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Biostimulation of Motor Engine Oil Polluted Soils in Benin City, Nigeria Using Organic and Inorganic Amendments

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Abstract

Waste Engine Oil (WEO) commonly called used automobile oils contain complexes of polycyclic aromatic hydrocarbon (PAHs) that are deleterious to human health. This study was carried out to determine the biostimulation potential of organic and inorganic amendments in the degradation of WEO polluted soils. WEO polluted soils were randomly collected from four LGA's in Benin City (Oredo, Uhunwonde, Egor and Ovia North East LGA's). The physicochemical and microbiological analysis followed standard chemical and microbiological methods respectively. Microcosm of 1.5 kg motor mechanic workshop soils (MMWS) polluted with WEO was divided into five groups per treatment and placed in a plastic container with a volume of 500 ml. After two days, 10% of all amendments used (cow dung, sludge, NPK and sawdust compost) was individually introduced into each WEO polluted soil and thoroughly mixed. The moisture content of samples were adjusted to 60% water holding capacity. Soil without amendment served as the control. The soils in the plastic containers were tilled twice a week for aeration and 60% water holding capacity was maintained throughout the period of experiment. Samples were collected once in two weeks from 0 – 14 weeks for total petroleum hydrocarbon (TPH) analysis. The result of the physicochemical parameters were not significant at $p<0.05$. The physicochemical parameters of the amendments revealed high phosphorus (mg/kg) for CD (572.5 mg/kg) and SLG (409.2 mg/kg). The total heterotrophic bacterial counts (THBC), total hydrocarbon utilizing bacterial counts before 14 weeks' treatment of soil samples ($THUBC^{bt}$) and total hydrocarbon utilizing bacterial counts after 14 weeks' treatment of soil samples ($THUBC^{at}$) revealed high significance ($p<0.05$) for $THUBC^{at}$ while THBC and $THUBC^{bt}$ values were not significant for Uhunwode LGA and Ovia N/E LGA MMWS. The percentage biostimulation potential of SDC and NPK was <30 % from week 2 to 14 whereas >30 % biostimulation potential was observed for soils amended with SLG. The coefficient of determination (R^2) for soils amended with CD and SDC were > 0.95(95 %) while soil amended with NPK and SLG were < 0.95 (95 %). The half-life ($t_{1/2}$ in weeks) of WEO removal from the soil revaealed that CD and SLG required between 9-17 weeks, while SDC and NPK required > 30 weeks for complete degradation.The performance of the organic and inorganic amendments followed this order, CD > SLG > SDC > NPK.

1. Introduction

Waste Engine Oil (WEO) commonly called used automobile oils contain complexes of poly-aromatic hydrocarbon (PAHs) such as benzo(k)fluoranthrene, benzo(a)pyrene, benzo(b)fluoranthrene, crysene, benzo(a)anthracene, pyrene, fluoranthrene, anthracene, phenathrene, florene, acenaphthalene and acenephthene. These PAHs are deleterious to plants, animals and humans, contributing significantly to the pollution of soils in specific locations within Benin metropolis. Waste oil can cause chronic/subacute toxicological effect, thereby promoting reduction in growth rate and reproduction causing alteration in population dynamics and natural communities in ecosystems [1]. Several efforts have been made by developed countries to recycle WEO from automobiles [2], nonetheless the indiscriminate disposal of WEO remain a major problem in the developing countries of the world where there are no government policies on WEO disposal [3] .

Successes recorded in biostimulation of hydrocarbon polluted soils depends solely on nutrient availability to the degrading microbial consortia. Lack of nutrient, nutrient exhaustion and limited HC degrading population remains a challenge to biostimulation approach. Indigenous soil microflora's can degrade organic contaminants effectively with higher tolerance to toxicity posed by the pollutant unlike exogenous microorganisms introduced through the bioaugumentation system [4, 5]. Organic amendments (cow dung and sawdust compost) are rich in essential mineral nutrients such as phosphorus, organic carbon and nitrogen required by indigenous bacteria to completely mineralized WEO. The addition of nitrogen and phosphorus-based amendments has been showed to facilitate biodegradation of petroleum in soil [6, 7]. The inorganic amendment (inorganic fertilizers and industrial effluent sludge) also contributes to nutrient balance in hydrocarbon degrading microcosm. The inorganic nutrient can cause a balance in C: N: P ratio, resulting to effective biostimulation of microflora's present in the degrading soil [8, 9].

This study was carried out to determine the rate of biostimulation of WEO polluted soil in four LGA's in Benin City, Nigeria, using organic (cow dung and sawdust compost) and inorganic (inorganic fertilizers and industrial effluent sludge) nutrient-based amendments.

2. Methodology

2.1 Study Area

The study was carried out in Benin City, South-South Nigeria. Motor engine oil polluted soils are scattered all over Benin metropolis because of sitting motor service stations within the City area. The Global Position System (GPS) coordinates of sampled area were Oredo position A: N6^0 19' 50.57'', E5^0 44' 42.78", B: N6^0 19' 49.45'', E5^0 44' 41.61"; Uhunwode position A: N6^0 19' 50.57'', E5^0 44' 42.78", B: N6^0 19' 49.93'', E5^{0}44' 42.1'', Egor position A: N6^0 22' 40.62", E5^0 35' 30.94", B: N6^0 22' 45.60", E5^0 35' 31.95"; Ovia N/E (Oluku) position A: N6^0 26' 0", E5^0 35' 55", B: N6^0 27' 44", E5^0 36' 0".

2.2 Sample collection

Soil samples were aseptically collected from these areas using soil auger and transported to the laboratory for further analysis.

2.3 Determination of Physicochemical Properties of Soils

Physicochemical parameters such as pH, electrical conductivity, moisture content, total organic carbon, total nitrogen, available phosphorus content, percentage nitrate, exchangeable cations

(K_2 and Ca), total hydrocarbon content (THC) and water holding capacity of soil were determined following the Association of Official Analytical Chemist methods [10].

2.4 Enumeration of Bacterial Isolates

The method described by Cheesbrough [11] was used for estimating total heterotrophic bacteria counts and total hydrocarbon utilizing bacterial counts. The discrete colonies on the nutrient agar and Bushnell Haas agar were selected and counted. The mean colony counts on the nutrient agar and Bushnell Haas plates of each given dilution was used to estimate the total viable count of samples in colony forming units per gram (cfu/g). The total hydrocarbon utilizing bacterial counts were carried out using the modified vapour-face transfer method [2].

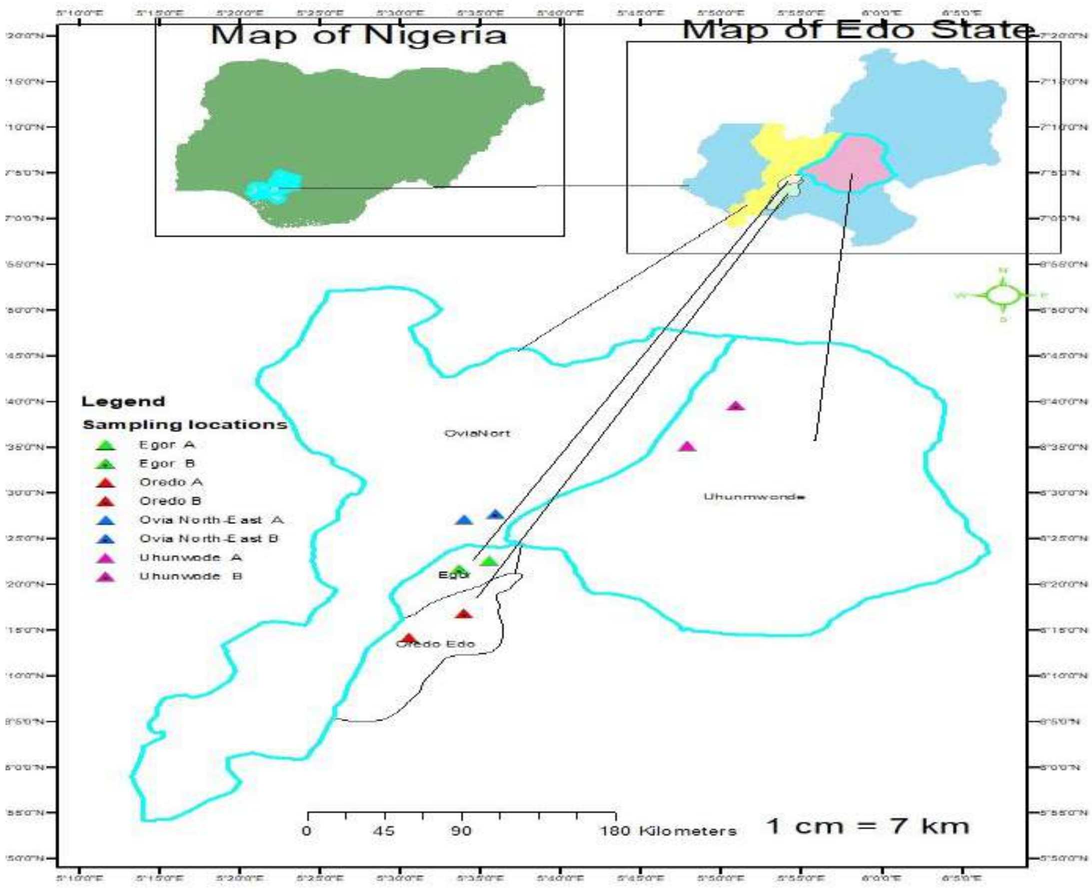


Figure 1: Map of Edo State showing the study area

2.5 Bioremediation set-up of Soil Microcosm

Soil from automobile mechanic workshop (1.5kg) was shared into five groups per treatment and placed in a plastic container with a volume of 500 ml. After two days, 10% of all amendments used (cow dung, sludge, NPK and sawdust compost) was individually introduced into each oil polluted soil and thoroughly mixed. The moisture content of samples was adjusted to 60% water holding capacity. Soil without amendment served as the control. The soils in the plastic containers were tilled twice a week for aeration and 60% water holding capacity was maintained throughout the period of experiment. Samples were collected once in two weeks from 0 – 14 weeks for analysis.

Table 1: Experimental set-up for treatment of soil polluted with WEO Organic amendment: cow dung and, inorganic amendment: NPK and Effluent treatment sludge

2.6 Determination of Biostimulation Potential

Groups	Set-up	Remarks
A	1.5kg soil from automobile workshop acting as control	Not Amended
B	1.5kg Soil + 10% cow dung	Amended
C	1.5kg Soil + 10% sawdust compost	Amended
D	1.5kg Soil + 10% NPK 15:15:15 fertilizer	Amended
E	1.5kg Soil + 10% Effluent treatment sludge	Amended

The Biostimulation Potential (BP) in percentage was calculated using the formula below. The rate of reduction in THC was assayed using the spectrophotometric methods (Spectronic Camspec M501). The decrease in concentration of THC in mg/kg was monitored for 14 weeks' treatment period.

$$\% \text{ Biostimulation Potential (BP)} = \frac{\text{THC}_{\text{ctrl}} - \text{THC}_{\text{amended}} \times 100}{\text{THC}_{\text{ctrl}}} \quad - \quad - \quad (1)$$

THC_{ctrl} : total hydrocarbon content of control groups
$THC_{amended}$: total hydrocarbon content of soils amended with organic and inorganic nutrients

2.7 Kinetics and half-life of WEO removal

The rate constant (R^2) was calculated from the scattered plot of concentration (TPH^{-1} or Ln TPH) of WEO after degradation study against time (weeks). The linear plot was used to determine if the reaction is first order (Ln TPH) or second order (TPH^{-1}) as showed in Fig. 5 to 8. The rate constant of soil from all sample collected was plotted to obatin the line of best fit in order to determine R^2 for all amendments (CD, SDC, NPK and SLG) used.

First order [13, 14] kinetics model and second order [15] kinetics model was used for this study depending on the line of best fit of scatagram plot of concentration of THC. The half-life [16] for first and second order kinetics were derived from the initial kinetic equations.

$$C_a = C_0^{(-kt)} \quad \text{(first order)} \quad - \quad - \quad - \quad - \quad - \quad - \quad (2)$$

C_a: concentration of WEO (mg/kg)
C_o: initial contration of the soil (mg/kg)
k: the rate constant for first order kinetic model
t: time of the raction

$$\frac{1}{[A_t]} - \frac{1}{[A_0]} = kt \quad \text{(second order)} \quad - \quad - \quad - \quad - \quad - \quad - \quad (3)$$

$[A_t]$: concentration of A at time t
$[A_o]$: concentration of A at time 0
k: rate constant for second order kinetic model
t: time of the reaction
half-life ($t_{1/2}$):

$t_{1/2} = \frac{Ln2}{k}$ 1st order - - - - - - - (4)

$t_{1/2} = \frac{1}{k[A_0]}$ 2nd order - - - - - - - (5)

2.8 Gas Chromatography Operating Procedure.

The Gas chromatography with Flame Ion Ionization detector (GC-FID) model 6890 series and 6890 plus was used to elucidate the aliphatic and aromatic profile of WEO according to the method of [17] . Sample was analyzed at a temperature of 250^0C, detector temperature 300^0C, equilibration time 0.1 minutes, oven temperature 60^0C, Initial time of 2 minutes, rate 1: 10^0C per min., intermediate value of 300^0C at 10mins, rate 2: 25^0C /min with final value 310^0C, final time of 3 minutes and purge /Valve On 1 minute.

3. Results and Discussion

Table 2**:** Physicochemical properties of soil from motor mechanic workshops soil in Benin City

Locations	**Moisture %**	**pH**	**EC (µS/cm)**	**Org. C (%)**	**N (%)**	**P (mg/kg)**	**K (mg/kg)**
Oredo^A	23.54±0.79	5.57	87.0±10.2	7.43±0.04	0.82±0.08	3.48±0.60	7.57±0.51
Oredo^B	18.77±1.20	5.21	57.00±6.7	4.87±0.07	0.54±0.04	2.28±012	4.96±0.34
Uhunwode^A	31.87±1.09	5.46	121±20.5	6.11±0.07	0.67±0.04	4.84±0.70	10.53±0.61
Uhunwode^B	36.23±0.73	5.35	79.0±15.5	3.68±0.04	0.40±0.03	3.16±0.84	6.87±0.92
Egor^A	38.92±2.56	5.33	143±41.8	6.16±0.44	0.68±0.08	5.72±0.04	12.44±1.29
Egor^B	23.55±3.85	5.26	84.0±34.5	3.81±0.25	0.42±0.05	3.36±0.02	7.31±0.86
Ovia N/E^A	14.62±0.70	5.57	137±13.5	4.09±0.22	0.45±0.01	5.48±0.23	11.9±1.20
Ovia N/E^B	11.84±0.46	5.34	82.0±12.4	1.57±0.01	0.17±0.09	3.28±0.11	7.13±0.80
p-values	0.069	0.926	0.659	0.386	0.385	0.659	0.66

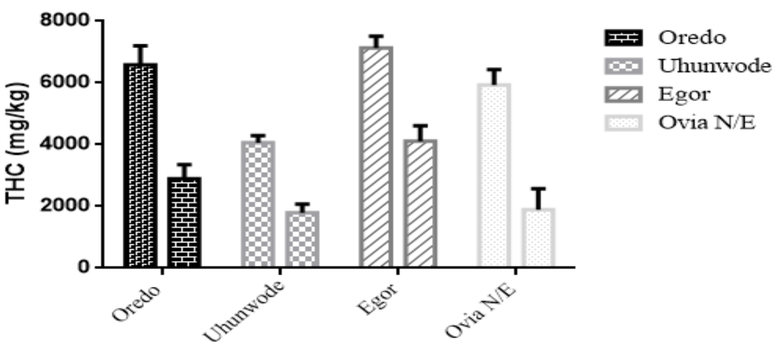


Figure 2: Total hydrocarbon content of soils from sampled LGA

Table 3: Physicochemical parameters of organic and inorganic amendment

Parameters	CD	SDC	NPK	SLG
pH	7.95±0.01	6.92±0.05	6.45±0.02	7.50±0.05
organic carbon (%)	8.01±0.02	6.45±0.05	3.20±0.09	6.52±0.04
Phosphorus (mg/kg)	572.5±0.09	98.23±0.03	120.0±0.07	409.2±0.12
Total nitrogen (%)	3.46±0.03	3.40±0.07	9.81±0.09	7.21±0.01

The physicochemical parameters investigated for soil samples from motor mechanic workshop were moisture, pH, electric conductivity (EC), organic carbon (C), organic magnesium (M), nitrogen (N), phosphorus (P) and potassium (K) (Table 2). The result of the physicochemical parameters were not significant at $p<0.05$. The physicochemical parameters of amendment used (Table 3) showed high phosphorus (mg/kg) for CD (572.5 mg/kg) and SLG (409.2 mg/kg). The EC values influenced soil properties such as cation exchange capacity (CEC), salinity, organic matter and sub-soil properties [18, 19]. The EC is a major pollution index for soil pollution and richness. Soil physicochemical properties such as pH, EC, organic carbon, Nitrogen, magnesium and other essential element were altered by prolonged stay of spent waste engine oil in motor mechanic workshop soils [20-22] .

The result of the mean total heterotrophic bacterial counts (THBC), total hydrocarbon utilizing bacterial counts before 14 weeks' treatment of soil samples ($THUBC^{bt}$) and total hydrocarbon utilizing bacterial counts after 14 weeks' treatment of soil samples ($THUBC^{at}$) from motor mechanic workshop soils (Table 4) revealed high significance ($p<0.05$) for $THUBC^{at}$ while THBC and $THUBC^{bt}$ values were not significant for Uhunwode LGA and Ovia N/E LGA motor mechanic workshop soils. The result of the THBC values is in line with the report of other studies [23-25].

The percentage biostimulation potentials of amendments (CD, SDC, NPK and SLG) used for this study showed progressive increase in percentage biostimulation potentials across the sampled points and sampled locations for soils treated with 14 weeks period. CD recorded % biostimulation potentials for week 2 to week 14; 8 to 63 % for soil samples from Oredo LGA motor mechanic workshop, 9 to 60 % for soil samples from Uhunwode LGA motor mechanic workshop, 10 to 60% for soil samples from Egor LGA motor mechanic workshop, 5 to 65 % for soil samples from Ovia N/E LGA motor mechanic workshop. The percentage biostimulation potential of SDC and NPK was <30 % from week 2 to week 14 for soils from motor mechanic workshop whereas >30 % biostimulation potential was observed for soils amended with SLG. The tendency to be utilized as nutrient for WEO removal in the order of performance were CD, SLG, NPK and SDC. The coefficient of determination (R^2) for soils amended are showed in Table 5. The result of this study revealed that Oredo LGA motor mechanic workshop soil recorded high percentage reduction for SDC (99.8 %) and low value for NPK (93.1%), Uhunwode LGA motor mechanic workshop recorded high value in SDC (99.80 %) and low value in NPK (93.1 %). The R^2 for soil from Egor LGA motor mechanic workshop recorded high value in CD (98.3 %) and low value against SDC (91.1 %). The R^2 for soil from Ovia N/E motor mechanic workshop recorded high value against CD (99.12 %) and low value against SDC (92.8 %). Abbassi and Shquirat [26] reported that microbial concentration influenced the value of kinetic constant.

Table 4: Total heterotrophic and total hydrocarbon utilizing bacterial counts of soil samples from motor mechanic workshop soils in four LGA's in Benin City

Sample code	Amend.	Oredo LGA			Uhunwode LGA			Egor LGA			Ovia North East LGA		
		THBC ($x10^5$ cfu/g)	$THUBC^{bt}$ ($x10^2$ cfu/g)	$THUBC^{at}$ ($x10^7$ cfu/g)	THBC ($x10^5$ cfu/g)	$THUBC^{bt}$ ($x10^2$ cfu/g)	$THUBC^{at}$ ($x10^7$ cfu/g)	THBC ($x10^5$ cfu/g)	$THUBC^{bt}$ ($x10^2$ cfu/g)	$THUBC^{at}$ ($x10^7$ cfu/g)	THBC ($x10^5$ cfu/g)	$THUBC^{bt}$ ($x10^2$ cfu/g)	$THUBC^{at}$ ($x10^7$ cfu/g)
1	CTR	1.4±0.19	1.8±0.09	0.10 ±0.22	1.1±0.18	1.5±0.09	1.1±0.18	1.3±0.09	1.6±0.15	0.04±0.02	1.8±0.08	2.3±0.18	0.18±0.06
2	CD	6.5±0.35	8.5±0.23	0.78±0.06	5.6±0.09	6.7±0.15	5.6±0.09	3.2±0.12	3.8±0.12	0.81±0.07	4.7±0.21	6.1±0.31	1.68±0.05
3	SDC	5.1±0.31	6.6±0.21	1.23±0.11	4.4±0.15	5.7±0.09	4.4±0.15	2.1±0.12	2.7±0.12	0.40±0.07	3.1±0.23	4.0±0.23	1.78±0.17
4	NPK	1.9±0.12	2.5±0.12	1.29±0.05	1.6±0.17	2.1±0.12	1.6±0.17	1.6±0.12	2.0±0.12	1.32±0.24	2.2±0.17	2.9±0.27	1.30±0.06
5	SLG	3.1±0.21	4.0±0.09	1.12±0.16	2.7±0.21	3.4±0.06	2.7±0.21	2.8±0.26	3.6±0.12	0.22±0.02	3.9±0.25	5.1±0.25	0.54±0.04
6	CTR	1.1±0.15	1.5±0.12	0.21±0.22	0.9±0.17	1.2±0.12	0.9±0.17	1.0±0.09	1.3±0.18	0.05±0.01	1.5±0.12	1.9±0.18	0.85±0.13
7	CD	5.4±0.19	7.0±0.19	1.95±0.63	4.5±0.23	5.9±0.09	4.5±0.23	2.6±0.19	3.4±0.12	0.03±0.01	3.9±0.09	5.1±0.21	3.53±0.11
8	SDC	4.2±0.25	5.5±0.12	1.45±0.05	3.5±0.23	4.6±0.12	3.5±0.23	1.7±0.06	2.3±0.12	0.01±0.02	2.6±0.09	3.3±0.09	3.43±0.09
9	NPK	1.6±0.22	2.1±0.15	1.50±0.23	1.3±0.15	1.7±0.09	1.3±0.15	1.3±0.15	1.7±0.15	0.10±0.14	1.8±0.15	2.4±0.09	3.53±0.14
10	SLG	2.6±0.09	3.3±0.10	1.25±0.04	2.1±0.09	2.8±0.12	2.1±0.09	2.3±0.06	3.0±0.24	0.03±0.00	3.2±0.15	4.2±0.09	2.95±0.12
	P-values	0.033*	0.025**	0.00***	0.393	0.343	0.000***	0.033*	0.025*	0.001***	0.480	0.468	0.001**

n=3, THBC: total heterotrophic bacterial count, $THUBC^{bt}$: total hydrocarbon utilizing bacterial count before treatment, $THUBC^{at}$: total hydrocarbon utilizing bacterial count after 14 weeks' treatment, the mean difference is significant at $p<0.05$, compared to one*, two**, three*** groups

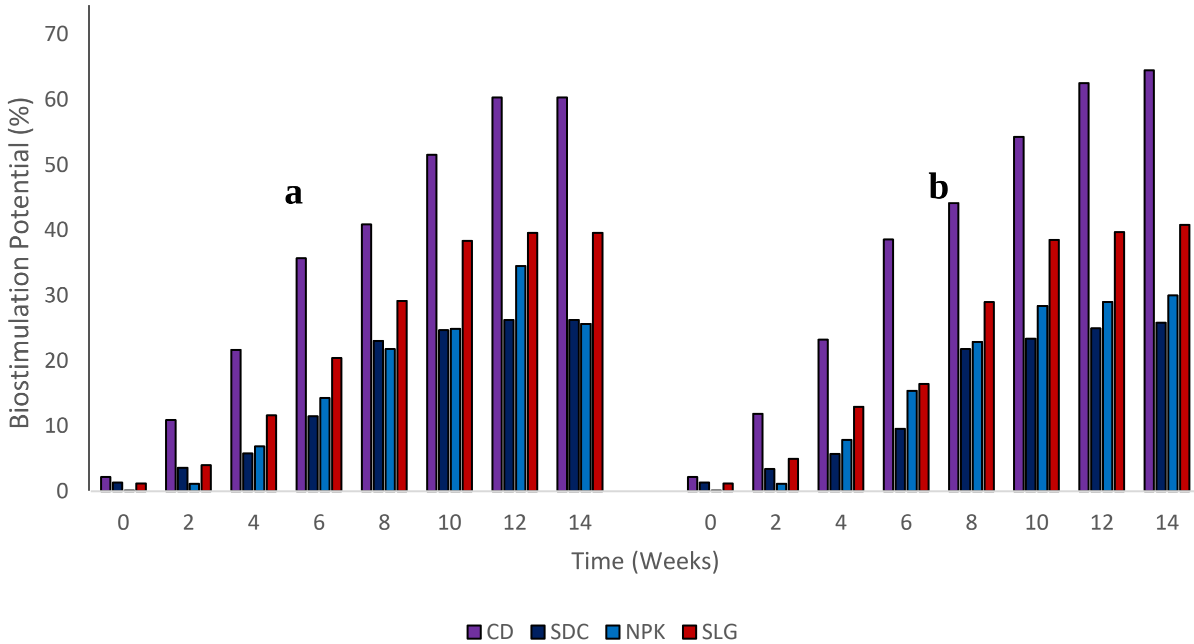


Figure 3: Biostimulation potentials of organic and inorganic amendments on soil samples from **Oredo LGA motor mechanic workshops**, **a**: N6^0 19' 50.57", E5^0 44' 42.78"; **b**: N6^0 19' 49.45", E5^0 44' 41.61".

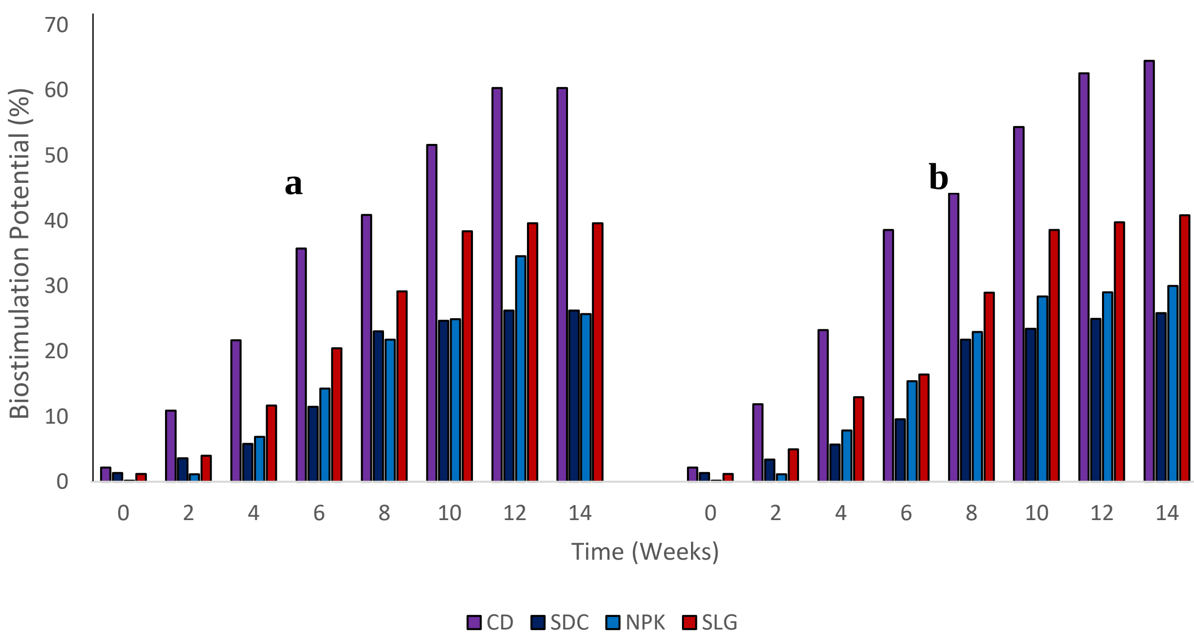


Figure 4: Biostimulation potentials of organic and inorganic amendments on soil samples from **Egor LGA motor mechanic workshop**, **a**: N6^0 22' 40.62", E5^0 35' 30.94"; **b**: N6^0 22' 45.60", E5^0 34' 31.95"

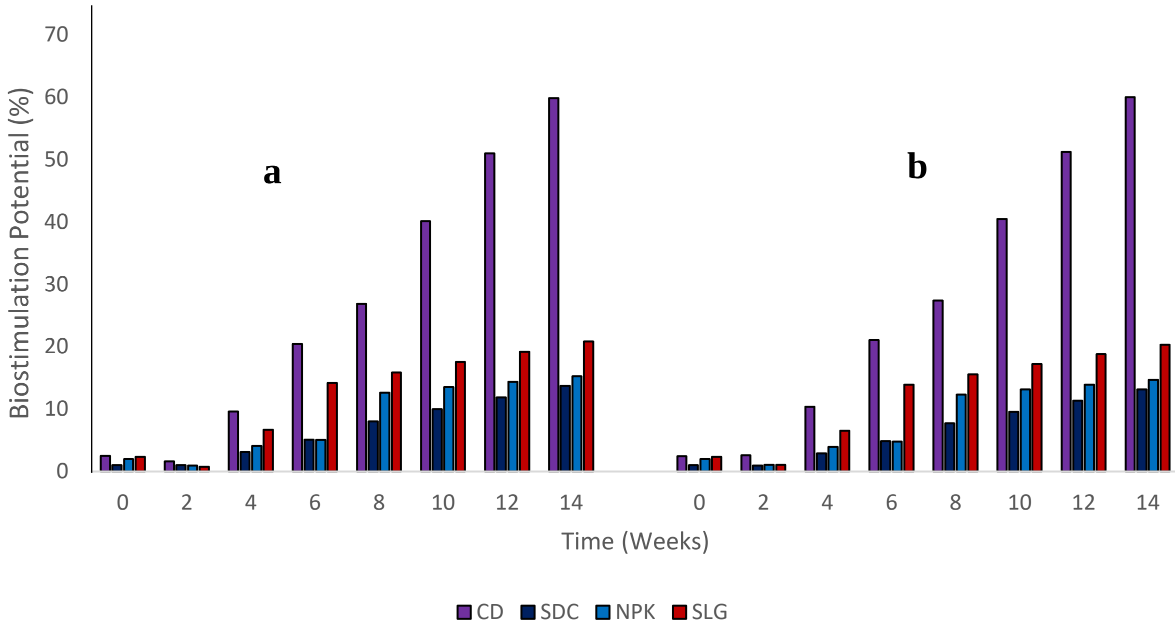


Figure 5: Biostimulation potentials of organic and inorganic amendments on soil samples from **Uhunwode LGA motor mechanic workshops**, **a**: N6^0 19’ 50.57”, E5^0 44’ 42.78”; **b**: N6^0 19’ 49.93”, E5^{0}44’ 42.1”

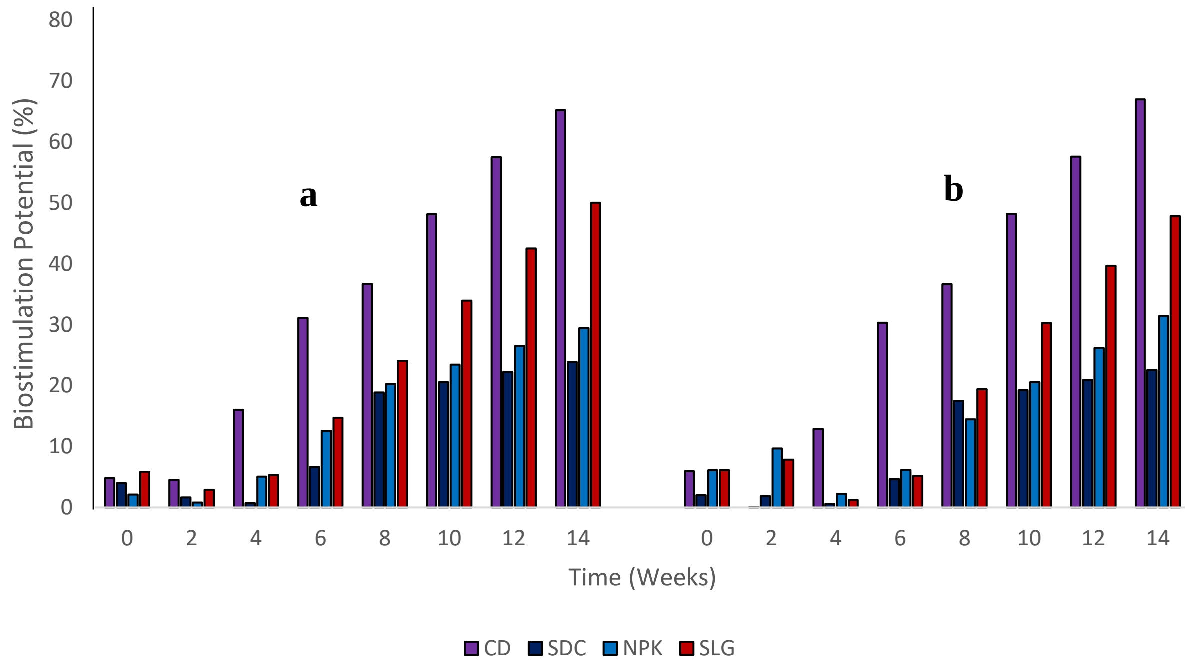


Figure 6: Biostimulation potentials of organic and inorganic amendments on soil samples from **Ovia North East LGA (Oluku) motor mechanic workshops**, **a**: N6^0 26' 0", E5^0 35' 55"; **b**: N6^0 27' 44", E5^0 36' 0"

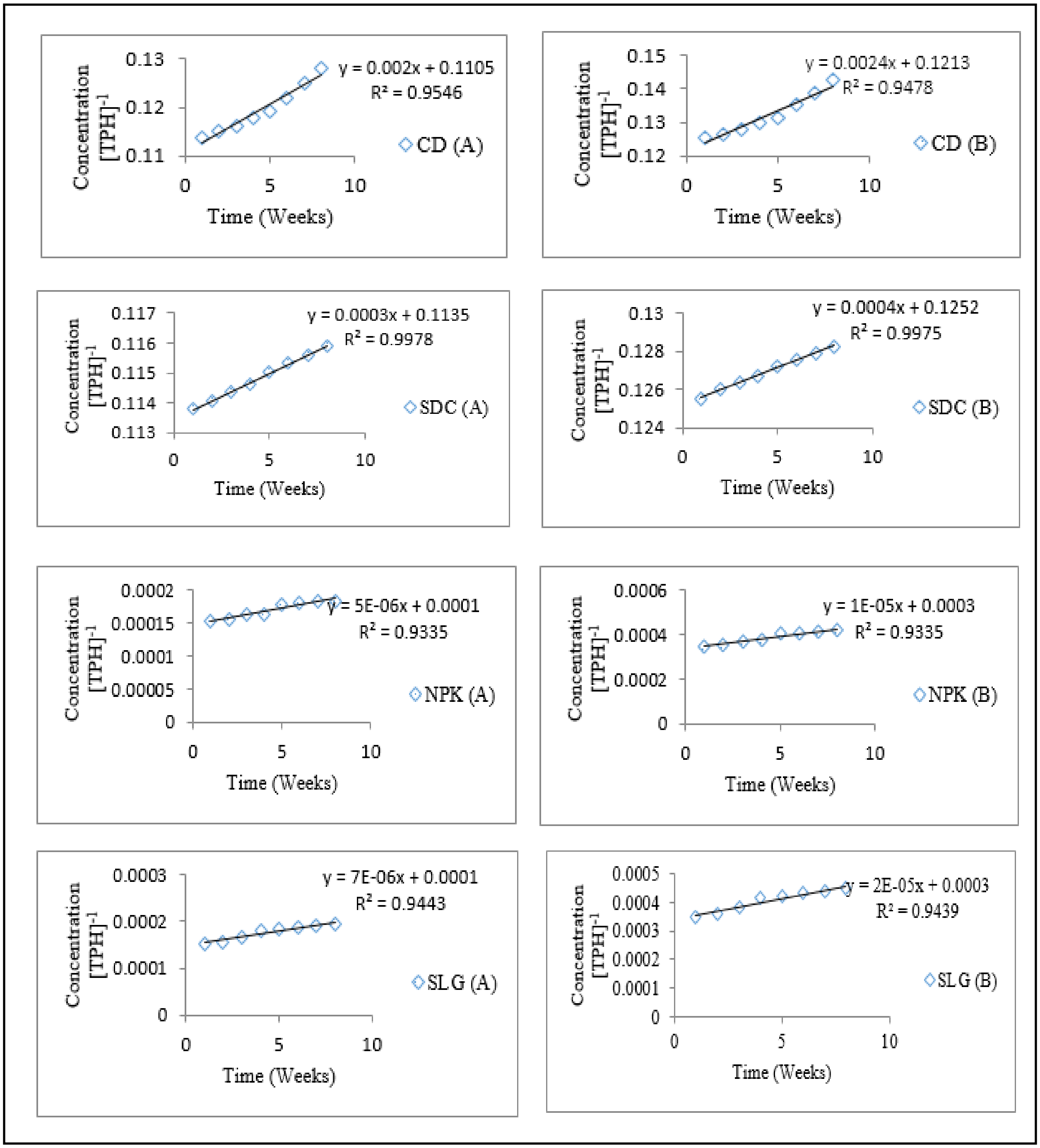


Figure 7: $[TPH]^{-1}$ vs Time to determine the rate constant of reaction for soil from **Oredo LGA motor Mechanic Workshop** amended with **CD, SDC, NPK and SLG**. The reaction is assumed to be second order

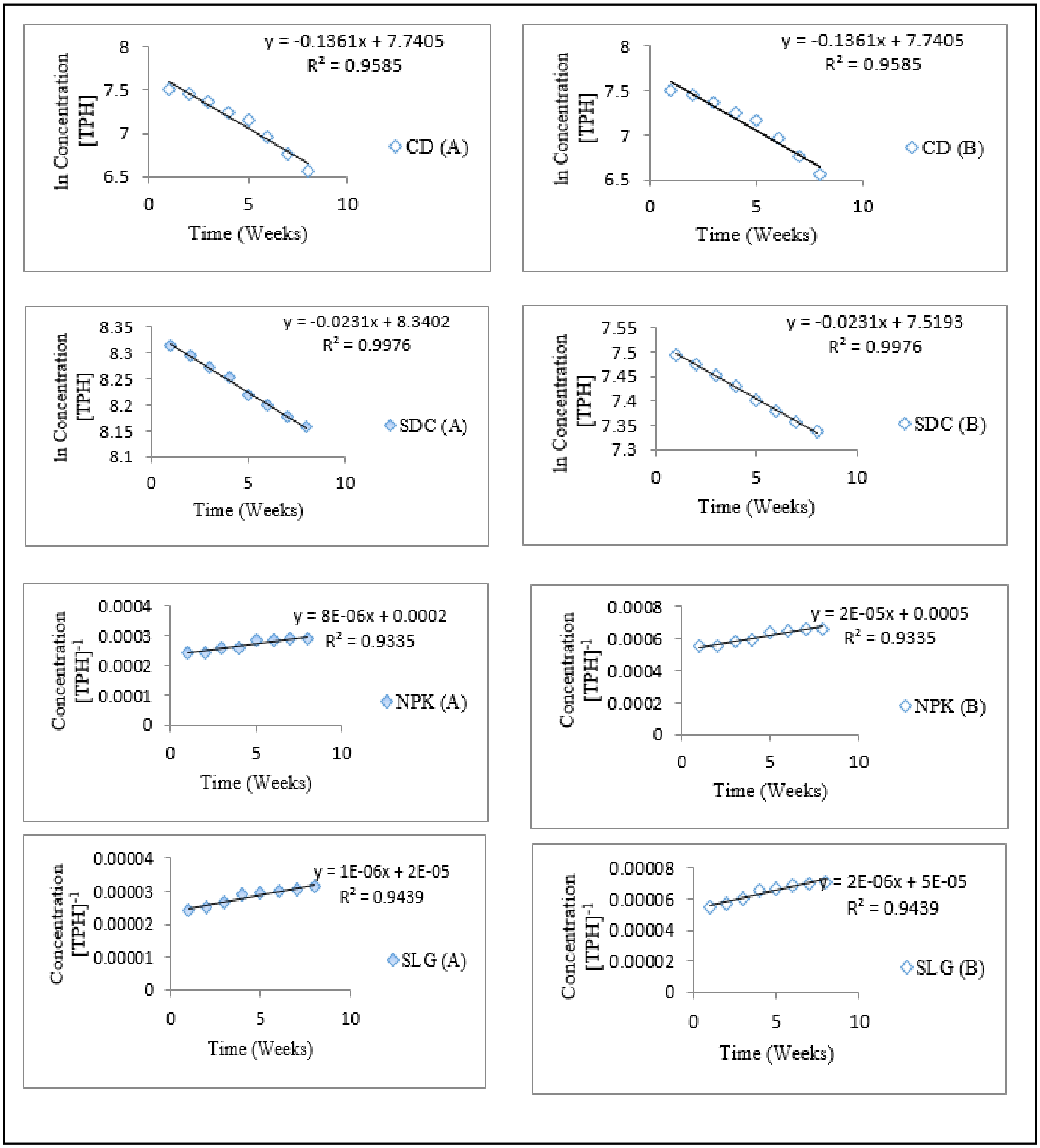


Figure 8: 1n TPH/[TPH]$^{-1}$ vs Time to determine the rate constant of reaction for soil from **Uhunwode LGA motor mechanic workshop** amended with **CD, SDC, NPK and SLG**. The reaction is assumed to be first or second order

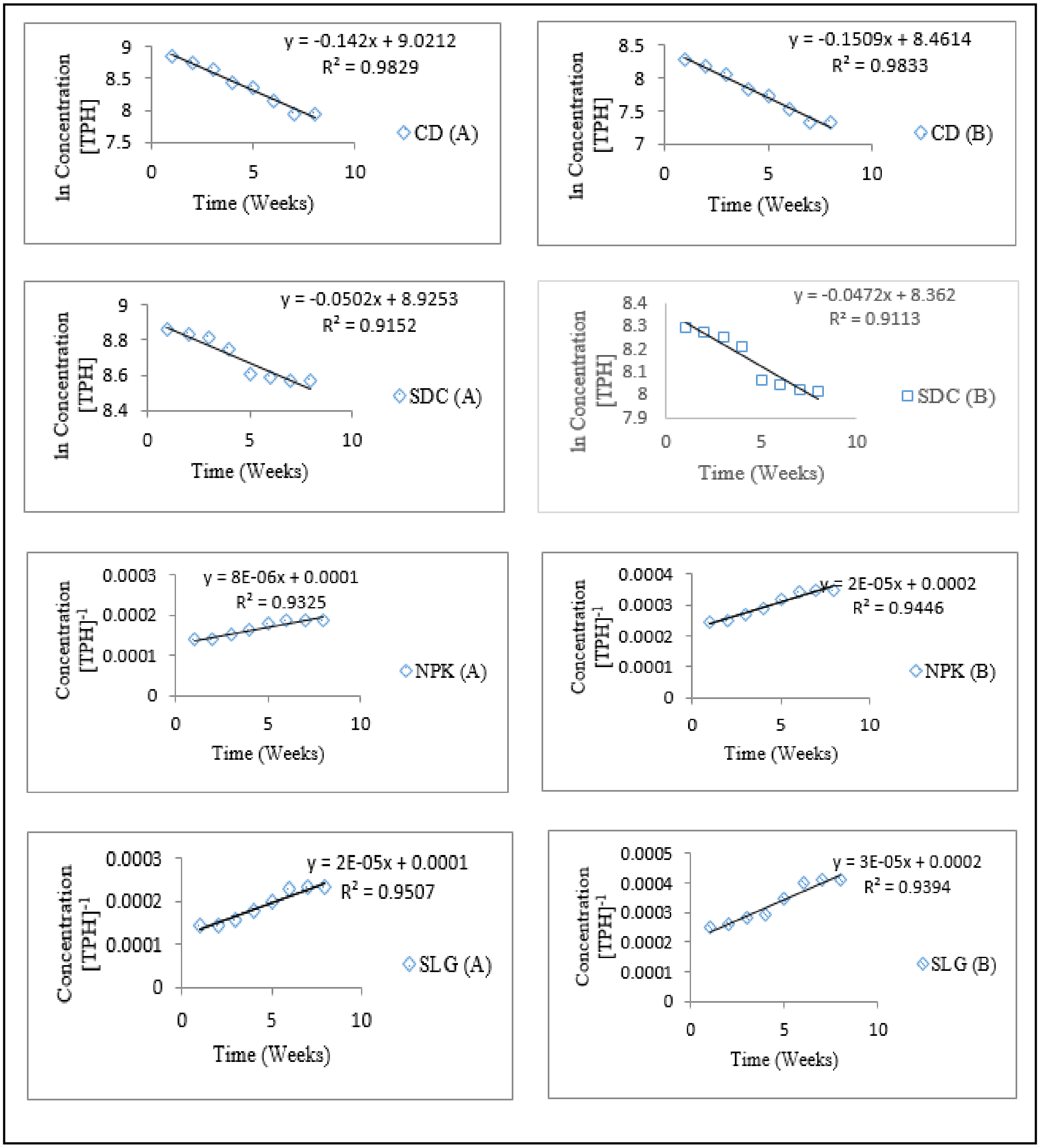


Figure 9: 1n TPH/$[TPH]^{-1}$ vs Time to determine the rate constant of reaction for soil from **Egor LGA motor mechanic workshop** amended with **CD, SDC, NPK and SLG**. The reaction is assumed to be first or second order

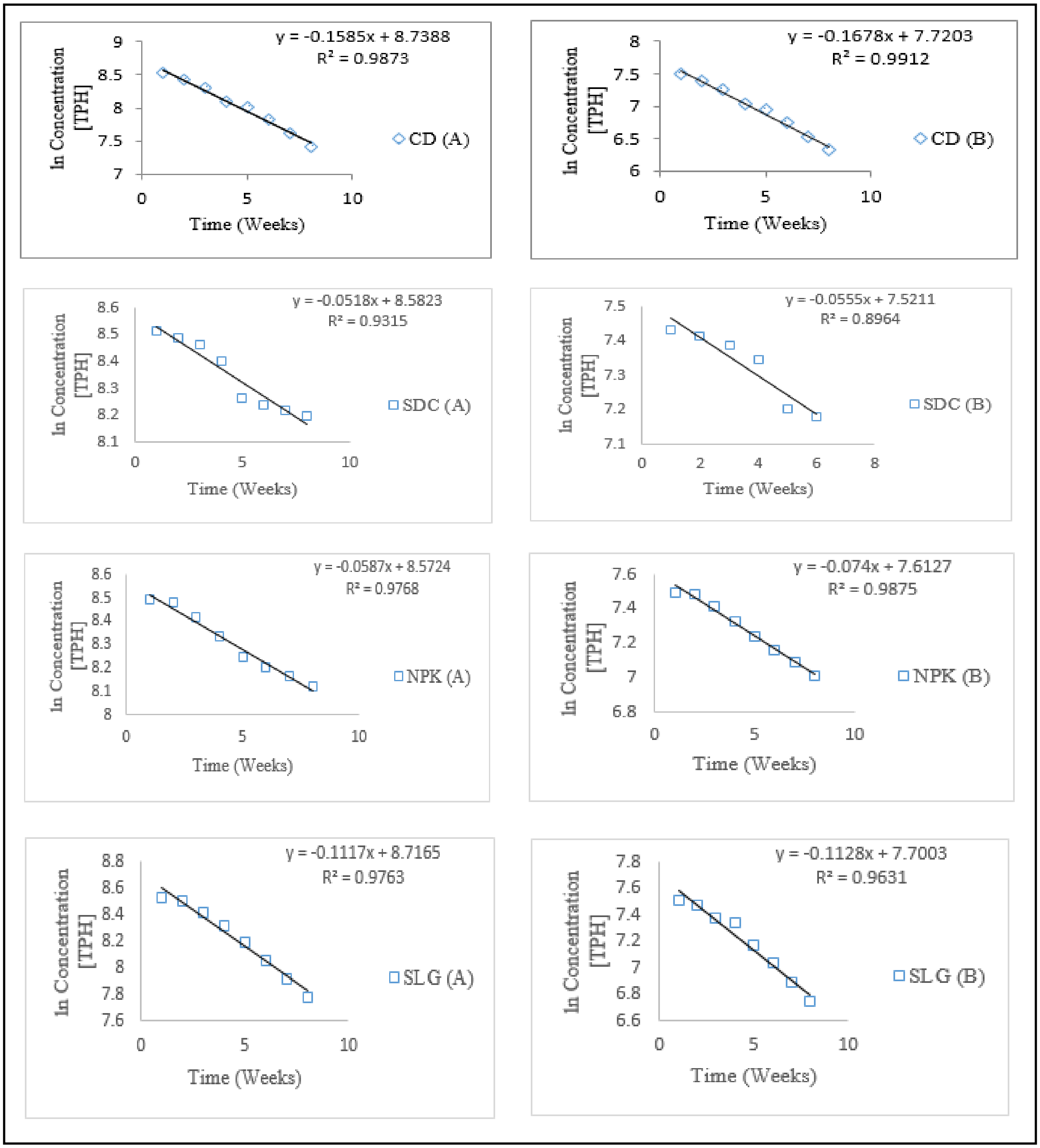


Figure 10: $[TPH]^{-1}$ vs Time to determine the rate constant of reaction for soil from **Ovia N/E LGA motor Mechanic Workshop** amended with **CD, SDC, NPK and SLG**. The reaction is assumed to be first order

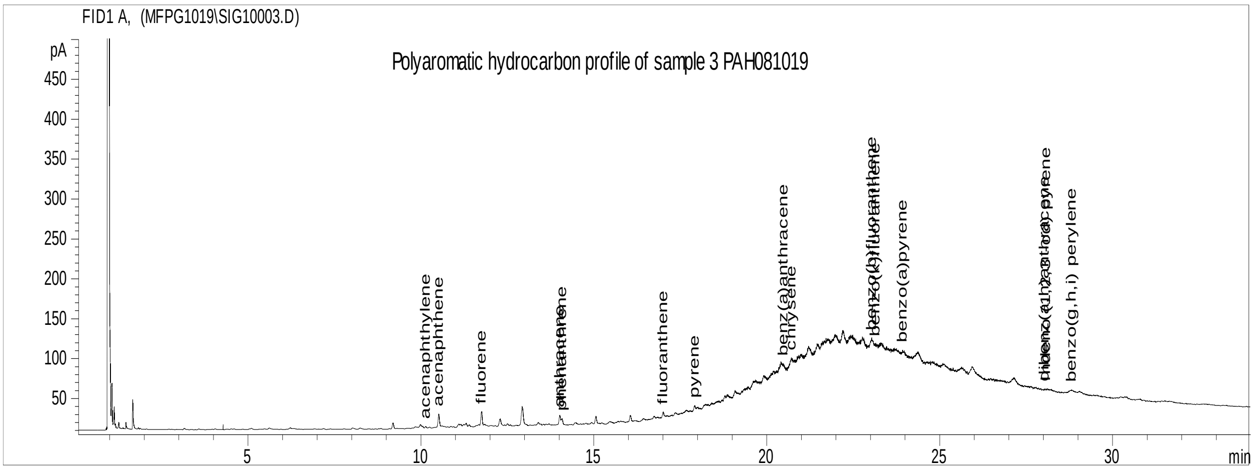
Table 5: Kinetics and half-life of contaminated WEO removal from motor mechanic workshop soils

Sample code	Amend.	Oredo LGA			Uhunwode LGA			Egor LGA			Ovia North East LGA		
		Biodegradation constant (k) weeks^{-1}	Half-life (weeks) $t_{1/2}$	Coefficient of determination (R^2)	Biodegradation constant (k) weeks^{-1}	Half-life (weeks) $t_{1/2}$	Coefficient of determination (R^2)	Biodegradation constant (k) weeks^{-1}	Half-life (weeks) $t_{1/2}$	Coefficient of determination (R^2)	Biodegradation constant (k) weeks^{-1}	Half-life (weeks) $t_{1/2}$	Coefficient of determination (R^2)
1	CTR	-	-	-	-	-	-	-	-	-	-	-	-
2	CD	0.07	9.902^{a}	0.967^{a}	0.07	9.902^{a}	0.956^{a}	0.07	9.902^{a}	0.983^{a}	0.08	8.664^{a}	0.987^{a}
3	SDC	0.01	69.31^{b}	0.998^{a}	0.01	69.31^{b}	0.998^{a}	0.02	34.66^{d}	0.912^{c}	0.03	23.10^{d}	0.932^{b}
4	NPK	0.02	34.66^{c}	0.931^{b}	0.02	34.66^{c}	0.931^{b}	0.03	23.10^{c}	0.928^{c}	0.03	23.10^{d}	0.977^{a}
5	SLG	0.02	34.66^{c}	0.934^{b}	0.02	34.66^{c}	0.933^{b}	0.04	17.33^{b}	0.954^{b}	0.06	11.55^{b}	0.976^{a}
6	CTR	-	-	-	-	-	-	-	-	-	-	-	-
7	CD	0.07	9.902^{a}	0.961^{a}	0.07	9.902^{a}	0.959^{a}	0.08	8.664^{a}	0.983^{a}	0.08	8.664^{a}	0.991^{a}
8	SDC	0.01	69.31^{b}	0.997^{a}	0.01	69.31^{b}	0.998^{a}	0.02	34.66^{d}	0.911^{c}	0.02	34.66^{e}	0.928^{b}
9	NPK	0.02	34.66^{c}	0.931^{b}	0.02	34.66^{c}	0.931^{b}	0.03	23.10^{c}	0.942^{b}	0.04	17.33^{c}	0.988^{a}
10	SLG	0.02	34.66^{c}	0.933^{b}	0.02	34.66^{c}	0.933^{b}	0.04	17.33^{b}	0.947^{b}	0.06	11.55^{b}	0.963^{c}

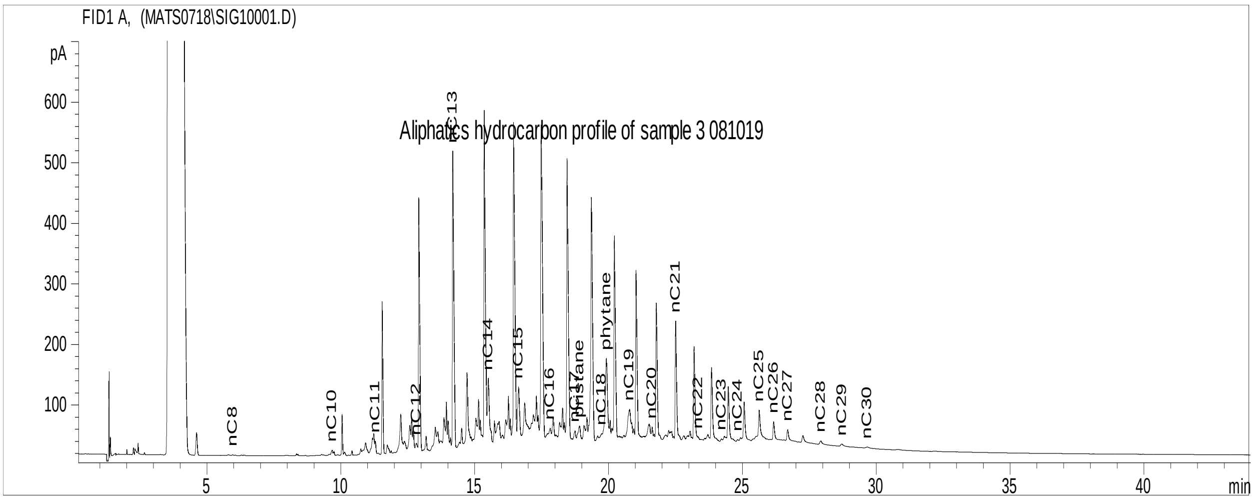
The mean difference is significant at $p<0.05$, similar letters in superscript across the columns indicate values that are not significantly different

Table 6: Poly-Aromatic and Aliphatic Hydrocarbon Content of waste engine oil

Components	Waste engine oil	PAHs
	(mg/L)	**(%)**
Naphthalene	0.000	0
Acenaphthalene	0.001	0.813
Acenaphthene	0.009	7.317
Florene	0.010	8.130
Phenathrene	0.005	4.065
Anthracene	0.008	6.504
Fluoranthene	0.004	3.252
Pyrene	0.004	3.252
Benzo(a)anthracene	0.015	12.20
Crysene	0.032	26.02
Benzo(b)fluoranthrene	0.014	11.38
Benzo(a)pyrene	0.005	4.065
Benzo(k)fluoranthrene	0.016	13.01
Indeno(1,2,3) perylene	0.000	0
Dibenzo(a,h)anthracene	0.000	0
Benzo(g,h,i) perylene	0.000	0
Total PAH (mg/L)	0.123	
Total Aliphatic (mg/L)	32.365	
Total THC (mg/L)	**32.489**	



a



b

Figure 11. GC-FID chromatogram of used Waste Engine Oil (WEO), a: poly-aromatic fraction, b: aliphatic hydrocarbon fraction

The GC-FID result for waste engine oil (WEO) (containing 60 mg/L of WEO) detected the presence of polyaromatic hydrocarbon fractions such as Acenaphthalene (0.001 mg/L), Acenaphthene (0.009 mg/L), Florene (0.010 mg/L), Phenathrene (0.005 mg/L), Anthracene (0.008 mg/L), Fluoranthene (0.004 mg/L), Pyrene (0.004 mg/L), Benzo(a)anthracene (0.015 mg/L), Crysene (0.032 mg/L), Benzo(b)fluoranthrene (0.014 mg/L), Benzo(a)pyrene (0.005 mg/L), Benzo(k)fluoranthrene (0.016 mg/L). Indeno(1,2,3) perylene, Dibenzo(a,h)anthracene and Benzo(g,h,i) perylene were not detected from the WEO sample. The WEO also recorded 32.365 mg/L aliphatic hydrocarbon fraction (Table 6, Figure 11). Studies have showed that exposure to polycyclic aromatic hydrocarbons such as anthracene, benzo(a)anthracene and benzo[*a*]-pyrene, is mainly by polluted air, e.g., through exposure to exhaust fumes of fossil fuel [27] . This study however revealed that anthracene (6.504 %), benzo(a)anthracene (12.20 %) and benzo[*a*]-pyrene (4.065 %) are also present in trace amount in waste motor engine oil. These compounds are immunosuppressive and affects humoral responsiveness [28] . Crysene was the highest PAH present in this study at 26.02 %. Crysene are deleterious in that they cause incidence of liver tumors, malignant, lymphomas, skin sarcomas and lung tumors in mice [29] .

4. Conclusion

The absence of recycling and reuse of WEO in developing nations has contributed significantly to WEO pollution of soils in different locations in Benin City. This study demonstrated the effectiveness of organic (cow dung, sawdust compost) and inorganic (NPK, industrial effluent treatment sludge) nutrient amendments in the biostimulation of WEO polluted soils in Benin metropolis. The first or second order kinetic data in this study revealed that soil amended with cow dung recorded highest rate of degradation potential compared to other amendments used. The performance of the organic and inorganic amendments followed this order, cow dung > effluent treatment sludge > sawdust compost.

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Conflict of Interest

There was no conflict of interest in the course of this study.

Nomenclature

WEO	Waste engine oil
CD	Cow dung
SLG	Industrial effluent treatment sludge
NPK	Inorganic fertilizer (NPK 15:15: 15)
SDC	Sawdust compost
THC	Total hydrocarbon content
TPH	Total petroleum hydrocarbon
THBC	Total heterotrophic bacteria count
$THUBC^{bt}$	Total hydrocarbon utilizing bacteria count before treatment
$THUBC^{at}$	Total hydrocarbon utilizing bacteria count after treatment
$[A_t]$	concentration of A at time t
$[A_o]$	concentration of A at time 0
k	rate constant for second order kinetic model
t	time of the reaction
$t_{1/2}$	half-life
C_a	concentration of WEO (mg/kg)
C_o	initial contration of the soil (mg/kg)
THC_{ctrl}	total hydrocarbon content of control groups
$THC_{amended}$	total hydrocarbon content of soils amended

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