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Inferences from TPHs removal kinetics during phyto- and myco-remediation of a soil heavily contaminated with crude oil

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ABSTRACT

Despite existing knowledge gaps such as the specific concentrations to which soils heavily contaminated with total petroleum hydrocarbons (TPHs) should be diluted for effective application of phyto and –myco-remediation agents and how enhancing agents influence TPHs removal rates at specific time intervals; kinetic modelling of TPHs removal during phyto- and myco-remediation can provide insightful inferences with practical implications. Therefore, in this study, soils heavily contaminated with crude oil were collected, and phyto- and myco-remediation carried out, both with and without the addition of Tween 80, over a period of 90 days, accompanied by concurrent soil analysis. The results revealed up to 445 grams of total petroleum hydrocarbons per kilogram of dry soil (g/kg dry soil), representing approximately 50 % TPHs contamination in the soil; and remediation efficiencies of 19, 68, 74, 87, and 88 %, for natural attenuation, sunflower, ferns, fermented palm wine, and the white rot fungus (*Pleurotus ostreatus*), respectively. Optimisation with Tween 80 increased the respective efficiencies to 31, 96, 93, 98 and 95 %. Kinetic modelling of the data revealed that natural attenuation of the highly contaminated soil proceeded predominantly by zero-order kinetics, which explains why natural attenuation is often ineffective for such soils. The phyto- and myco-remediation treatments shifted the removal kinetics from zero- order towards pseudo-first-order (PFO), and pseudo-second-order (PSO). The kinetic modelling, combined with tolerance limits, has been used to project the ideal initial (starting) concentrations for each agent. Thus, over the 90 days, the optimal initial concentrations are as follows: natural attenuation < 5 % of TPHs in soil; sunflower (*Helianthus annuus*) and ferns (*Dryopteris affinis*) < 9 %; fermented palm wine < 18 %; and *Pleurotus ostreatus* < 23 %. Finally, while appropriate dilution is necessary for optimal progression of natural attenuation and most phyto- and myco-remediation treatments, a thorough understanding of tolerance limits and removal kinetics will facilitate better decision-making during phyto- and myco-remediation.

1. Introduction

It is quite challenging to restore soils heavily contaminated with crude oil and its associated total petroleum hydrocarbons (TPHs) has such levels of pollution result in a colloidal mixture that significantly alters soil properties [1,2]. Over the years, efforts to remediate soils contaminated with crude oil have attracted considerable scientific interest, including methods such as phyto- and myco-remediation [3,4].

Phyto- and myco-remediation are considered sustainable methods for managing petroleum-contaminated soils [3,5,6]. However, compared to other conventional approaches, the techniques of phyto- and myco-remediation require a relatively longer period to achieve successful remediation. Over the years, various optimizations have been explored, including the use of surface-active agents such as Tween 80 [7]. Although the enhancing effects of Tween 80 are well documented, the removal kinetics of the process during phyto – and myco-remediation of soils heavily contaminated with total petroleum hydrocarbons (TPHs) remain largely unexplored.

Even with the enhancements, adapting phyto- and myco-remediation agents to soils that are heavily contaminated with crude oil remains a significant challenge. Several factors contribute to this difficulty, including the concentration of crude oil in the soil, and the

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ability of the agents to survive at such high concentrations [8,9]. Since most phyto- and myco-remediation agents do not thrive in soils with very high level of pollutants, the common practice is to dilute heavily contaminated soils to lower concentrations before applying the phyto- and myco-remediation agents [10,11]. This raises the question of the specific concentrations to which the soil should be diluted, for effective application of the agents. Existing studies often rely on trial and error to determine these concentrations. Therefore, there is a pressing need for a more scientific approach to inform this decision-making process.

Kinetic modelling has provided valuable insights into the removal of environmental pollutants [12]. These models have also been employed to elucidate microbial and physiological metabolism of chemical substances in the environment [13–15]. Nemati et al. [16] noted that pollutant kinetic analysis offers enhanced understanding of the removal process, aids in the development of effective remediation strategies, and allows for the estimation of residual pollutant concentrations at any given time. Furthermore, a comprehensive understanding of removal kinetics can provide information on the half-life of pollutants, which is useful for estimating pollutant concentrations at half-life, the rate of removal of the pollutants, the time duration of phyto- and myco-remediation, and how enhancing agents influence the removal rate. This knowledge can facilitate timely monitoring of the process to prevent undesirable outcomes.

Many studies have been conducted on the kinetic modelling of environmental pollutants [16–18]. However, information regarding the kinetic modelling of phyto- and myco-remediation processes for soils heavily contaminated with total petroleum hydrocarbons (TPHs) remains limited. Since these models provide invaluable insights into the mechanisms underlying the removal of chemical substances, it is essential to investigate the kinetic mechanisms associated with the phyto- and myco- remediation of TPHs-contaminated soils, particularly those that are significantly affected by crude oil.

Although kinetic modelling of pollutant removal is invaluable, the procedures for making decisions regarding model fit are not always straightforward. Various criteria for assessing the fit of kinetic models have been proposed, each leading to different inferences [12,19]. Thus, as in other cases, the decision-making process may be complex when considering the kinetics of phyto- and myco-remediation of complex mixtures such as TPHs in soils heavily contaminated with crude oil.

The aim of this study was two-fold:

(1) To assess the tolerance limits and the TPHs remediation efficiency of selected phyto- and myco-remediation agents, both with and without Tween 80, in a soil heavily contaminated with crude oil.
(2) To investigate whether the kinetics of total petroleum hydrocarbons (TPHs) removal can provide valuable insights to improve decision-making during the phyto- and myco-remediation of heavily contaminated soil.

2. Methods

2.1. Soil samples and glasshouse experiment

Petroleum-contaminated soil was collected from Ogale, Ogoniland, Nigeria (0295,428 N, 0533,596 E). Glasshouse experiments were carried out using the methods described by Dickson et al. [4] at the glasshouse facility of Nottingham Trent University, Brackenhurst Campus, United Kingdom (UK). The glasshouse remediation treatments included a control group (natural attenuation), and treatments involving sunflower (*Helianthus annuus* ''pacino gold''), the ferns (*Dryopteris affinis*), fermented palm wine (from *Elaeis guineensis*), and the white rot fungus, *Pleurotus ostreatus* (Table 1). The soil used was a silty loam, collected from Ogoniland, Niger Delta, Nigeria. The selection of this silty loam soil from Ogoniland, Nigeria, for this study was based on its prevalence in the study area and its use by local farmers for crop cultivation [20]. Ogoniland, located in the Niger Delta of Nigeria is well-known for its soils heavily contaminated with crude oil, making it an ideal source of soil samples for this research.

Prior to the commencement of the formal glasshouse experiments, preliminary trials were carried out to determine the level of soil dilution at which the agents would germinate and grow in the soil (that is the tolerance limit for each agent). Based on these findings, the soil samples were amended with cow manure to reduce the concentration of TPHs in the soil, accordingly. This amendment not only diluted the heavily contaminated soils but also provided essential nutrients for the growth of the agents [4]. Each of the treatments was replicated with the addition of the enhancement agent, Tween 80 (Table 1). The soil remediation experiment was conducted over a period of 90 days and monitored at the following time intervals (t): 0, 15, 30, 45, 60, 75, and 90 days. Further details regarding soil collection and glasshouse treatments are available in the Supplementary Material S.1.

At each sampling time (t = 0, 15, 30, 45, 60, 75, and 90 days), composite soil samples were collected from the glasshouse pots, and assessment for TPHs content was carried out to determine the

Table 1
Composition of glasshouse pots for the remediation treatment of the petroleum-contaminated silty loam soil from ogale, Ogoniland, Nigeria.

Soil Groups	Soil treatments	Soil (g)	Cow Manure (g)	Sunflower	Fern	Mushroom Substrate (g)	Mushroom Spawn (g)	Palm Wine (ml)
T1	Natural attenuation (Contaminated soil without any treatment)	**300**	-	-	-	-	-	-
T2	Natural attenuation with Tween 80: (The highly contaminated soil + Tween 80)	**300**	-	-	-	-	-	-
T3	Sunflower alone (Soil + sunflower)	**300**	**50**	**1 Sunflower plant**	-	-	-	-
T4	Sunflower + Tween 80 (Soil + sunflower + Tween 80)	**300**	**50**	**1 Sunflower plant**	-	-	-	-
T5	Ferns alone (Soil + ferns alone)	**300**	**50**	-	**1 fern plant**	-	-	-
T6	Ferns + Tween 80 (Soil + ferns + Tween 80)	**300**	**50**	-	**1 fern plant**	-	-	-
T7	Fermented Palm wine alone (Soil + fermented palm wine alone)	**300**	**50**	-	-	-	-	**250**
T8	Fermented palm wine + Tween 80 (Soil + fermented palm wine + Tween 80)	**300**	**50**	-	-	-	-	**250**
T9	*P. ostreatus* alone (Soil + *P. ostreatus* alone)	**300**	**50**	-	-	**20**	**10**	-
T10	*P. ostreatus* + Tween 80 (Soil + *P. ostreatus* + Tween 80)	**300**	**50**	-	-	**20**	**10**	-

percentage remediation according to methods reported by Dickson et al. [4]. The determination of soil texture and analysis for TPHs levels in the soil samples were also performed as reported in Dickson et al. [4].

2.2. TPHs concentrations, % remediationefficiency,and TPHs removalratesin the treatedsoils

The concentrations of TPHs at the start of the experiment and at various sampling times during the remediation ($t = 0$, 15, 30, 45, 60, 75, and 90 days) were analysed and reported accordingly. From these data, the % remediation efficiency, and the TPHs removal rates in the treated soils were also evaluated (ST1, supplementary material).

2.3. Kinetic modellingof the TPHs removal in the soiltreatments

The concentrations of TPHs at the start of the experiment and at various sampling times during remediation ($t = 0$, 15, 30, 45, 60, 75, and 90 days), were fitted to kinetic models of zero-order, pseudo-first-order (PFO), and pseudo-second-order (PSO) [21,22]. Additional details can be found in the supplementary material (S.2, SF1, ST1).

Several criteria have been recommended for selection of the most suitable model, and this is usually with recourse to statistical analysis, particularly regression, correlation, and error analysis [23,24]. Based on these criteria, the statistical analyses employed in this study for the decision-making included R-squared values (derived from the kinetic modelling and regression analysis), the Pearson correlation coefficient, standard error, and the root mean squared error (RMSE), in addition to a paired two-sample *t*-tests for means. These tests were carried out on pairs of fitting data for each model, such as $[A_t]$ vs T, for zero-order; ln $[A]_0$ vs T, for PFO; and $1/[A]_t$ vs T for PSO. The uniqueness of each data set to fit to the models, as indicated by the shape of the linear plot, and inferences from the statistical analysis were used to determine the associated TPHs removal kinetics for the various treatments (raw data available in ST1, supplementary material).

2.4. Data quality and scope of the study

Each phyto- and myco-remediation experiment, along with the laboratory and instrumental analyses was carried out in triplicate. For the collection of soil samples during the treatments, composite soil samples that had been thoroughly homogenised were used for analysis. Samples were taken on the exact day of remediation evaluation and were prepared and analysed within 24 h to prevent the loss of TPHs due to continuous biodegradation. The statistical analyses performed on the data included paired *t*-tests, regression, correlation, and error analysis.

Generally, for kinetic modelling, several studies have recommended a minimum of at least three measured experimental data points [25–27]. All the modelling conducted in this study made use of 7 measured experimental data points, taken at $t = 0$, 15, 30, 45, 60, 75, and 90 days.

The applicable concentration range for the kinetic models falls within the levels associated with various treatments during the phyto- and myco-remediation of the soil. These concentrations (in grams of TPHs per kilogram dry soil) are as follows: 359 to 445 for T1, 277 to 404 for T2, 84 to 261 for T3, 10 to 241 for T4, 61 to 233 for T5, 13 to 177 for T6, 41 to 312 for T7, 6 to 294 for T8, 28 to 230 for T9, and 9 to 191 for T10.

2.5. Statistical analyses

Statistical analyses in the present study were carried out using MS Excel (2017) with the XLSTAT add-in. XLSTAT is highly regarded by users and analysts for its extensive features and seamless integration with Microsoft Excel. It provides a robust alternative to other statistical software, offering a user-friendly interface that does not require coding [28]. The statistical analyses performed included paired t-tests, regression analysis, Pearson correlation, and error analysis (both standard error and relative mean squared error).

The paired t-test was used to evaluate whether there were significant differences in TPHs concentrations in the soil for each treatment set and time interval. Specifically, the t-test was conducted for each treatment (e.g., T1, T2, etc.) by comparing the initial concentrations (at $t = 0$ days) with concentrations at subsequent time intervals (e.g., $t = 15$, 30, 45 days). This assessment was necessary to determine whether the differences in TPHs concentrations across these intervals were statistically significant. Regression analysis, Pearson correlation, and error analysis were employed alongside R-squared values to identify the best-fitting kinetic model [23,24].

3. Results and discussion

3.1. Results

3.1.1. Soil particle size analysis, and TPHs concentrations, and tolerance limits of the agents

Particle size analysis revealed that the soil from Ogale, Ogoniland, Niger Delta, Nigeria, is silty loam (ST1, in supplementary material). According to USDA soil taxonomy, the soil from the study area can be categorized as Ultisols [29]. There were no significant differences ($p = 1.00$) in the soil textural properties among the soil samples used in the remediation experiments.

The highest concentration of TPHs (445 g/kg dry soil) was recorded in the control soil T1 (untreated petroleum-contaminated soil). The initial concentrations of TPHs in the soils for each treatment, determined from preliminary tolerance limit experiments, were 261 g/kg for the sunflower, 233 g/kg for the fern, and 229 g/kg for P. ostreatus. The fermented palm wine demonstrated the ability to function at a higher initial concentration of 312 g/kg of TPHs in the soil (Fig. 1; additional details are provided in ST1 of the supplementary material).

3.1.2. TPHs removal and remediation efficiency of the agents

The trend in the TPHs removal and the remediation efficiency of the agents were evaluated based on the corresponding treatment period. Here, the treatment periods were categorized into those of the main intervals 0–15 days, 0–30, 0–45, 0–60, 0–75, and 0–90 days, and the sub-intervals 15–30 days, 30–45, 45–60, 60–75, and 75- 90 days. The sub-intervals help to evaluate the remediation progress between each interval, for example, $t = 15$ to $t = 30$, etc.; while the main intervals evaluate the progress between the starting concentrations for each treatment at $t = 0$ and those at the treatment intervals of $t = 15$, 30, 45, 60, 75, and 90 days.

For the main intervals (0–15, 0–30, 0–45, 0–60, 0–75, and 0–90 days), there was a general increase in TPHs removal efficiency with the addition of Tween 80. However, there were instances where the addition of Tween 80 was associated with a decrease in TPHs removal efficiency. Such cases included the treatments T1 and T7 at 0–15 days; and T3 at 0–30 days. After a 60-day interval, all of the treatments (except T1 and T2) had achieved TPHs removal efficiencies greater than 60 %, although only some treatments attained remediation efficiencies of at least 50 % at intervals shorter than 60 days. It was also generally observed that the TPHs removal efficiency of natural attenuation (T1 and T2) increased over time (Fig. 1), although these efficiencies consistently remained below 50 % at any given time.

At the sub-intervals (15–30, 30–45, 45–60, 60–75, and 75–90 days), several notable trends were observed. Although there was a decrease in TPHs remediation efficiency during the interval 0–15 days for natural attenuation (T1 against T2), for the same natural attenuation, an increase was observed at the interval 15–30. However, the decrease in TPHs remediation efficiency for T7, against T8 during the interval 0–15 days remained unchanged during the 15–30 days. After 30 days, there was a general increase in TPHs removal efficiency with the addition of Tween 80 across the sub- intervals. In all cases, the TPHs remediation efficiencies were nearly the same for both the main and sub-intervals

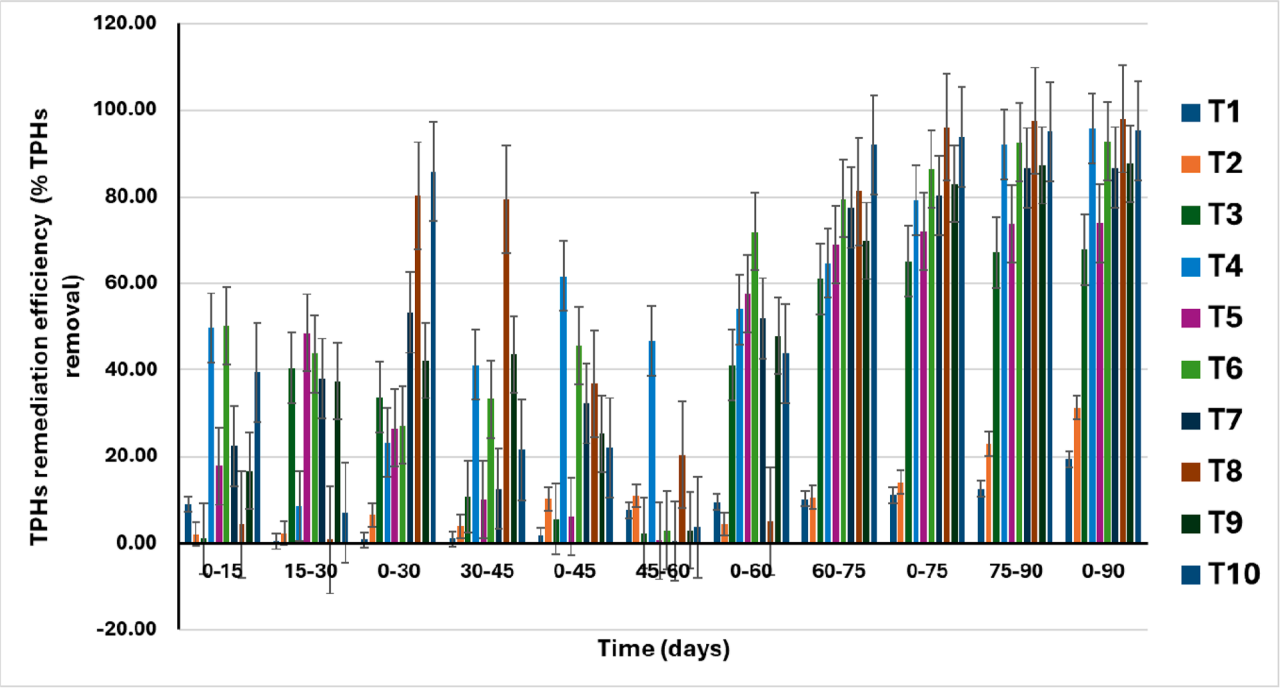

Fig. 1. TPHs remediation efficiency of the Treatments (raw data in supplementary material T1, T2, etc. represent each treatment e.g. T1=natural attenuation without Tween 80, T3 = sunflowers without Tween 80, respectively as already mentioned in Table 1). The vertical bars indicate standard errors.

after the 60-days treatment period. These patterns were also observed in the natural attenuation treatments (T1, T2).

Without Tween 80, the highest mean removal efficiency at 90 days was observed in the soil samples treated with P. ostreatus (T9) with TPHs removal efficiency of 88 %; followed by fermented palm wine (T7), 87 %; the ferns (T5), 74 %; sunflower (T3), 68 %; and lastly natural attenuation (T1), 19 %. On addition of Tween 80, the highest mean removal rate at 90 days was observed for the soil samples treated with fermented palm wine(T8), 98 %; followed by sunflower (T4), 96 %; P. ostreatus (T10), 95 %; the ferns (T6), 93 %; and lastly natural attenuation (T2), 31 %. (Fig. 1, details on ST1 in supplementary material).

The addition of Tween 80 had significantly enhanced impact on the remediation efficiency of the agents, reducing the differences between treatments, such that the treatment with the phyto- and myco-remediation agents were all above 90 %, at 90 days.

Statistical analysis using a paired *t*-test revealed that the concentrations of TPHs in all treatments were significantly different at each treatment interval compared to the levels at the start of the remediation process (Table 2).

3.1.3. *TPHs removal rate during the soil treatments*

The TPHs removal rate, evaluated as changes in the concentration of TPHs over a specified time period, revealed varying rates of TPHs removal for each of the treatments across the different time intervals. For instance, for T10, the TPHs removal rate was up to 5000 mg of TPHs per day at 0–15 days, 545 at 15–30 days, 2800 at 0–30 days, 6200 (at 30–45 days), 3900 (at 0–45 days), 220 (at 45–60 days), 3000 (at 0–60 days), 790 (at 60–75 days), 2400 (at 0–75 days), 23 (at 75–90 days), and 2000 (at 0–90 days). Generally, the highest and lowest TPHs removal rates (6200, and 23 mg of TPHs per day, respectively) for T10, was

Table 2
Statistical analysis (*t*-test) for TPHs concentrations in the soil for each treatment, and time interval. The *t*-test was performed for each treatment by comparing the initial concentrations (e.g. Concentrations at $t = 0$ days against other concentrations at the different time intervals e.g. $t = 0$ days, against $t = 15$, 30, 45, etc. **Note:** If p is <0.05, the differences in the values tested are considered statistically significant.

	Control soil (natural attenuation)		Soil treated with sunflowers		Soil treated with ferns		Soil treated palm wine		Soil treated with P. ostreatus	
Time frame (days)	**T1 P values for *t*-test for the intervals $t = 0$ against other treatment intervals (e.g. $t = 15$, 30, etc. for T1**	**T2 P values for *t*-test for the intervals $t = 0$ against other treatment intervals (e.g. $t = 15$, 30, etc. for T2**	**T3 P values for *t*-test for the intervals $t = 0$ against other treatment intervals (e.g. $t = 15$, 30, etc. for T3**	**T4 P values for *t*-test for the intervals $t = 0$ against other treatment intervals (e.g. $t = 15$, 30, etc. for T4**	**T5 P values for *t*-test for the intervals $t = 0$ against other treatment intervals (e.g. $t = 15$, 30, etc. for T5**	**T6 P values for *t*-test for the intervals $t = 0$ against other treatment intervals (e.g. $t = 15$, 30, etc. for T6**	**T7 P values for *t*-test for the intervals $t = 0$ against other treatment intervals (e.g. $t = 15$, 30, etc. for T7**	**T8 P values for *t*-test for the intervals $t = 0$ against other treatment intervals (e.g. $t = 15$, 30, etc. for T8**	**T9 P values for *t*-test for the intervals $t = 0$ against other treatment intervals (e.g. $t = 15$, 30, etc. for T9**	**T10 P values for *t*-test for the intervals $t = 0$ against other treatment intervals (e.g. $t = 15$, 30, etc. for T10**
$t = 0$ vs $t = 15$	0.0007	0.004328	1.07E-06	5.92E-13	5.92E-10	2.37E-07	3.6E-07	9.55E-09	3.28E-09	1.79E-12
$t = 0$ vs $t = 30$	0.0006	0.002032	0.000311	2.15E-13	6.94E-14	6.61E-12	6.61E-11	9.67E-09	1.83E-10	1.93E-10
$t = 0$ vs $t = 45$	0.0005	9.19E-05	*4.4E-08*	*8.4E-11*	*1.04E-11*	*5.9E-11*	*4.3E-14*	1.52E-11	7.92E-14	2.58E-11
$t = 0$ vs $t = 60$	0.0002	0.000346	4.91E-08	1.32E-13	1.15E-14	2.12E-15	2.05E-11	1.15E-11	6.75E-14	3.11E-08
$t = 0$ vs $t = 75$	0.0005	6.11E-05	7.05E-08	6.03E-14	2.08E-14	1.6E-15	2.47E-08	1.76E-08	3.44E-08	2.7E-08
$t = 0$ vs $t = 90$	0.0004	0.000159	8.1E-13	3.46E-08	1.70E-08	1.89E-15	2.25E-08	1.74E-08	2.33E-11	2.7E-08

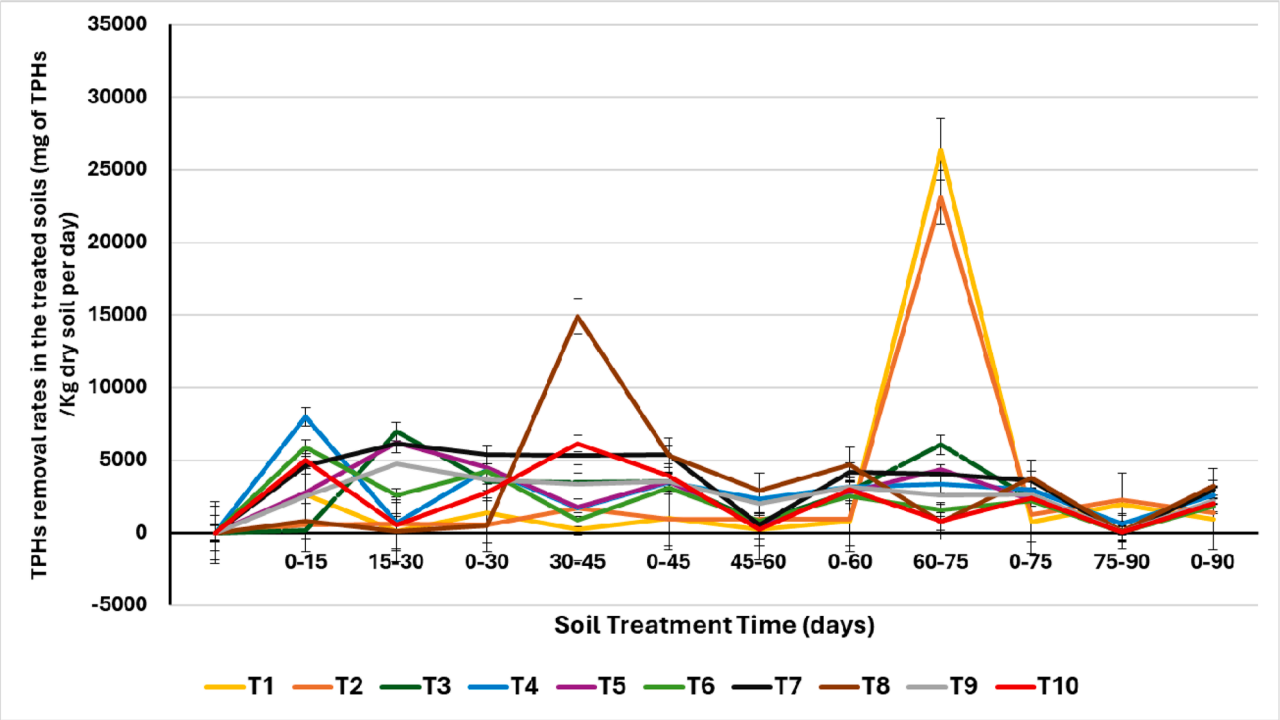

Fig. 2. Rate of TPHs removal in the treated soils (mg of TPHs /Kg dry soil per day). T1, T2, etc. represent each treatment e.g. T1=natural attenuation without Tween 80, T3 = sunflowers without Tween 80, respectively as already mentioned in Table 1.

obtained during the sub-interval, 30–45 days, and 75–90 days, respectively.

The addition of Tween 80 also enhanced the TPHs removal rate; for instance, the increase in the TPHs removal rate curve (Fig. 2) was more pronounced for T10 at most instances, compared to that of T9 where the TPHs removal curve remained relatively flat at most intervals (Fig. 2).

For natural attenuation (T1, T2), the TPHs removal rate remained relatively stable and generally below 2000 mg of TPHs per day, yet peaked up steeply at the interval 0–60 days, 60–75 and 0–75 days to a maximum of 26,000 and 23,000 mg of TPHs per day, respectively, and plunged thereafter to 2300 mg of TPHs per day at 75–90, and falling below 1500 mg of TPHs per day at 90 days (Fig. 2).

3.1.4. Kinetic modelling of the Phyto- and myco-remediation of the soil samples

Kinetic modelling of the phyto- and myco-remediation data established that the control treatments (T1 & T2), progressed predominantly by zero order kinetics (Table 3). The model for T1 exhibited a better zero-order kinetics compared to those of PFO, and PSO (Table 1, S.2, Figure SF1 in supplementary material). However, in T2 (which is T1 optimised with Tween 80), the observations revealed the kinetics of PFO and PSO setting in Therefore, T2 will be of mix kinetics, but zero order will still predominate (Table 3; S.2, Figure SF1 in supplementary material).

T3 and T4 advanced by PFO kinetics (Table 3). Here, it was observed that while the determining parameters (R- squared values from kinetic modelling and regression analysis, Pearson correlation, and error analysis -Standard Error and root mean squared error), were favourable for zero-order and PSO kinetics [30,32], that of PFO was the most feasible. The results indicated that some components may also progress by PSO and zero-order kinetics. Therefore, T3 and T4 will be of mix kinetics, but will predominantly follow PFO. For T5 and T6, the determining parameters proffered a mix of PFO and PSO, which will predominantly progress by PFO, in both cases. The kinetics for T7 revealed a mix of zero, PFO and PSO, that will predominantly advance by PFO, while that of T8 was a mix of zero, PFO and PSO, that will predominantly follow PSO. T9, demonstrated a mix of Zero, PFO and PSO, that will predominantly progress by PFO, while T10, revealed zero order & PSO, that will predominantly follow PSO (Table 3, S.2, Figure SF1 in supplementary material).

Generally, the removal kinetics of the phytoremediation agents (sunflower and fern), progressed by PFO, both with and without the addition of Tween 80, while the treatments involving the mycoremediation agents (fermented palm wine, and *P. ostreatus*) without the addition of Tween 80 followed PFO, but on the addition of Tween 80, progressed to PSO (Table 3).

3.2. Discussion

3.2.1. Soil texture and TPHs concentrations

The silty loam soil demonstrated the capacity to absorb and retain up to 445 g of TPHs per kg of dry soil, representing approximately a 50:50 soil-TPHs mix. These values were significantly higher than the 107 mg/kg reported by Sari & Trihadiningrum [33], but comparable to the 420 g/kg obtained by Kim et al. [34] from petroleum-contaminated soils in China. The data from Kim et al. [34] and the results of this study were obtained from long-term contaminated soils (soils that have been contaminated with crude oil for many years). Therefore, the duration of crude oil contamination can contribute to the retention and accumulation of very high levels of TPHs concentrations in soils. At such high concentrations, the soils would be so soaked, with the contaminant severely impacting plant growth and soil microbial activity. Gupta et al. [35] noted that microbial activity plays a crucial role in the removal of organic pollutants. Bisht & Chauhan [36] and Xiang et al. [37] both linked high concentrations of organic pollutants to increased soil toxicity, disrupted soil aeration, limited nutrient availability, and reduced activities of soil microbes, which hinders natural attenuation. This also explains why most of the treatment agents (plants and fungi) could not thrive and initiate the remediation of TPHs in the soil under such high concentrations, as observed in this study. In the present study, the tolerance limits of the agents in the silty loam soil heavily contaminated with crude oil were as follows: sunflower (*Helianthus annuus*), 261

Table 3
Kinetic modelling of Phyto- and myco-remediation of the soil samples, with statistical analysis.

Soil Treatment	Kinetic models	R- Square values from plotted graphs R- Square Values form plotted graphs	R- Square values from regression analysis			Pearson Correlation (PC)	Standard Error (SE)	Relative mean squared error (RMSE)**	Comments * R-square values greater than 0.8 are considered indicative of a good fit [30,31] ** The SE values indicate a measure of precision, while the RMSE values are a measure of the accuracy of each model [32]
			R Square	Multiple R	Adjusted R Square				
T1	**Zero Order**	**0.8138**	**0.8138**	**0.9021**	**0.7765**	**−0.8631**	**15.3174**	**0.3693**	The R-squared values for zero-order kinetics are the highest, same with the other statistical analysis parameters. R-squared values for PFO and PSO are below the 0.8 threshold* and are therefore not considered a good fit. In this case, the R-squared value alone is sufficient to determine the best removal kinetic model, as the other models do not meet the 0.8 threshold. Also, the RMSE values for the zero-order kinetics revealed good accuracy, while the SE is lowest for the zero order. PC also favours the zero-order kinetics. Thus, the removal kinetics here, is deemed to predominantly follow zer-order**.**
	PFO	0.7172	0.7172	0.8469	0.6465	−0.9730	16.6853	0.0018	
	PSO	0.7202	0.7202	0.8487	0.6503	−0.8694	16.5956	0.1370	
T2	**Zero Order**	**0.9442**	**0.9442**	**0.9717**	**0.9331**	**0.9812**	**8.3825**	**0.0274**	The R-squared values for zero order kinetics are the highest here; however, the R-squared values for the PFO & PSO models are above the 0.8 threshold and thus also considered a good fit. Again, SE & RMSE values for the zero-order kinetics reveal good precision and accuracy. Here the R-squared and PC values are sufficient to determine the most appropriate removal kinetic model. Thus, the removal kinetics are considered to be of mix ed, but will predominantly follow zero order**.** The significant increase in the R-squared values for PFO and PSO in T2 signifies that the kinetics progressively deviate from the zero order in T1, upon the application of Tween 80.
	PFO	0.9275	0.9275	0.9631	0.9094	0.9736	8.4454	0.0019	
	PSO	0.9213	0.9213	0.9599	0.9017	0.9733	8.8002	0.1479	
T3	Zero Order	0.8216	0.8216	0.9064	0.7859	0.8487	14.9940	0.2731	Although the R-squared values of the zero-order kinetics and PSO are above the 0.8 threshold, that of PFO is the highest. Again, the fact that the rate of zero-order kinetics is comparatively slower than the higher orders, makes the higher order kinetics (PFO, PSO) to have preference over zero-order, when all of such satisfy the 0.8 threshold. Also, the SE, RMSE & PC values favour PFO than the zero-order and PSO. Here the significance of the R-squared values for both zero & PSO is acknowledged. Thus, the removal kinetics will be a mix of zero, PFO and PSO, but will predominantly follow **PFO.**
	PFO	**0.9126**	**0.9126**	**0.9553**	**0.8907**	**0.9336**	**9.2770**	**0.1709**	
	PSO	0.8769	0.8769	0.9364	0.8461	0.9064	11.0088	0.1500	
T4	Zero Order	0.8795	0.8795	0.9378	0.8554	−0.9865	29,986.2331	2.5062	The R-squared values for zero-order and PFO are all above the 0.8 threshold, unlike that of PSO. The highest R-squared value of the PFO gives PFO a preference here. Furthermore, the PC, SE and RMSE values also favour PFO. Thus, the removal kinetics will be a mix of zero, and PFO, but will predominantly follow **PFO.**
	PFO	**0.9218**	**0.9218**	**0.9601**	**0.9022**	**−0.9340**	**8.7756**	**0.0196**	
	PSO	0.7679	0.7679	0.8763	0.7098	−0.9186	15.1170	0.5786	
T5	Zero Order	0.7798	0.7798	0.8830	0.7357	0.8115	16.6585	0.2755	The R-squared values for **PFO** is the highest here. Again, PC value favours PFO. Here the significance of the R-squared values for PFO & PSO is acknowledged. Thus, the removal kinetics will be a mix of PFO and PSO but will predominantly follow **PFO.**
	PFO	**0.9021**	**0.9021**	**0.9498**	**0.8776**	**0.9103**	**9.8176**	0.0142	
	PSO	0.8503	0.8503	0.9221	0.8129	0.9165	12.1388	0.2537	
T6	Zero Order	0.7661	0.7661	0.8753	0.7193	0.9307	17.1684	0.5403	The R-squared values, PC and other statistics favour **PFO,** yet the R-squared values for PSO is also above the 0.8
	PFO	**0.9863**	**0.9863**	**0.9931**	**0.9829**	**0.9943**	**3.6688**	**0.0092**	

(*continued on next page*)

Table 3 (*continued*)

Soil Treatment	Kinetic models	R- Square values from plotted graphs R- Square Values form plotted graphs	R- Square values from regression analysis			Pearson Correlation (PC)	Standard Error (SE)	Relative mean squared error (RMSE)**	Comments * R-square values greater than 0.8 are considered indicative of a good fit [30,31] ** The SE values indicate a measure of precision, while the RMSE values are a measure of the accuracy of each model [32]
			R Square	Multiple R	Adjusted R Square				
	PSO	0.9289	0.9289	0.9638	0.9111	0.9614	8.3683	3.2790	threshold. Thus, the removal kinetics will be a mix of PFO and PSO but will predominantly follow **PFO.**
T7	Zero Order	0.8638	0.8638	0.9294	0.8366	0.8949	13.0984	0.3575	The R-squared values for zero-order, PFO and PSO are all above the 0.8 threshold.
	PFO	**0.9705**	**0.9705**	**0.9851**	**0.9631**	**0.9772**	**5.3890**	**0.0109**	The highest R-squared value of the PFO gives PFO a preference here. Furthermore, the PC SE and RMSE values favour PFO. Thus, the removal kinetics will be a mix of zero, PFO and PSO, but will predominantly follow **PFO.**
	PSO	0.9571	0.9571	0.9783	0.9463	0.9643	6.5004	0.1095	
T8	Zero Order	0.8260	0.8260	0.9089	0.7912	0.8814	14.8063	0.5531	The R-squared values for zero-order, PFO and PSO are all above the 0.8 threshold.
	PFO	0.8784	0.8784	0.9372	0.8480	0.9656	10.9423	0.0486	The highest R-squared value of the PSO, the PC, SE and RMSE all give PSO a preference here. Here the significance of the R-squared values for zero, & PFO is acknowledged. Thus, the removal kinetics will be a mix of zero, PFO and PSO, but will predominantly follow **PSO.**
	PSO	**0.9195**	**0.9195**	**0.9589**	**0.8993**	**0.9857**	**8.9037**	**0.4849**	
T9	Zero Order	0.9010	0.9010	0.9492	0.8812	0.9207	11.1708	0.3071	The R-squared values for zero-order, PFO and PSO are all above the 0.8 threshold.
	PFO	**0.9826**	**0.9826**	**0.9912**	**0.9782**	**0.9955**	**4.1444**	**0.0089**	The highest R-squared value of the PFO gives PFO a preference here. Furthermore, the PC SE and RMSE values favour PFO. Thus, the removal kinetics will be a mix of zero, PFO and PSO, but will predominantly follow **PFO.**
	PSO	0.9597	0.9597	0.9796	0.9496	0.9754	6.3023	0.1836	
T10	Zero Order	0.8201	0.8201	0.9056	0.7841	0.8566	15.0567	0.4855	The R-squared values for **PSO** is the highest, and the R-squared values for both zero order & PSO are quite above or within the 0.8 threshold. Again, the PC values favour PSO. Thus, the removal kinetics will predominantly follow **PSO.**
	PFO	0.8975	0.8975	0.9474	0.8719	0.9246	10.0427	0.0372	
	PSO	**0.9217**	**0.9217**	**0.9600**	**0.9021**	**0.9394**	**8.7799**	**0.4011**	

g of TPHs per kg dry soil; fern (*Dryopteris affinis*), 233 g of TPHs per kg dry soil; fermented palm wine (from *Elaeis guineensis*), 312 g of TPHs per kg dry soil; and the white rot fungus (*Pleurotus ostreatus*), 229 g of TPHs per kg dry soil.

As previously discussed, most studies employed trial and error to determine the initial concentrations to which heavily contaminated soil samples are diluted before phyto- and myco-remediation. In this study, the first approach was to establish the tolerance limits of each of the treatments. This approach is logical because if the agents can germinate and grow effectively at certain concentrations, then such will be capable of initiating remediation effectively. Therefore, rather than randomly diluting such soils, the tolerance limits of the agents can serve as a determining factor for the initial concentration to which heavily contaminated soil should be diluted, during a phyto-and myco-remediation process.

Thus, based on these findings, the following principles have been established:

(1) (1). A phyto– or myco-remediation agent must first be able to survive and adapt to its environment before it can initiate the remediation process.
(2) (2). The maximum concentration at which an agent can survive is crucial, and identifying this threshold is a significant step during phyto – or myco-remediation.

3.2.2. *TPHs remediation efficiency and removal rate of the agents*

The pattern of TPHs removal and remediation efficiency revealed that each agent possesses a unique mechanism that contributes to its overall effectiveness in the remediation. This observation corroborates the findings of Zhang et al. [38], who suggested that different plants utilise various mechanisms—such as phytotransformation, rhizosphere bioremediation, phytostabilization, phytoextraction, phytodegradation, or rhizofiltration—for the removal of environmental pollutants. According to Terek et al. [39], such mechanisms are related to the agents' physiological adaptations, including various biochemical and morphological responses, the development of a robust root system, changes in phytohormonal balance, stimulated increases in abscisic acid levels, alterations in the quantity and quality of root exudates, and the activation of antioxidant enzymes such as catalase and peroxidase. Other mechanisms associated with TPHs remediation efficiency by plants include changes in the flux of carbon to the root, enhancement of mineralization at the root–soil interface [40], as well as modifications in the associated soil and rhizosphere microflora [41,42].

Soil attenuation is facilitated by soil microflora in conjunction with various other factors [43]. However, heavily contaminated soils often lack these functional microflorae, which diminishes its efficiency in remediating soil pollutants. This phenomenon explains why the heavily contaminated soil exhibited low remediation efficiency during natural attenuation.

Each of the phyto- and myco-remediation agents demonstrated potential for total petroleum hydrocarbons (TPHs) remediation, achieving an efficiency of at least 40 % at 60 days, and at least 60 % at 90 days (Fig. 1, Table ST2 in the supplementary material). On enhancements with Tween 80, all the agents exhibited a significant increase in the remediation efficiency at 60 days, with even greater improvements observed at 90 days. In contrast, the efficiency of the natural attenuation treatment remained low, even with the addition of Tween 80. This observation suggests that there is a greater potential to enhance the efficiency of agents that already possess remediation capabilities. Notably, at the 90-day mark, the addition of Tween 80 increased the remediation efficiency of natural attenuation by 12 %, the sunflower by 28 %, the fern by 19 %, fermented palm wine by 11 %, and that of *P. ostreatus* by 8 %. Most importantly, the addition of Tween 80 to all the agents (except natural attenuation), effectively reduced the time required to achieve a 90 % TPHs removal efficiency to <90 days (Table ST1 in supplementary material). The progressive increase in TPHs remediation efficiency of the agents on the addition of Tween 80 is consistent with the findings of other researchers, including Agnello [44], Almansoory, *et al.* [45], and Liao *et al.* [46].

The time-dependent removal efficiency of the agents is further illustrated by the varying TPHs removal rates associated with the different intervals during the process. The TPHs removal rates demonstrated that each time period during the treatment period is crucial and should be closely monitored in detail to evaluate the various chemical substances, including the different classes of hydrocarbons and their intermediate products, removed during the process. In contrast to most studies on TPHs removal, which primarily focus on the overall efficiency of the process, the results of this study demonstrate that the TPHs removal rates vary throughout the process, and there are instances where the removal rates are significantly high, as well as instances where the removal rate are notably low. A thorough understanding of these rates will facilitate the implementation of enhancement strategies, by specifically identifying conditions that can accelerate the process during periods of low removal rates, thereby improving overall efficiency.

3.2.3. Deductions from the TPHs removal kinetic modelling

The treatments, T1 and T2, served as controls; therefore, the process followed natural attenuation. Panchenko et al. [47] reported that natural attenuation took over 5 years to achieve the same remediation efficiency as the phytoremediation agents in their study. In this study, only 19 % of TPHs removal was achieved by natural attenuation, compared to the other agents. The associated zero-order kinetics, which predominantly characterise the natural attenuation treatments (T1 and T2), explain why it takes a significantly longer period for natural attenuation to be effective in such heavily contaminated soil.

Studies such as Udo et al. [48] and Zoghi and Mafigholami [49] have reported a first-order removal kinetics for TPHs in contaminated soils. The study by Zoghi and Mafigholami [49], however, employed soil washing and 7 data points (collected at 0, 10, 15, 20, 40, 50 min of the washing contact time) in the kinetic modelling. During the study, it was also observed that optimisation of the soil washing technique resulted in an increased overall TPHs removal rate, occasioned by an increased rate constant (k). Falciglia et al. [12] demonstrated kinetic modelling during the remediation of petroleum hydrocarbon-contaminated marine sediments by thermal desorption, using both 3 and 4 data points. A similar report have also been presented by Zahed et al. [50], where first-order kinetics described the biodegradation of crude oil. In the present study, 7 data points at intervals of 0, 15, 30, 45, 60, 75, and 90 days were used for the phyto- and myco-remediation treatments. The similarity in the removal kinetics of first order and the increase in the rate constant (k), on optimisation of the phytoremediation agents in the present study corroborate the findings of Zoghi and Mafigholami [49] and other literatures, and demonstrates that the modelling with the experimental data is consistent.

The TPHs removal kinetics in this study for the phytoremediation agents progressed by first- order kinetics, and on optimisation with Tween 80, the kinetics was still by first-order kinetics. However, there was a significant increase in the rate constants of the optimised process (with Tween 80) compared to the unoptimized process (Figure SF1, Table ST3 in supplementary material). PFO kinetics of phytoremediation have been previously reported [51] and appear to be the predominant kinetic mechanism during phytoremediation. Therefore, the kinetic modelling results obtained in this study are consistent with these findings.

This study has hereby, also, establish that TPHs removal kinetics of the mycoremediation agents in the heavily contaminated soil, proceeds by PFO, and with optimisation can proceed to PSO. According to Srinivasan [52], the rate constant of a reaction provides a measure of the speed. Additionally, for second-order kinetics, the rate is exponentially related to the concentration [53]. Therefore, the relatively higher rate constants of the kinetics associated with the mycoremediation agents and PSO kinetics further invariably explains why the mycoremediation of TPHs contaminated soils was achieved within a comparatively shorter time frame.

3.2.4. Practical implications of the TPHs removal kinetics

3.2.4.1. Inferences from the zero-order kinetics of the heavily contaminated soil. The integrated rate law for zero-order kinetics is expressed in Eq. (1) [54].

$$[A] = [A]_0 - kt \quad \text{..... (1)}$$

Where

[A] = The concentration of total petroleum hydrocarbons (TPHs) at any given time (t)

$\mathbf{[A]_0}$ = Initial TPHs concentration at $t = 0$

$\boldsymbol{K}$ = zero-order rate constant

$\boldsymbol{t}$ = time

From Eq. (1), the concentration at any given time during the removal process is directly proportional to the initial concentration. This implies that if the initial concentrations of the TPHs are high, then the corresponding concentration at any stage of the process will also remain elevated, which is consistent with the findings of this study. Furthermore, in a zero-order process, an increase in the initial reactant concentration will extend the half-life of the process (Eq. (2)). Consequently, it will take a significantly longer time for half of the original TPHs concentration to be removed [55].

The half-life of Zero-order kinetics is expressed as:

$$t_{1/2} = [A]_0/2k \quad \text{..... (2)}$$

From the above, both the concentration at any given time and $t_{1/2}$ are directly proportional to the initial concentration. This explains why it will take a significantly longer time for high concentrations of TPHs in soils to be removed through natural attenuation. The removal process will be further exacerbated by the fact that such high concentrations are associated with soil toxicity, which adversely affects the activity of soil microbes and other agents, thereby contributing to the delayed remediation.

Considering the concentration of the TPHs in the untreated silty loamy soil (445 g/kg), the estimated time for half of the concentrations to be removed will be

$$t_{1/2} = [445{,}189]/(2k)$$

$\boldsymbol{k} = \mathbf{707.9}$ (ST3, Supplementary material)

Therefore, it will take at least 314 days for half the original TPHs concentrations in the soil to be removed through natural attenuation, and the process may take longer in real life scenarios with recurring pollution. Given the fact that the concentration at $\mathbf{t_{1/2}}$ is 50 % of the original concentration, this will amount to about (445/2 g/kg), or 223 g

Table 4
Limiting concentrations for the effective removal of TPHs in the soil by the agents (Details on Table ST4 in the supplementary material).

Soil treatment	Agents used in the soil treatments	Recommended upper limits of TPHs concentration in the soil (mg/Kg) for optimal remediation efficiency with the agents	Recommended upper limits of TPHs concentration in the soil (in %) for optimal remediation efficiency with the agents	Effect of further soil dilution (below the initial starting concentration, or tolerance limit of the agents), on TPHs remediation efficiency of the agents
T1	Natural attenuation	44,519 - 89,031	5–9 %	Further dilutions, down to the 5 % TPHs concentrations in the soil will lead to enhance efficiency of the Natural attenuation.
T2	Natural attenuation with Tween 80	133,552	13 %	Further dilutions, down to at least 13 % TPHs concentrations in the soil will lead to enhance efficiency of the Natural attenuation.
T3	Sunflower alone	44,519–89,031	5–9 %	Further dilutions, down to the range of 5–9 % TPHs concentrations in the soil will lead to enhance efficiency of the Sunflower.
T4	Sunflower + Tween 80	133,552	13 %	Further dilutions, down to at least 13 % TPHs concentrations in the soil will lead to enhance efficiency of the Treatment (Sunflower + Tween 80).
T5	Ferns alone	44,519–89,031	5–9 %	Further dilutions, down to the range of 5–9 % TPHs concentrations in the soil will lead to enhance efficiency of the Fern.
T6	Ferns + Tween 80	178,071	18 %	No further dilutions, will be required for this treatment (Ferns + Tween 80)
T7	Fermented palm wine alone	178,071	18 %	Further dilutions, down to the at least 18 % TPHs concentrations in the soil will lead to enhance efficiency of the Fermented Palm wine.
T8	Fermented palm wine + Tween 80	293,794	29 %	No further dilutions, will be required for this treatment (Fermented Palm + Tween 80)
T9	*P. ostreatus* alone	229,461	23 %	No further dilutions will be required for the treatment with P. ostreatus.
T10	*P. ostreatus* + Tween 80	191,336	19 %	No further dilutions, will be required for this treatment (P. ostreatus + Tween 80)

of TPHs per kg dry soil.

The values obtained for the tolerance limits of the phyto- and myco-remediation agents (sunflower, ferns, and *P. ostreatus*) in this study were in the range of 221 to 261 g/kg. Therefore, the half-life concentration of the TPHs in the soils (223 g/kg), lies within the tolerance limits of the agents. This implies that an understanding of the removal kinetics can also assist in estimating the initial concentrations to which soil samples heavily contaminated with TPHs should be diluted for optimal application of remediation agents. Since the half-life concentration (50 % of the initial concentration) of the soil in this study corresponds approximately to the tolerance limits of the agents, the ideal recommended starting up (initial) TPHs concentrations in the soil samples should be below the 50 % of the TPHs concentration in the soil. This information, combined with the kinetics associated with the different remediation agents, will be utilised in the section that follows, to project the ideal initial concentrations of the TPHs in the soil for optimal remediation efficiency of each agent.

3.2.4.2. Projection of TPHs removal efficiency of the agents based on the below 50 % of the initial TPHs concentrations of the heavily contaminated soil. Based on the inferences drawn from the zero-order kinetics of the highly contaminated soil and the estimated time required to reach the soil TPHs threshold level of 10,000 mg/Kg [56], the ideal initial (starting) concentrations for the various agents have been projected for the various below 50 % concentrations of TPHs in the soil, using the removal kinetics associated with each agents. Specifically, 40, 30, 20 and 10 % of the initial TPHs concentration of T1 (445 g/kg), in the contaminated soil were considered. These resulting concentrations were fitted into the various kinetic models of the PFO and PSO associated with each of the treatments, (Tables ST4, ST5 in the supplementary material), and further deductions were made (section S.3, in supplementary material). The projections revealed that the ideal initial (starting) concentrations of the TPHs for optimal phyto- and myco-remediation process in

the heavily contaminated silty loamy soil can be as low as 5 %, depending on the agents (Table 4).

3.2.4.3. Effect of soil dilution on TPHs remediation efficiency of the agents. From the analysis, a comparison of the natural attenuation at high concentrations, with those at the various concentrations below the 50 % of the initial TPHs concentration in the contaminated soil (T1) (ST4 in supplementary material), established that natural attenuation will progress more effectively at much lower concentrations of the TPHs in the soil. The general observation is that further dilution of the soil worked best for natural attenuation, with and without the addition of Tween 80 (Table ST4 in the supplementary material).

Similar procedures and projections have been used to project the ideal upper limits or limiting TPHs concentrations in the soil for optimal efficiency of the different agents (Table 3; details in Table S.3 in the supplementary material).

From this, it is established that, over the 90-day treatment period, further dilutions will favour the soil treatments T1 and T2 (natural attenuation), T3 and T4 (sunflower both without and with Tween 80), T5 (fern without Tween 80), and T7 (fermented palm wine). Conversely, the treatments T6 (fern with Tween 80), T8 (fermented palm wine without Tween 80), and T9 and T10 (*P. ostreatus* both without and with Tween 80), will not require further dilutions.

3.3. Scope and wider implication of the study

It is important to note that the group composition of the total petroleum hydrocarbons (TPHs) in the contaminated soil evaluated in the present study were those of the gasoline-diesel range (C6 to C12, and C8 to C26), consisting of the gasoline range and the diesel range organics [57,58], and also those of the Mineral Oils in the range of C15 to C50 [57]. Both the gasoline-range and the diesel-range organics in petroleum hydrocarbons have been implicated in human health risks, including short-term exposure effects such as fatigue, headache, nausea, and drowsiness, and long-term effects such as permanent damage to the central nervous [57,59].

The N—Hexane found within this range has been reported to cause a nerve disorder called peripheral neuropathy, characterised by numbness in the feet and legs and, in severe cases, paralysis [60]. Other compounds, such as benzene, toluene, and xylene, which are found in the gasoline range, can affect the human central nervous system and may lead to death at sufficiently concentrations [57]. Additional effects of TPHs components in the gasoline-diesel range and the mineral oil organics include impacts on the nervous system (headaches, dizziness, and peripheral neuropathy), Blood (leukaemia and other haematologic neoplasms), damage to the liver and kidneys, irritation of the skin and eyes, gastritis, changes in semen quality, and elevated levels of serum creatinine [61]. Compounds such as benzene, benzo[a]pyrene, and gasoline have also been reported as carcinogenic or probably carcinogenic [62].

Therefore, the successful removal of TPHs in the gasoline-diesel range organics and mineral oils from petroleum-contaminated soils during the treatment also demonstrates the broad applicability of these phyto- and mycoremediation agents, particularly in reducing the associated health risks. However, further studies are required to profile the individual compounds within the TPHs gasoline-diesel range at every stage of the phyto- and myco-remediation process. Although Patterson et al. [63] had reported that petroleum biodegradation metabolite mixtures are less harmless, additional research is necessary to evaluate the toxicological profiles of the remediation by-products generated during the phyto- and myco-remediation.

4. Conclusion

The silty loamy soil exhibited a significant capacity to absorb and retain large amounts of crude oil, resulting in approximately a 50:50 ratios, with 50 % of the soil volume occupied by the contaminating crude oil. At such high concentrations, soil health will be impaired; and the expected ecosystem functions, disrupted. This study revealed that such high concentrations of crude oil in the soil is dominated with zero-order TPHs removal mechanisms, which explains why the natural attenuation process requires a longer duration for effective remediation. The phyto- and myco-remediation agents -sunflower, ferns, palm wine, and P. ostreatus - all demonstrated significant efficacy in remediating the TPHs in the soil, both with and without the addition of Tween 80. The remediation efficiency of these agents was accompanied by a progressive shift in the removal kinetics, progressively from zero-order towards PFO, and PSO.

It has also been revealed that the initial concentrations of the TPHs in the soil are a limiting factor in the application of phyto- and myco-remediation. Therefore, the starting concentrations should be such that supports both the survival and optimal performance of the remediation agents. This study has demonstrated that a better insight of the ideal starting concentrations for the application of phyto- and myco-remediation agents, can be derived from knowledge of both the tolerance limits of the agents and the associated removal kinetics.

The TPHs removal kinetics revealed that mixed kinetic mechanisms operate among the agents during the phyto- and myco-remediation of TPHs in soil. These kinetic models have been used to determine the effective initial concentration to which heavily contaminated soil can be diluted for the optimal application of the phyto- and myco-remediation agents. Thus, for a 90-day remediation period, the initial concentration limits for optimal remediation of the agents in the silty loamy soil were as follows: natural attenuation, $\leq$ 5 % of TPHs in soil, sunflower (*Helianthus annuus*) $\leq$ 9 %, ferns (*D. affinis*) $\leq$ 9 %, fermented palm wine $\leq$ 18 %, and *P. ostreatus* $\leq$ 23 %. Similar projections can be derived for other remediation time frames e.g. 60 days, 120 days, and so forth.

CRediT authorship contribution statement

Udeme John Dickson: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ferdinand Giadom:** Visualization, Validation, Supervision, Resources, Project administration. **Robert John George Mortimer:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Marcello Di Bonito:** Writing – review & editing, Validation, Supervision, Resources. **Nicholas Ray:** Writing – review & editing, Visualization, Supervision, Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nexres.2026.101427.

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Further reading

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