



Novel Mathematical Models for Prediction of Spent Engine oil Interactions with *Vernonia amygdalina* Leaf Extract

Unyeawaji Brownson Ntesat ^a,
Okogbule-Wonodi Achinike ^a, Chinwendu Emeka ^{a*}
and Mgbowaji Zacchaeus ^b

^a Department of Agricultural and Environmental Engineering, Faculty of Engineering, Rivers State University, Port-Harcourt, Nigeria.

^b World Bank African Centre for Excellence for Oil Field Chemicals Research, University of Port Harcourt, Choba, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJARR/2024/v18i6665

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/116117>

Original Research Article

Received: 18/02/2024
Accepted: 22/04/2024
Published: 04/05/2024

ABSTRACT

Aims: The michaelis-menten biokinetic model was used for the determination of key biokinetic parameters; maximum specific rate constant (μ_{max}) and michaelis-menten constant (K_m) including the total polycyclic aromatic hydrocarbon (TPAH) degradation rate equation in the biostimulation of spent engine oil (SEO) – contaminated soil using *vernonia amygdalina* leaf (bitter leaf) extract.
Study Design: Single factor experiment in a randomized complete block design (RCBD) was used in this study.

*Corresponding author: Email: chinwendu.emeka1@ust.edu.ng;

Place and Duration of Study: Research farm, Rivers state university, Port-Harcourt, Nigeria, between April 2023 and May 2023.

Methodology: TPAH was quantified by the standard method, according to USEPA method using gas chromatography-mass spectrometry (GC-MS). The experiment consisted of 4 reactors replicated thrice including the control (Labelled A to D) comprised of soil, SEO and bitter leaf extract in the ratios of 8:1:0.5, 8:1:1, 8:1:1.5, and 8:1:0, respectively, after 8 weeks of treatment.

Results: The results obtained shows that TPAH concentration dropped by 42.3 – 63.9% from 98.73mg/kg⁻¹ with an average reduction of 52% was achieved in all treatment reactors. The average of μ_{max} was 4.4mg/kg-week and K_m was 79.2mg/kg while the TPAH degradation was deduced as $r = -4.4S/(79.2 + S)$ at any TPAH concentration. The validation of the developed model indicates a high coefficient of determination (r^2) ranging from 0.927 to 0.992 and a low root mean square error of 9.08 to 20.81mg/kg. This statistical tool effectively shows the potential of the model to predict TPAH concentration in the biostimulation of spent engine oil-polluted soil using bitter leaf extract

Conclusion: Up to 63.9% TPAH reduction was achieved for the stated period. The evaluation results show the potential of the model to predict TPAH in the biostimulation of SEO-contaminated sandy loam soil using bitter leaf extract.

Keywords: Bioremediation; biodegradation; biokinetics model; pollution; total polycyclic aromatic hydrocarbon; spent engine oil.

1. INTRODUCTION

Soil is an integral part of the natural ecosystem and suggest that environmental sustainability depends largely on it [1]. Any nation that destroys its soil has destroyed itself [2-4], because soil provides a growing medium for plant roots, mineral and nutrients, exchanges oxygen and gases, protects against erosion and speeds up the natural decomposition process of organic matter [5]. Thus, healthy soil is essential for the well-being of humans, animals and plants. Soils in most parts of the world is polluted. Petroleum hydrocarbon contamination of soil is a global environmental issue, which results in environmental hazards and human health problems [6,7]. Besides, it strongly affects soil physicochemical properties as well as the population and activity of soil microorganisms [8]. Petroleum hydrocarbon fills soil pore spaces and inhibits effective soil aeration, strips the soil of its natural nutrients, and inhibits plants from absorbing appropriate nutrients, which eventually leads to plant mortality, depletes oxygen in the soil, destroying soil microorganisms useful for plant growth and toxicity as well as reduces soil fertility [5].

Engine oil is a bye-product of crude oil, the used engine oil is also known as the spent engine oil and the chemical composition of the engine oil varies widely and depends on the original crude oil, the refining procedures, the kind and efficiency of the engine the oil is lubricating, the combustion products produced by gasoline, the

additions made to both the fuel and the original oil, and the amount of time the oil is left in the engine. The oil usually contains the following compositions: 11–15% monoaromatic hydrocarbons, 2-5% diaromatic hydrocarbons, 4-8% polyaromatic hydrocarbons, and 73–80% weight/weight aliphatic hydrocarbons (mostly alkanes and cycloalkanes with 1–6 rings) [9]. Spent engine oil (SEO) disposed into gutters and water drains, In Nigeria, it is common for motor mechanics in particular to leave unused farmland and plots exposed. This oil, also known as waste engine oil or spent lubricant, is typically acquired from car and generator engines that have been drained after servicing [10], with a large portion of the oil being dumped into the ground. It has been demonstrated that the effects of used motor oil contamination on the environment are more pervasive than those of crude oil contamination [11]. For example, it was stated that Nigeria produces around 87 million liters of spent oil waste each year, and that its disposal has not received enough attention [10]. Because of the influence of SEO in the soil, removing SEO-polluted soil is a global issue, especially in the majority of undeveloped and emerging nations. The environmental problems caused by hydrocarbons have greatly increased. The primary route by which PAHs enter the atmosphere [12], PAHs are carcinogenic, mutagenic and teratogenic persistence organic pollutants formed by the fusion of two or more benzene ring [13]. It might pose a serious threat to living organisms and humans through different pathways [14]. However, several cost-ineffective

and environmentally unfriendly methods like the physicochemical technique including thermal desorption, incineration, soil washing soil vapor, disposal in landfills, excavation, etc. have been used.

Presently, the biological treatment (Bioremediation) techniques for the treatment of SEO- polluted soil has been demonstrated to be affordable and eco-friendly. Bioremediation is simply the use of microbes to detoxify pollutants into non-toxic forms [15]. Its affordability and environmental friendliness have led to its adoption over chemical technologies [16]. Some well-known biological treatment technologies include biostimulation, biosparging, bioventing, bioaugmentation, and phytoremediation.

Adding minerals or fertilizers often referred to as amendments, in order to boost the development and metabolic activity of native bacteria is known as biostimulation [17]. Most polluted locations are often low in phosphorus and nitrogen, which makes it difficult for native microbes to get enough resources for their survival and degradation processes. The existing microbial population's ability for degradation is increased when rate-limiting nutrients are added to the system. Both organic and inorganic fertilizers contain nutrients that are used as biostimulants [17]. Thus, organic fertilizers could provide nutrients for the biostimulation of soil contaminated by wasted engine oil in a way that is safer for the environment due to the fact that it nourishes the soil and gives bacteria nourishment, making them more productive [18].

Vernonia amygdalina is a widely grown and unique medicinal plant in the East and West of Africa. [19]. It is used as an active anti-cancer [20], anti-bacteria, anti-malarial, and anti-parasitic agent [21]. This plant has complex active ingredients with potential applications in pharmacology. In ethnomedicine, the roots and

leaves are used to cure fever, hiccups, kidney issues, and stomach discomfort.

Hamowia and Saffaf [22] Chew sticks made from the stem and root of the bark are used in several West African countries, including Nigeria, Ghana, and Cameroon. The leaves of *Vernonia amygdalina* are among the most commonly consumed leafy vegetables. (See Fig. 1). Despite the medicinal importance of the leaf, the extract which is often discarded as waste can also be utilized for remediation of petroleum hydrocarbon contaminated sites. However, there is dearth of information on the use of *vernonia amygdalina* leaf extract for bioremediation of TPAH in spent engine-contaminated soil.

2. MATERIALS AND METHODS

2.1. Materials

Top-soil (0 -30cm in depth) was collected using clean shovel from the research farm, Rivers state university, Port-Harcourt, Nigeria. The spent engine oil was collected from mechanic workshop in the Niger Delta region of Nigeria. The uncontaminated soil sample was collected from the demonstration farm, Rivers State University, Port-Harcourt with clean shovel at 0-30cm depth. Fresh bitter leaf was bought at fruit garden market, D-line, Port-Harcourt, the leaves were sorted, washed several times with water and rinsed with distilled water. Then, it was placed into a portable electric blender. After which, the solution obtained was purified by filtration (See Fig. 2) using whatman No. 1 filter paper [23], and further sterilized by filtration through a millipore membrane filter of 0.45 μm pore size [24]. The sterile extracts were kept chilled in sterile capped bottles until it was used to treat the soil. The extract was then mixed with the soil and crude oil. Aliquots were collected using bottles for laboratory analysis at 2 weeks interval until a period of 8 weeks to determine the rate of hydrocarbon degradation.



Fig. 1. *Vernonia Amygdalina* Leaf (Bitter Leaf)

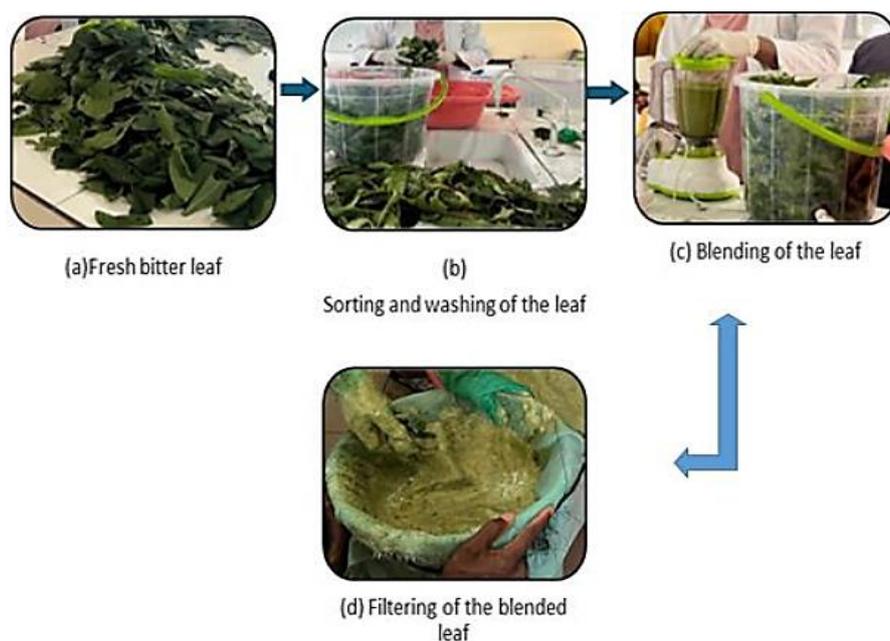


Fig. 2. Schematic of the bitter leaf extract process

2.2. Methods

2.2.1 Experimental design

Single factor experiment in a randomized design (RCBD) was used in this study. In such experiment only single factors varies while others are kept constant. The treatment consists solely of the different levels of single variable factors. All other factors are uniformly applied in all reactors at a prescribed level [25]. The treatment consisted different levels of the leaf extract (0, 250, 500, and 750). All other factors such as the quantity of soil and SEO were applied uniformly to all plastic reactor a single prescribed level. The experimental design consisted of 4 treatments with 2 replicates each making a total of 8 treatment reactors for SEO-contaminated soils. The samples were collected after contaminating the soil with spent engine oil (SEO), and every 2weeks until the end of the study period for laboratory analysis to determine the residual total polycyclic aromatic hydrocarbon (TPAH) for a period of 56 days. They were labelled A, B, C including the control (D) with two replications as shown in Table 1. Randomizations was achieved using the draw lot approach as described in several literatures [25].

2.2.2 Analysis of sample

The pH, electrical conductivity (EC), and temperature of the sample were determined

using a HI9812-5 pH/EC/TDS/Temperature Meter (Hanna Instruments, USA). MC (w/w), on dry basis, was determined by the oven-drying method with an oven maintained at 105 ± 5 °C for 24 h, pH was determined by dipping the electrode into a 1:2.5 sample suspension that had been stirred and allowed to equilibrate for 1 h. The hydrometer method was used to determine the PSD while the soil texture was determined by the USDA soil textural classification. TPAH quantification method, instrument calibration, and quality control measures adopted are as described by Emeka et al. [2]. Organic carbon was determined by the standard operating procedures based on British Standard 7755 Section 3.8:1995 [26]. Total nitrogen was determined by the Kjeldahl digestion technique. Total phosphorus was determined by the ascorbic acid reduction method. PAH analysis was carried out in line with EPA 8270 method on an Agilent 6890 GC /MSED 5973 equipped with a split / splitless injector, J and W 30 meter DB-5 column and mass selective detector. A 5g of soil sample was dried chemically using 5g sodium sulphate anhydrous. Extraction was carried out separately using 10ml of Dichloromethane, a combination of extraction solvent and sample were agitated by shaking for 45 minutes with vortex mixer. Glass wool/glass funnel were used to decant extract. The extract was allowed to concentrate to 1ml before taken to GC for PAH analysis.

Table 1. Experimental material mix proportion

Reactor	Material mix proportion	Material mix ratio
A	4000g of soil sample +500g of SEO+250g BLE	8:1:0.5
B	4000g of soil sample +500g of SEO+500g BLE	8:1:1
C	4000g of soil sample +500g of SEO+750g BLE	8:1:1.5
D (Control)	4000g of soil sample +500g of SEO+0g BLE	8:1:0

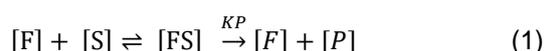
2.2.3 Bio-kinetic model

Rate of TPAH depletion = $-rV$ (4)

The bitter leaf extract in spent engine contaminated soils, which nurtures the microorganisms, enzymes and the substrate medium undergo the reaction as shown in equation 1.

Rate of TPAH accumulation = $-\frac{dN}{dt}$ (5)

Substituting equations (2) through (5) into the mass balance expression in Fig. 3 gives equation (6)



$$FoS_o = FS - rV - \frac{dN}{dt} \quad (6)$$

Assumptions

- I. The simple substrate controls the velocity of reaction.
- II. Enzymes formed react with substrate forming enzyme substrate complex.
- III. The enzymes substrate decomposed to form product and enzymes.

For batch reactor,
 Rate of inflow of mass = 0
 Rate of outflow of mass = 0
 Rate of formation = 0

Hence, equation (3.8) reduced to

$$-\frac{dN}{dt} = -rV \quad (7)$$

The biodegradation rate of residual soil total polycyclic aromatic (TPAH) as remediated by bitter leaf extract was modeled by the Michaelis-Menten kinetics is a well-known model used to simulate the kinetics of enzymes, explaining how the concentration of the enzymes and their substrate affects the rate of an enzyme-catalyzed process. Because the experiment was carried out in batches, the decay rate model was first integrated with the batch reactor's rate equation. The resulting equation was utilized to predict the TPAH concentration in spent engine oil-contaminated soil at any given time. As shown in Fig. 3, applying the mass balance expression in Fig. 3, the batch model can be deduced as follows.

By expressing equation (37) in terms of concentration, we have equation (8).

$$-\frac{d(\frac{N}{V})}{dt} = -\frac{ds}{dt} = r \quad (8)$$

Thus, equation (8) becomes equation (9)

$$\frac{ds}{dt} = -r_s \quad (9)$$

Where:

- S_o = Initial concentration of TPAH (mg/kg)
- S = Instantaneous concentration of TPAH (mg/kg)
- F_o = Inlet flow rate (L/week)
- F = Outlet flow rate (L/week)
- V = Volume of reactor (L)
- N = Mass of TPAH (mg)
- t = Time of remediation (week)

Assumptions

- The reactor above is batch.
- There is no inflow and outflow of mass.
- There is no longitudinal and lateral flow of mass.

As shown in Fig. 2, applying the mass balance expression in Fig. 3. We obtained as follows:

$$\text{Inflow of TPAH into reactor} = F_o S_o \quad (2)$$

$$\text{Outflow of TPAH from reactor} = FS \quad (3)$$

But the growth rate decay is expressed as:

$$-r = \frac{\mu_{max}}{K_m + S} \quad (10)$$

Where:

r = TPAH degradation rate (mg/kg-week)
 μ_{max} Maximum specific rate constant (mg/kg-week)
 K_m = Constant (mg/kg)

$$k_3 = hf \left(t(i) + \frac{1}{2} h, S(i) + \frac{1}{2} k_2 \right) \quad (16)$$

$$k_4 = hf \left(t(i) + h, S(i) + k_3 \right) \quad (17)$$

Substituting equation (3.29) into (3.30), we will have as follows:

$$-r = \frac{dS}{dt} = \frac{\mu_{max}}{K_m + S} \quad (11)$$

To obtain the constants in equation (11), we take the inverse of both sides to ascertain the Michaelis-Menten equation as shown in equation (12)

$$-\frac{1}{r} = \frac{K_m}{\mu_{max}} \left(\frac{1}{S} \right) + \frac{1}{\mu_{max}} \quad (12)$$

Equation (12) is the equation of a straight line, which plots for $\frac{1}{r}$ versus $\frac{1}{S}$, known as the double reciprocal plot or Linweaver-Burk plot, gives the slope of the graph as $\frac{K_m}{\mu_{max}}$, while the intercept represent $\frac{1}{\mu_{max}}$. The two constants, μ_{max} and K_m were experimentally by measuring r at different TPAH concentration and plotting $\frac{1}{r}$ versus $\frac{1}{S}$, then, μ_{max} and K_m were substituted into equation (11), which was used to predict the degradation rate of TPAH (r) in respect to the TPAH content for each treatment reactor. Equation (11)

is an ODE (ordinary differential equation) and it is very complex to resolve, hence, MATLAB computer program was used to solve the Runge-Kutta, which is expressed as follows.

$$S(i+1) = S(i) + [k_1 + 2(k_2 + k_3) + k_4] / 6 \quad (13)$$

Where:

$$k_1 = hf(t(i), S(i)) \quad (14)$$

$$k_2 = hf \left(t(i) + \frac{1}{2} h, S(i) + \frac{1}{2} k_1 \right) \quad (15)$$

Where

h = Step size
 t = time
 S = TPAH concentration
 K_1, k_2, k_3, k_4 = slopes
 $i = 1, 2, 3,$

2.3 Evaluation of Model Performance

Single factor experiment in a randomized complete block design (RCBD) was carried out using one-way analysis of variance, data analysis toolbox in Microsoft excel-365 (Microsoft Inc. USA). The validity (goodness of fit) of the biokinetic models were tested by comparison with the experimental and the efficiency of the model was evaluated using the following statistical tool; coefficient of determine (R^2) and root mean square error (RMSE).

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (\text{Measured}_i - \text{Predicted}_i)^2} \quad (18)$$

$$R^2 = 1 - \frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \quad (19)$$

Where n is the total number of evaluated samples,
 y_i is the i th measured output,
 \hat{y}_i is the i th predicted output, and
 \bar{y} is the mean observed output.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics of Uncontaminated Soil and Bitter Leaf Extract

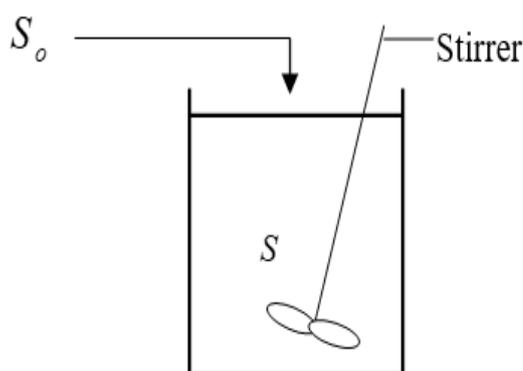
The values of key parameters of the uncontaminated soil used is composed of 59.7% sand, 28.0% and 12.3% clay and is classified as sandy loam soil. The pH was 6.18, which is slightly acidic. Moisture content, electrical conductivity, organic matter and organic carbon of the uncontaminated soil were 22%, 41 μ s/cm, and 0.86% and 1.73%, respectively. The TPAH concentration in the uncontaminated soil was within the normal background level found in soil.

It is observed that nutritional values of the organic fertilizer (bitter leaf extract) were 0.52mg/kg of nitrogen, 24.41mg/kg of phosphorus and 37.46mg/kg of potassium.

3.2 Variation of Soil TPAH with Time in SEO-Contaminated Soil Treated with BLE

The average TPAH concentration in the contaminated media after mixing the soil and spent engine oil was 98.73mg/kg in all reactors (See Fig. 4). This corresponds to the 0 week after contaminating the soil. The changes in the TPAH concentration during the remediation period of 8 weeks in each reactor. After 2 weeks, there were decrease in TPAH, which varied according to the different treatments. The reduction in treatment A, B, and C with 250, 500, and 750g of BLE including the control with 0 BLE. There was high significant difference from each other and it fell within (4.1 – 44.9%). After 4 weeks, there were further reduction in TPAH in all the reactors (A, B, and C). The TPAH

concentration dropped from an initial of 98.73mg/kg at the commencement of remediation work to 70.5, 64.5 and 48.4mg/kg for treatment A, B, and C, representing 27.3, 33.6 and 49.75% reductions in TPAH. This confirms that the organic fertilizer would be a promising amendment for the reclamation of spent engine oil-contaminated soil. TPAH degradation across the reactors was quite high except for the control that was relatively low (Up to 20.7%). It is similar to the findings of Okparanma et al. [27], who stated that much of residual soil TPH was degraded within 14 days using water treatment residual. The TPAH degradation among the different material mix in various reactors over 8 weeks remediation period. It was obvious that TPAH varied with material mix in the reactors. Reactor C experienced the highest amount of TPAH degradation (Up to 63.9%), while reactor D experienced the least amount of TPAH degradation (39%) is shown in Fig. 4. This suggested the reason for the incorporation of amendment for the contaminated soils.



Schematic Batch Reactor with Stirrer

$$\left(\begin{array}{c} \text{Rate of} \\ \text{TPAH} \\ \text{flow into} \\ \text{reactor} \end{array} \right) = \left(\begin{array}{c} \text{Rate of} \\ \text{TPAH out} \\ \text{flow from} \\ \text{reactor} \end{array} \right) + \left(\begin{array}{c} \text{Rate of} \\ \text{TPAH} \\ \text{depletion} \\ \text{with} \\ \text{reactor} \end{array} \right) + \left(\begin{array}{c} \text{Rate of} \\ \text{TPAH} \\ \text{accumulati} \\ \text{on with} \\ \text{reactor} \end{array} \right)$$

Fig. 3. Mass Balance of the batch reactor

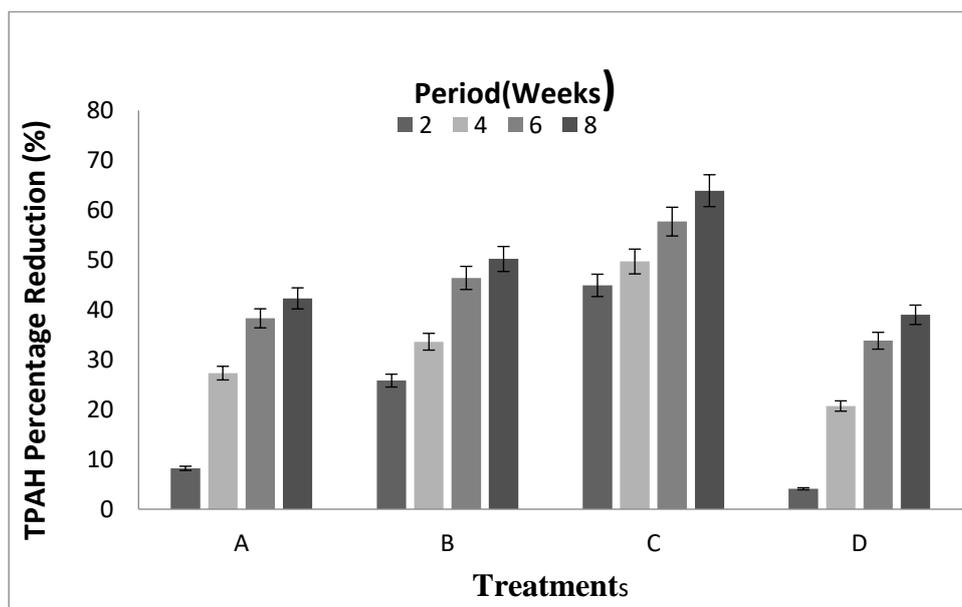


Fig. 4. TPAH Concentrations percentage Reduction (A, 250g of Bitter Leaf Extract; B, 500g of Bitter Leaf Extract; C, 500g of Bitter Leaf Extract; D, and Control with 0g of Bitter Leaf Extract), Error Bar represents standard bar

3.3 Model Parameters

Lineweaver-burk plot shown in Fig. 5 was used to determine the maximum specific rate constant (μ_{max}) and the michaelis-menten constant (K_m). The coefficient of determination (r^2) ranged from 0.952 to 0.997, with an average of 97.6% across the treatment reactors. This shows that the michaelis- menten model fitted the experimental data with 97.6% of explained variance, leaving on 2.4% unaccounted for (as shown in Fig. 5), K_m remained fairly constant at about 79.2mg/kg, which confirms that $\frac{1}{K_m}$ remained fairly unchanged as $\frac{1}{\mu_{max}}$ decreased with increase in the amount of BLE applied [27]. This is an indication of non-competitive inhibition where

inhibitor will bind to an enzyme at its allosteric site [27-29], its presence is demonstrated in the lineweaver –burk plot by changes that occurs in the y – intercept ($\frac{1}{\mu_{max}}$) is shown in Table 2. The TPAH degradation rate equations for the three treatment reactors were obtained by the substitution of K_m and μ_{max} with an average. The TPAH degradation rate equation for the biostimulation is $r = -4.4S/(79.2 + S)$, which can be adopted for the estimation of the rate of TPAH degradation at any concentration in the removal of spent engine oil- contaminated sandy loam soil treated with bitter leaf extract. The differences in the reactor material mix may be most likely be the cause of the differences in the μ_{max} and r as observed in Table 2.

Table 2. Summary of the Michaelis - Menten model parameters deduced from the Lineweaver- Burk Plots

Reactor	μ_{max} (mg/kg-week)	K_m (mg/kg)	r (mg/kg-week)
A	5.695	97.050	$r = -\frac{5.695(S)}{97.050 + S}$
B	4.297	80.404	$r = -\frac{4.297(S)}{80.404 + S}$
C	3.212	60.202	$r = -\frac{3.212(S)}{60.202 + S}$
Average	4.401	79.219	$r = -\frac{4.401(S)}{79.219 + S}$

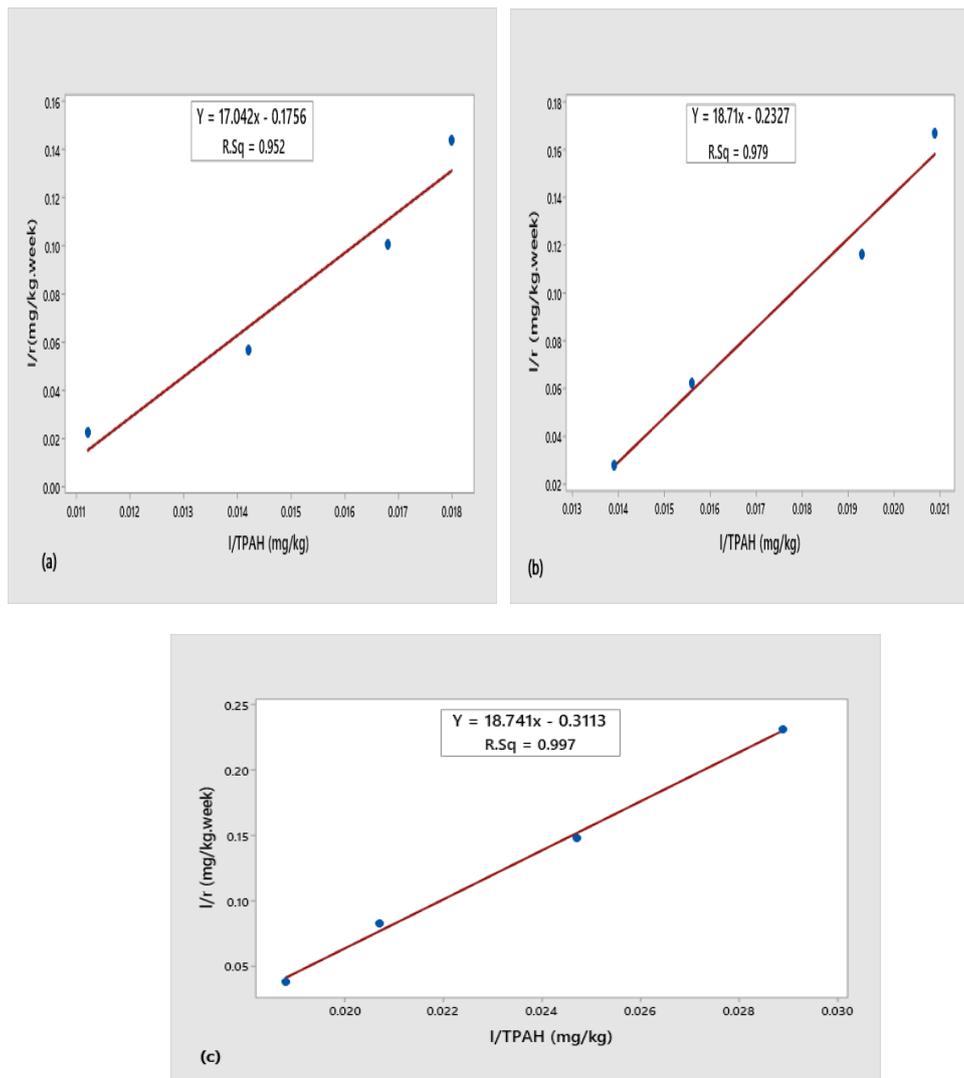
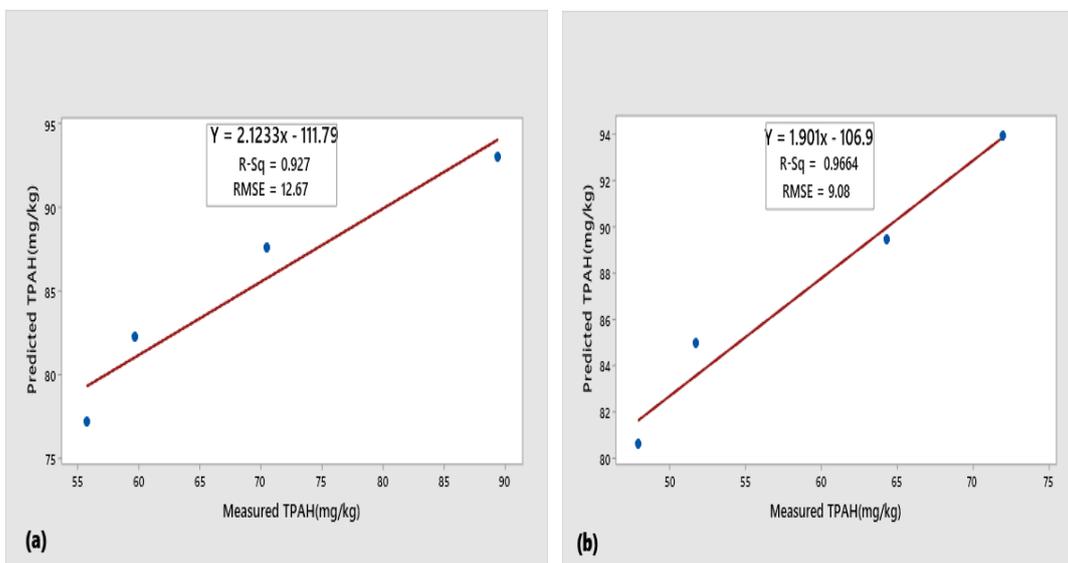


Fig. 5. Lineweaver-Burk plot for TPAH for SEO - Contaminated Soil Treated with; (a) 250g of BLE (b) 500g of BLE and (c) 750 BLE



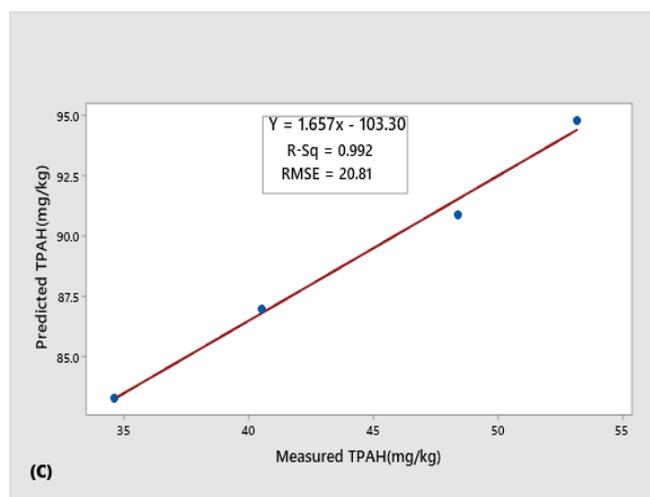


Fig. 6. Predicted vs measured TPAH for SEO - Contaminated Soil Treated with; (a) 250g of BLE (b) 500g of BLE and (c) 750 BLE

3.3 Model Prediction Ability

The model prediction ability was tested by comparing the predicted data results with measured experimental data after the rate constant have been determined from the lineweaver-burk plots. The validation r^2 of over 96.2% and average RMSE of 14.2mg/kg for all the treatment reactors (Shown in Fig. 6) indicates the potential of the developed model to predict TPAH in SEO – contaminated soil treated with bitter leaf extract.

4. CONCLUSION

This study revealed that 63.9% TPAH reduction was achieved after 8 weeks of treatment using bitter leaf extract. The key kinetic parameters of michaelis – menten model, known as the maximum specific rate constant (μ_{max}) and michaelis-menten constant (K_m). The average of μ_{max} was 4.4mg/kg-week and 79.2mg/kg-week while the TPAH degradation rate equation was deduced as $r = -4.4S/(79.2 + S)$ at any TPAH concentration, with r^2 of over 96.2% and average RMSE of 14.2mg/kg. The evaluated results indicated the potential of the model to predict TPAH in the biostimulation of spent engine oil–contaminated sandy loam soil using bitter leaf extract.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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