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# Investigation of the Solvent Extraction Using Alcohol and Water on *Bryophylum pinnatum* Leaves for Total Petroleum Hydrocarbon Degradation on Loamy Soil

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***Abstract***

*Investigation of the solvent extraction using alcohol and water on Bryophylum pinnatum leaves for total petroleum hydrocarbon degradation on loamy soil was monitored for the purpose of evaluating the Micheal's Menten functional parameters as well as the coefficients. The maximum specific rate of the total petroleum hydrocarbon (TPH), which was expressed as $V_{max}$ and the rate of the dissociation of the TPH, which is denoted as $K_m$ was computed in relationship to the effect of volume of solvent dosage. The performance of the two solvents used which is alcohol and water has demonstrated that both solvents are useful in the extraction of the necessary nutrients from the Bryophylum pinnatum leaves for crude oil remediation. The performance of the extracts obtained using the different solvent were rated good because of the high rate of the TPH reduction in concentration within 42 days.*

**Keywords:** Investigation, solvent extraction, alcohol, water, *Bryophylum pinnatum*, leaves, TPH

## INTRODUCTION

Crude oil contains toxic compounds and radioactive elements that are serious health concerns, and also affects plant growth [1, 2]. Bioremediation is a method that used to treat a polluted environment by adding microorganism or bio-stimulants such as enzymes, biomass to remove contaminants from contaminated environment through metabolic process [3]. The use of bioreactor ensures the growth of microbes necessary for the degradation of contaminants are controlled and monitored, thereby providing the necessary conditions to achieve an optimum process performance [4]. This study adopted the bio-stimulation method, implemented in batch reactor for bioremediation of polycyclic aromatic hydrocarbons (PAHs) contaminant in polluted (loamy and clay) soils using *Bryophylum pinnatum* plants extract [5–7].

Soil pollution arising from increasing demand for petroleum and its products has become a common problem in recent years [8]. Crude oil contamination in soil, particularly in clay and loamy soils poses a significant environmental challenge with far-reaching implications for the ecosystems, agriculture, and human health. Clay and loam soils, characterized by their distinct physical and

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chemical properties, interact with crude oil in ways that hindered remediation efforts [9–11]. Remediation technology and biotechnology advancements which has been extensively reviewed in recent times have diverse composition and physicochemical properties, the specific underlying mode of biostimulant action of individual plants that is cost effective and eco-friendly is often unclear and still under investigation [12, 13].

Several researchers have demonstrated different methods and experimental design in remediating contaminated soil. The study on the degradation of crude oil polluted soil using magnetic nano-particles [14]. In their study, two different samples (loamy and clay) were artificially polluted with crude oil and their investigation was based on degradation of polycyclic aromatic hydrocarbon (PAH) and total petroleum hydrocarbon (TPH) for four weeks remediation period [15]. Similar study on algae biostimulants: a critical look at microalgal biostimulants for sustainable agricultural practices [16]. Also, the performance of *Mangifera indica* seed biomass that yielded varying percentage reduction of total petroleum hydrocarbon was investigated [17, 18].

Hence, the application of biostimulants (*Bryophylum pinnatum* plant) increase nutrient and help plants tolerate abiotic stresses which both enhance the quality and yield of crops [19, 20]. Therefore, the degradation potential of *Bryophylum pinnatum* plants on total petroleum hydrocarbon (TPH) in crude oil polluted loamy and clay soils was investigated. It is important to note that in this study *Bryophylum pinnatum* plant contained the needed nutrients that improve the available microorganisms in a stress environment for treatment.

## MATERIALS AND METHODS

### Experimental Layout of Crude Oil Contaminated Soil Treated with *Bryophylum pinnatum* Leaves Extracts of Water

- $T_3$: Addition of 100 ml of crude oil on 2000 g of loamy soil mix with 100 ml *Bryophylum pinnatum* leaves extracts of water.
- $T_4$: Addition of 100 ml of crude oil on 2000 g of loamy soil mix with 200 ml *Bryophylum pinnatum* leaves extracts of water.
- $T_5$: Addition of 100 ml of crude oil on 2000 g of loamy soil mix with 300 ml *Bryophylum pinnatum* leaves extracts of water.

### Experimental Layout of Crude Oil Contaminated Soil Treated with *Bryophylum pinnatum* Leaves Extracts of Ethanol

- $T_9$: Addition of 100 ml of crude oil on 2000 g of loamy soil mix with 100 ml *Bryophylum pinnatum* leaves extracts of ethanol.
- $T_{10}$: Addition of 100 ml of crude oil on 2000 g of loamy soil mix with 200 ml *Bryophylum pinnatum* leaves extracts of ethanol.
- $T_{11}$: Addition of 100 ml of crude oil on 2000 g of loamy soil mix with 300 ml *Bryophylum pinnatum* leaves extracts of ethanol.

### Determination of Total Petroleum Hydrocarbon

Samples were analyzed in line with USEPA 8015 method using Gen Tech Master G equipped with a split/split less injector, J and W 30-meter DB-5column and an FID detector. The carrier gas, Helium was set at 20 ml/min. The injector temperature set as 240℃ and the detector temperature at 300℃. The total run time at 1 NI injection volume through the capillary column with a slow ramp rate 40℃ to 310℃ was 45 mins. The gas chromatograph (GC) was calibrated using three working standards prepared from a stock solution of Accu standard 500 mg/ml in chloroform hydrocarbon window defining standard. About 5 g of soil sample was used for the analysis. Sample was dried chemically using 5 g sodium sulphate anhydrous, extraction was carried out separately by using 10 ml Dichloromethane, and o-Terphenyl was used as surrogate. Combination of extraction solvent and sample was agitated by shaking for 45 mins with vortex mixer and glass wool/ glass funnel was used to

 

decant extract. The extract was allowed to concentrate to 1 ml before taken to the GC for TPH analysis. The glass electrodes were standardized after every 10 determinations. The electrodes were dipped in distilled water when not in use and the reference electrode was ensured to always contain saturated potassium chloride in contract with solid potassium chloride crystals, 3 to 4 drops of toluene were added in standard buffer solution to prevent growth of mould.

The residual TPH percentage at any time was calculated using Eq. (1).

$$TPH_R\% = \frac{TPH_i - TPH_f}{TPH_i} \times 100\% \quad (1)$$

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

## RESULTS AND DISCUSSION

### Evaluation of Michael-Menten Constants of TPH Degradation on Loamy Soil

Figure 1 explains the maximum specific degradation rate constant values using a well-established equation ($V_{max}$) and Michaelis-Menten constant ($K_m$) that were determined using $\frac{V_{max} x\ S}{K_m + S}$ for 100, 200, and 300 ml of water biostimulant on loamy soil including the control. The linearized results from the control T1 showed high level of reduction possibly due to volatilization of hydrocarbon content during day 42 with the best line of fit $R^2 = 0.9671$. Equation of line of best fit had y=-110.5x +48671 for the calculated values of $V_{max}$ and $K_m$ in the T1 control for loamy soil were $R = \frac{0.2418126\ x\ S}{1.01\ x10 + S}$ at 95% confidence level.

Figure 2 illustrates the maximum specific degradation rate constant values ($V_{max}$) and Michaelis-Menten constant ($K_m$) that were determined using 100 ml of water extracts in bioreactor T3 containing loam soil. From the baseline results of TPH 48291.13530 (ppm), it was suspected that indigenous microbial activity in the densely polluted soil may be insufficient for bioremediation. Hence, the mixed proportion of water biostimulant added to the soil stimulate microbial functions for bioremediation. The linearized results from T3 showed the performance of treatment on hydrocarbon degradation with equation of the line had y=-683.96x + 48874 and regression of best fit $R^2 = 0.9975$ representing 99.75%. The calculated values for $V_{max}$ and $K_m$ in the T1 control for loam soil were $R = \frac{0.2252005\ x\ S}{1.01\ x10 + S}$.

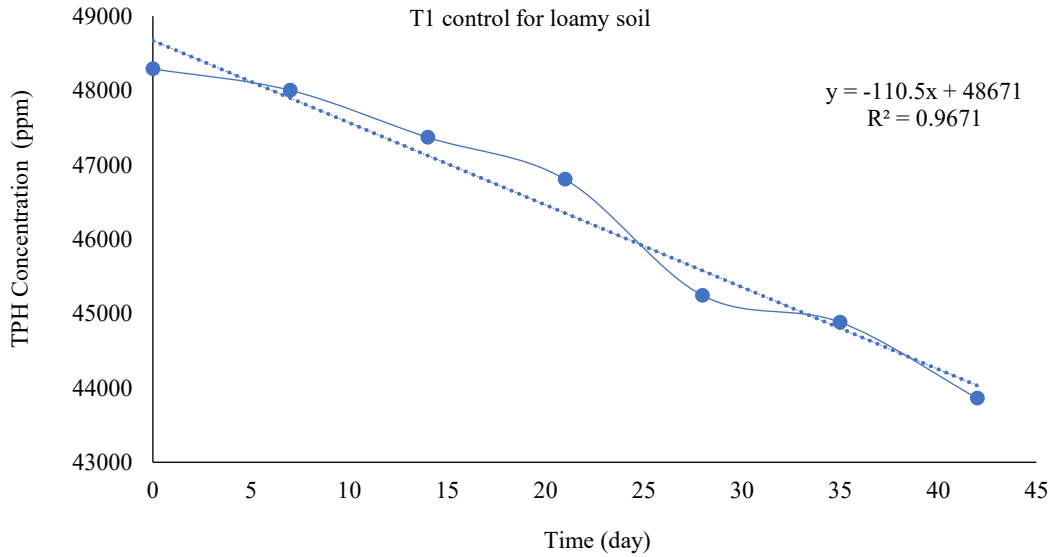


**Figure 1.** TPH concentration versus Time for control in loamy soil.

 

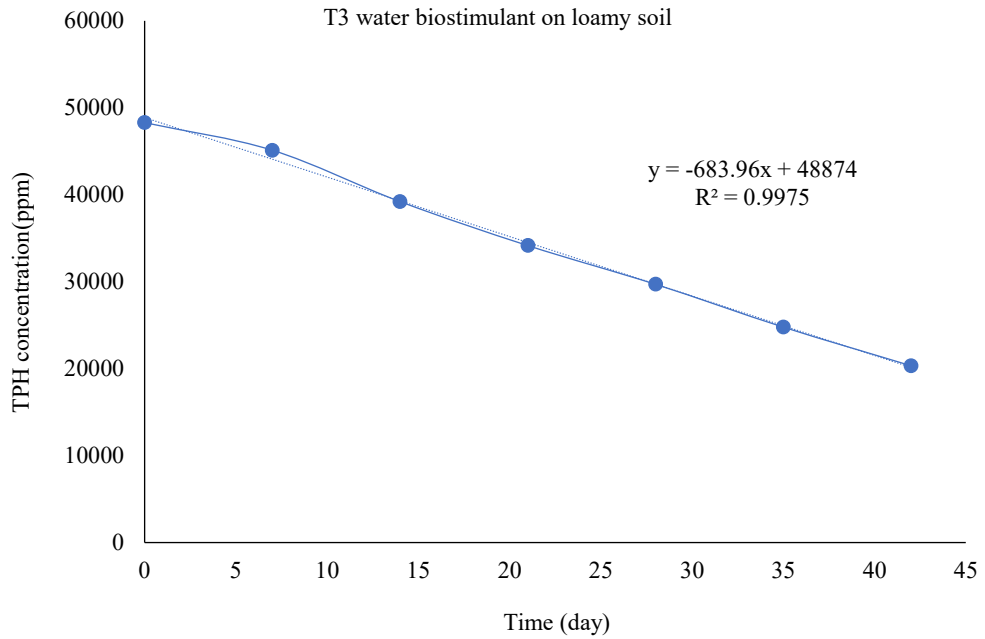


**Figure 2.** TPH concentration versus Time in T3 water biostimulant in loamy soil.

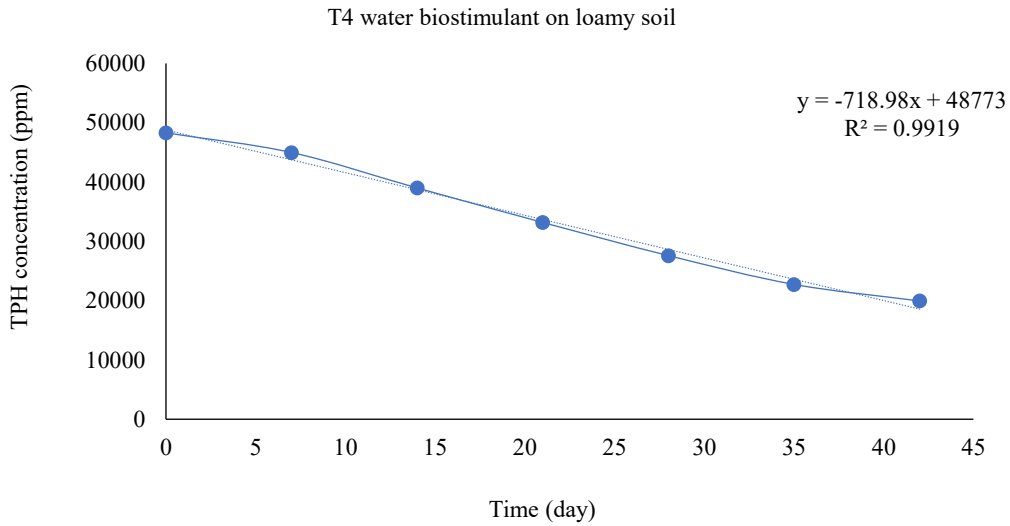


**Figure 3.** TPH concentration versus Time for T4 water in loamy soil.

Figure 3 revealed slight reduction in TPH concentration in bioreactor T4. The maximum specific degradation rate constant values ($V_{max}$) and Michaelis-Menten constant ($K_m$) that was determined using 200 ml of water extracts in bioreactor T4 containing loamy soil. The linearized results from the calculated values for $V_{max}$ and $K_m$ showed that $R = \frac{0.224327 \text{ x } S}{1.01 \text{ x} 10 + S}$ which demonstrate high performance with square root of best fit $R^2 = 0.9919$ with regression equation of y=-718.98x +48773.

Figure 4 revealed high performance of *Bryophylum pinnatum* leaves in the reduction of TPH concentration in bioreactor T5. The maximum specific degradation rate constant values ($V_{max}$) and Michaelis-Menten constant ($K_m$) that was determined using 300 ml of water biostimulant in bioreactor T5 containing loamy soil. The linearized results from the calculated values for $V_{max}$ and $K_m$ showed that $R = \frac{0.2234878 \text{ x } S}{1.01 \text{ x} 10 + S}$ which demonstrate high reliability with square root of best fit $R^2 = 0.9956$ with regression equation of y=-779.18x + 48927.

 

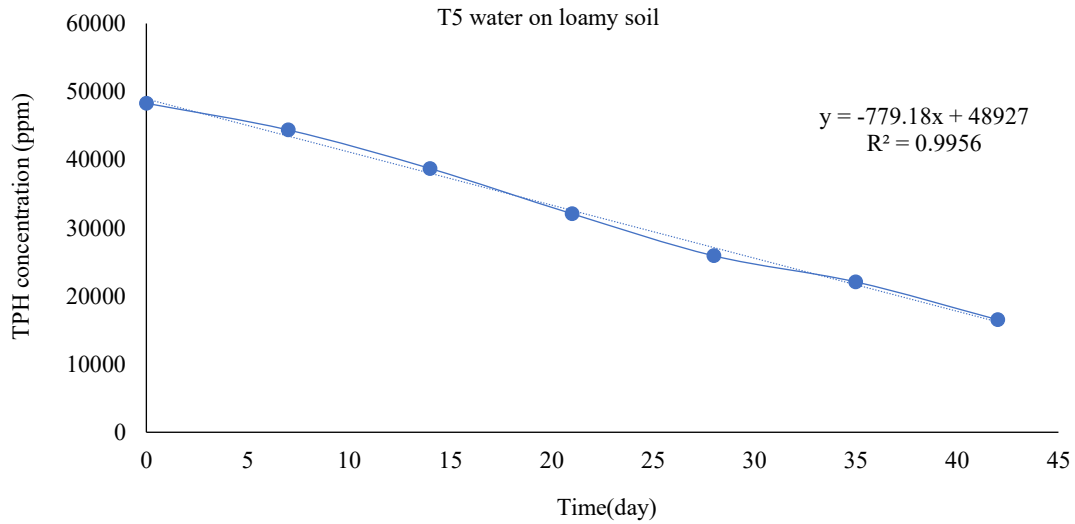


**Figure 4.** TPH concentration versus Time in T5 water in loamy soil.

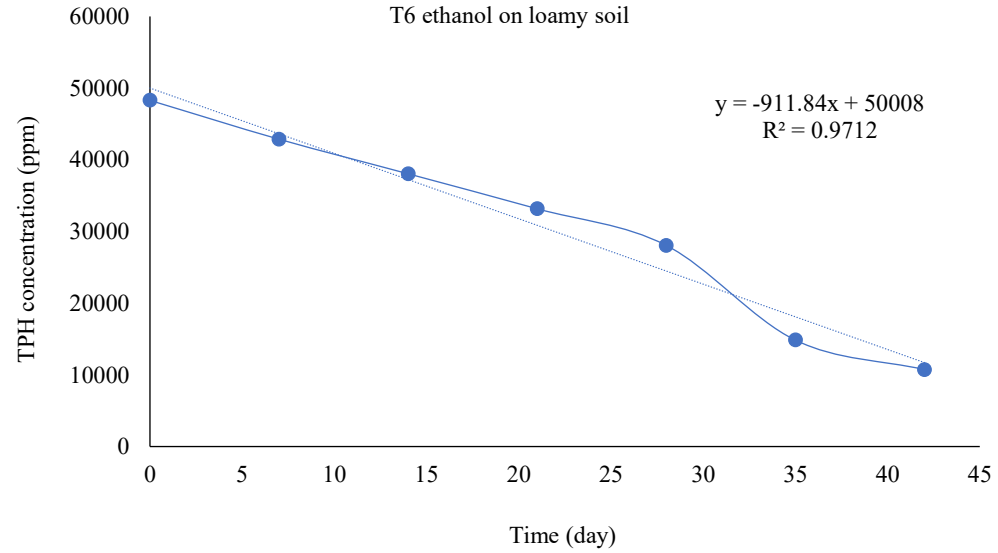


**Figure 5.** TPH concentration versus Time in T6 ethanol in loamy soil.

Figure 5 depicts the reduction in TPH concentration due to nutrient composition of *Bryophylum pinnatum* leaves using ethanol extracts revealed high performance of TPH degradation in bioreactor T6. The maximum specific degradation rate constant values ($V_{max}$) and Michaelis-Menten constant ($K_m$) that was determined using 100 ml of ethanol extracts in bioreactor T6 containing loam soil. The linearized results from the calculated values for $V_{max}$ and $K_m$ showed that $R = \frac{0.2240148 \times S}{1.01 \times 10 + S}$. The equation of the line is expressed as y = -911.84x + 50008 with the square root of best fit $R^2 = 0.9712$ representing 97.12% displaying high reliability curve.

Figure 6 depicts the reduction in TPH concentration due to nutrient composition of *Bryophylum pinnatum* leaves using ethanol revealed high performance of TPH degradation in bioreactor T7. The maximum specific degradation rate constant values ($V_{max}$) and Michaelis-Menten constant ($K_m$) that was determined using 200 ml of ethanol extracts in bioreactor T7 containing loamy soil. The linearized results from the calculated values for $V_{max}$ and $K_m$ showed that $R = \frac{0.2226548 \times S}{1.01 \times 10 + S}$. The equation of the line is expressed as y = -967.56x + 49629 with the square root of best fit $R^2 = 0.983$ representing 98.3% displaying high reliability of the experimental data.

 

The results obtained in Figure 7 reveals the TPH behaviour with increase in Biostimulant in bioreactor T8. Decrease in TPH concentration occurred due to increased rate of biostimulant that enhanced microbial action on soil environment. The linearized equation is given as y = -1025x + 49596 and $R^2$ value 0.9841. This suggests that 98.41%, which is near 1 in T8 has strong relationship with the measured data over time. However, report on high reliability of model on crude oil remediation using plant extract of Guava leaves. The maximum specific degradation rate constant values ($V_{max}$) and Michaelis-Menten constant ($K_m$) that was determined using 300 ml of ethanol extracts in bioreactor T8 containing loam soil had high performance rating of hydrocarbon degradation. The linearized results from the calculated values for $V_{max}$ and $K_m$ showed that $R = \frac{0.2221895 \times S}{1.01 \times 10 + S}$ .

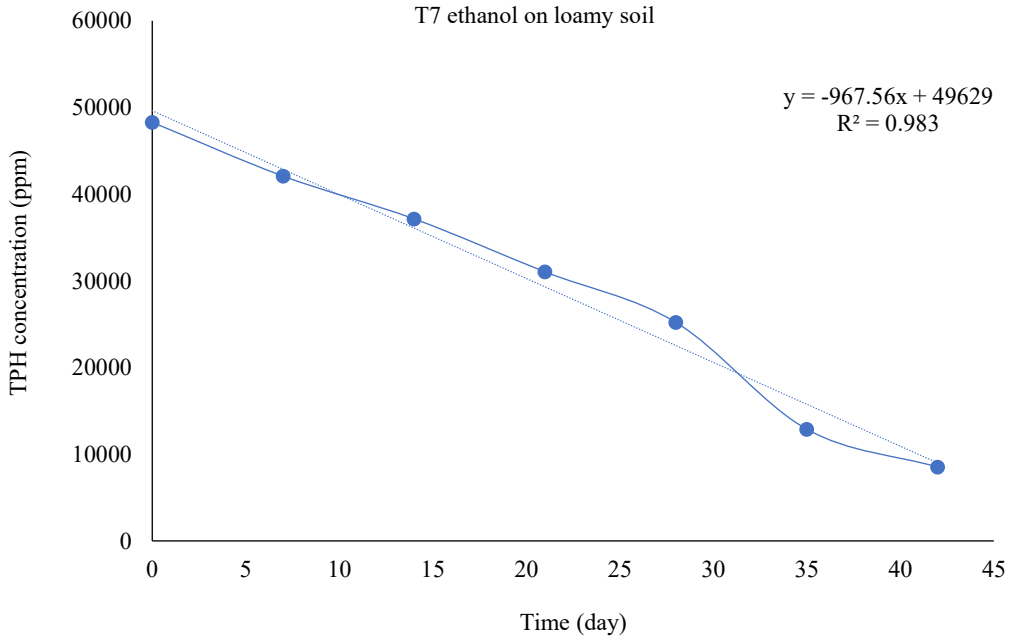


**Figure 6.** TPH concentration versus Time in T7 ethanol in loamy soil.

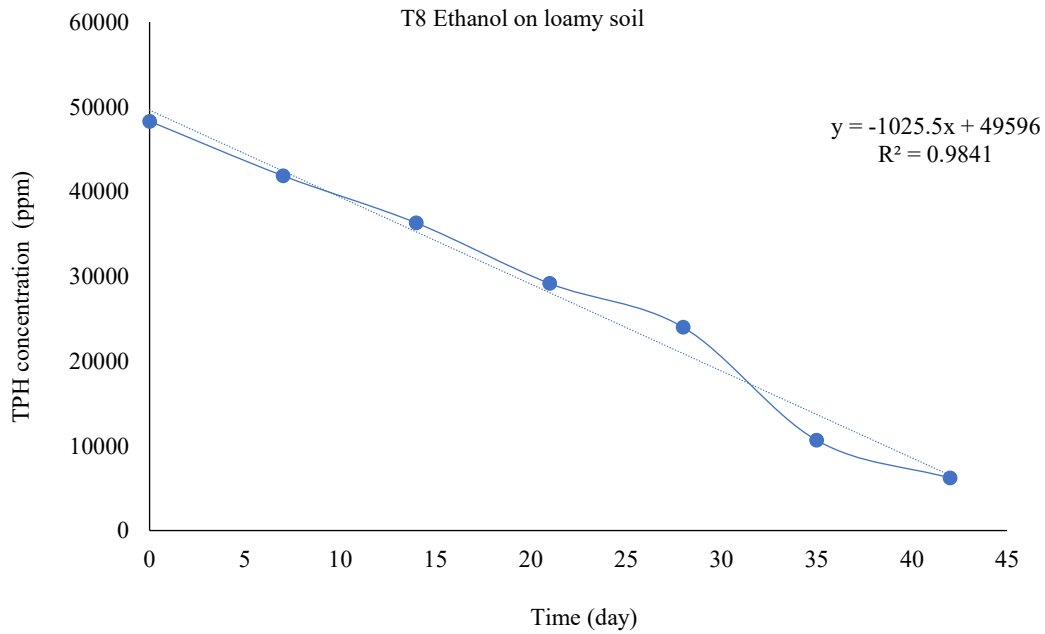


**Figure 7.** TPH concentration versus Time in T8 ethanol in loamy soil.

 

## CONCLUSION

The following conclusions were made:

- This study demonstrates the degradation of TPH concentration using *Bryophylum pinnatum* leaves in fermented water and ethanol biostimulant on crude oil polluted soil.
- The linearized Polymath was effective for prediction of total petroleum hydrocarbon on loamy soil using *Bryophylum pinnatum* leaves in fermented water and ethanol extract.
- The integrity of the plant extracts in this case was only examined in alcohol and water medium as a solvent.
- The kinetic parameters of the Micheal Menten concept were determined as computed based on the obtained data from the plots as demonstrated in this research.

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