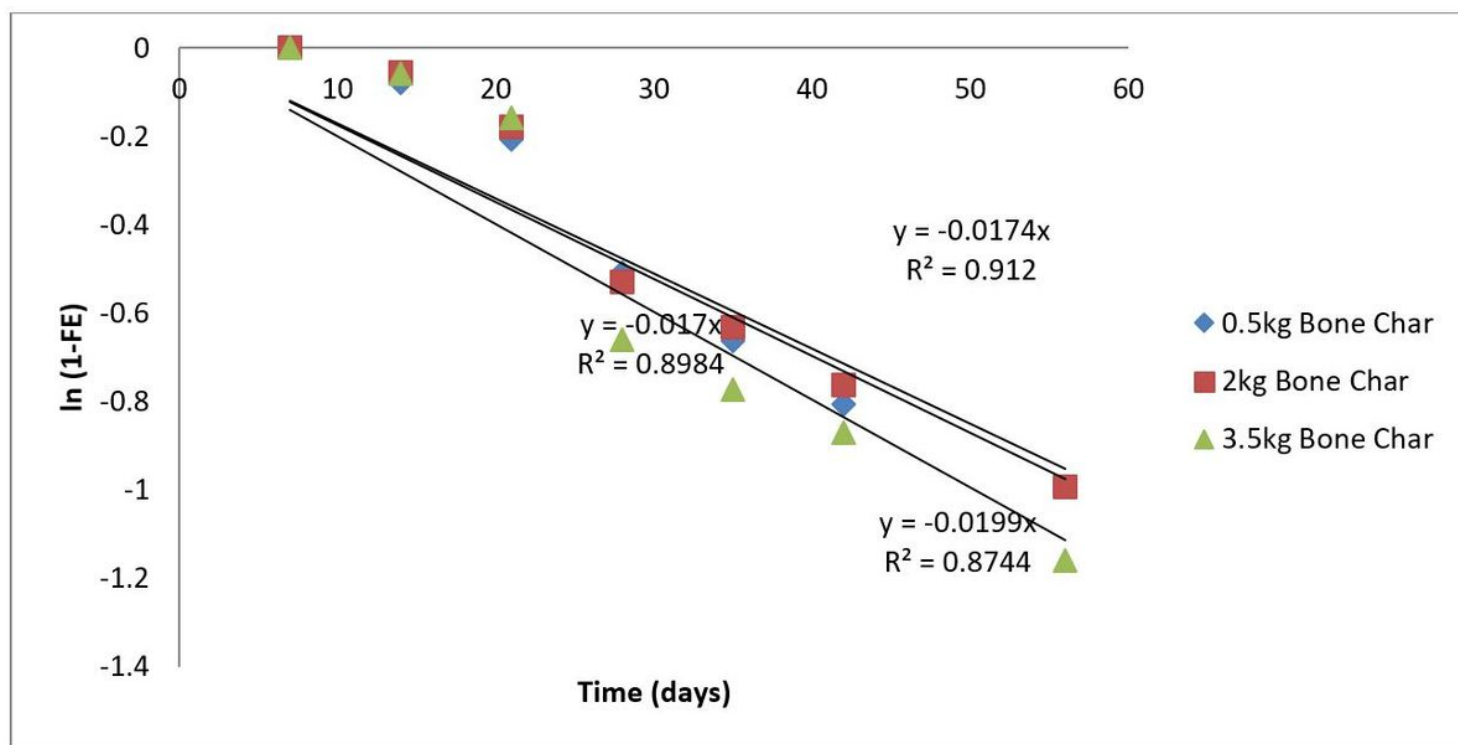


Figure 13

First Order Kinetic Pattern of TOC reduction for Bone Char Treated Soil Samples

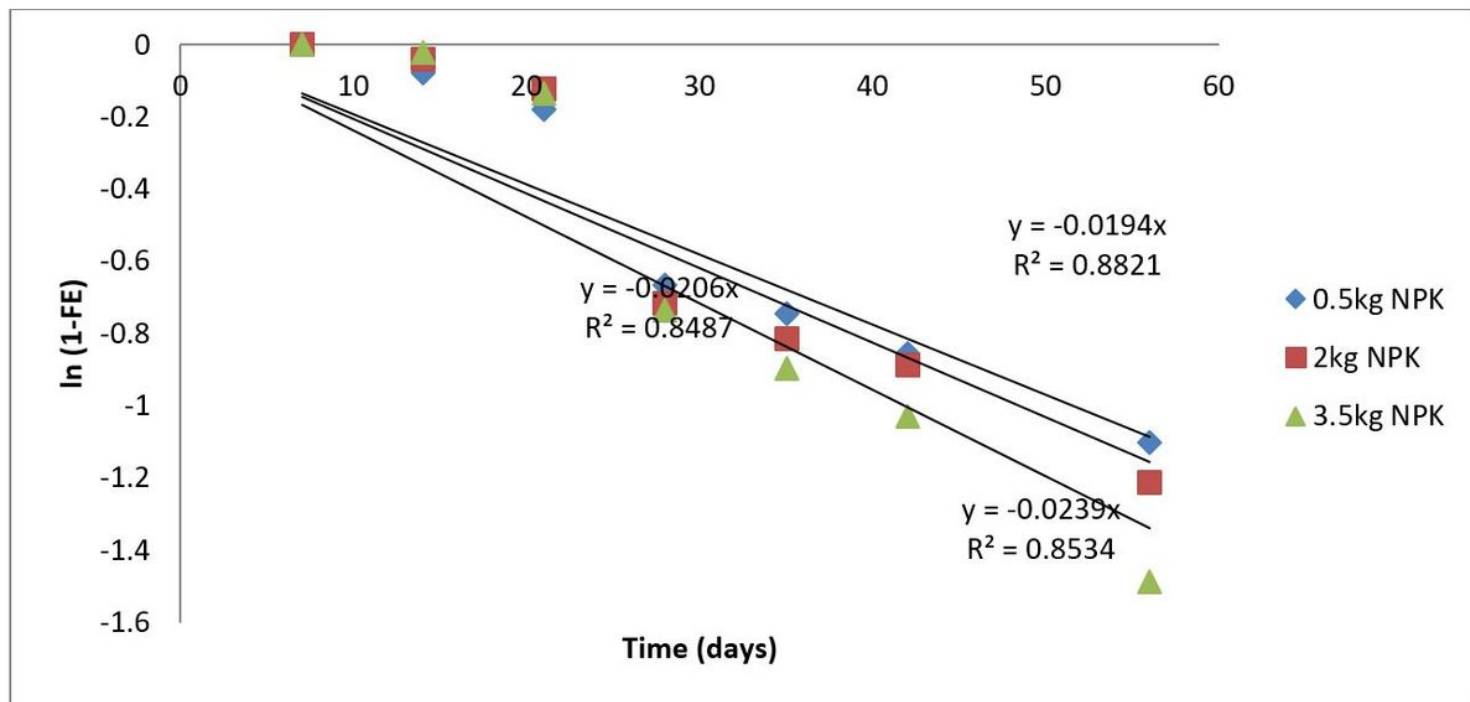


Figure 14

First Order Kinetic Pattern of TOC reduction for NPK Treated Soil Samples



A Delineated portion contaminated with crude oil



B Crude oil contaminated soil with dead plants (2weeks after contamination)



C Remediation treatment with Bone Char/NPK



D 14 days after remediation treatment



E 30 days after remediation with growths



F 45 days after remediation



G 60 days after remediation

Figure 15

Pictorial views of experimental site at various stages of treatment