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AMELIORATION OF DIFFERENT SOIL TYPES CONTAMINATION WITH SPENT HYDROCARBON

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Abstract
A simulated bioremediation study was carried out to evaluate the biodegradation of hydrocarbon fractions in soil contaminated with used lubricating oil using microbial consortium (Amnite P1300) under laboratory conditions for 90 days. Artificial contaminated soil types (clayey S1 and sandy S2) at a loading rate of 30,000 and 45,000 mg/kg in a 300 g modeled with used lubricating oil that was amended with commercially available hydrocarbon-degrading microbial consortium: contaminated soil samples were amended with commercially available hydrocarbon-degrading microbial consortium: Amnite P1300 as the bioaugmentation (T1), other treatments consist of nutrients amendments - (NH4)2SO4 and K2HPO4 (NPK) as biostimulation (T2), unamended soil - natural attenuation as (T3) and the control soil treated with sodium azide (NaN3) as (T4). These were evaluated on the microbial community and the degradation level of this used lubricating oil after contamination. Replicated three microcosms flasks per treatment were incubated, and the performance of each treatment was examined by monitoring the biodegradation of aliphatic and polycyclic hydrocarbons present in the used lubricating oil. After 90 days of incubation, Naphthalene, Acenaphthylene, and Acenaphthene were depleted in all the treatments. The control samples in both soils and the contamination levels were observed below the detection limit of 0.5 mg/kg, thus indicating abiotic removal, due to volatilization. It was observed that the shorter-chain aliphatic hydrocarbons are more degraded than the longer-chain aliphatic hydrocarbons in both soils.

Keywords: *Biodegradation, hydrocarbon fractions, Polycyclic Aromatic Hydrocarbons, Aliphatic hydrocarbons, soil types, used lubricating oil*

Introduction

The rise in consumption of automotive lubricating oil is a worldwide problem and has increased the volume of waste. In most of the developing countries, the problems tend to worsen with economic and population growth, rapid industrialization, and disregard for environmental guideline ethics, particularly with engine oil. The main component of the base oil is cyclic alkanes (c-alkanes). Long-chain hydrocarbon and c-alkanes are known to be recalcitrant to microbial degradation. The engine oil contains C16-C36 hydrocarbon and more than 75 percent c-alkanes. The ring numbers of c-alkanes in the base oil are from 1 to 3 and each ring contains 5 or 6 members. Most of the c-alkanes in the base oil have long alkyl side chains (Zhang et al., 2024). After a period of usage of engine oil, it contains more metals and heavy polycyclic aromatic hydrocarbons (PAHs), that is used to lubricate parts of an automobile engine, to smoothen engine operations (Lucas et al., 2024), which could lead to chronic hazards including mutagenicity and carcinogenicity (Opinath et al., 2024). Prolonged exposure and high oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow, and an increased risk of cancer (Saikat et al., 2022). In addition, PAHs have a widespread occurrence in various ecosystems that contribute to the persistence of these compounds in the environment (Gopinath et al., 2024). The damage caused by petroleum hydrocarbon contamination will depend on the type and concentration of the contaminants. It has generally been accepted that the susceptibility of hydrocarbons to microbial attack increases in the following order: cyclic alkanes < low-molecular

weight aromatics < branched alkanes < n-alkanes (George et al., 2020).

The used engine oil is hardly degraded by microorganisms in nature, the introduction of cultured microorganisms is encouraged because the degradation of long-chain hydrocarbons, which are solid at temperatures less than 10 °C, is hindered by their limited bioavailability (Pietro et al., 2024). In addition, the recalcitrance of hydrocarbons, especially the aliphatic and aromatic fractions, inhibits the proliferation of microorganisms by minor ingredients in used engine oil. The vulnerability of these hydrocarbons to microbial degradation varies with the quality of the hydrocarbon molecule. For instance, aliphatic of intermediate chain length (C10–C24) are rapidly and easily degraded, while branched and very long chain alkanes are increasingly resistant to microbial degradation (Pietro et al., 2024). The cooperation of more than one single species of microorganisms is required in the biodegradation of complex hydrocarbons; particularly pollutants that are made up of many different compounds, such as crude oil or petroleum. Individual microorganisms can metabolize only a limited range of hydrocarbon substrates, so mixed cultures with broad enzymatic capacities are required to increase the rate of petroleum biodegradation in the polluted soil. This study studied the biodegradation of hydrocarbon fractions in the used engine oil of two contrasting Brazilian soils. This was carried out by modeling microcosms in the laboratories for 90 days to confirm the utility of these indices in evaluating bioremediation monitoring techniques in the field to reduce environmental hazards.

Material and Methods

Soil sampling

Samplings of soils within the layers of (0 – 20 cm) were collected in four sampling points in two locations (Sêtê Lagoas and Três Marias), in Minas Gerais State of Brazil. The geographical locations of the sampling points were as presented in (Adeyemo, 2019). The study sites were characterized by annual rainfall of (1272 and 1226 mm) and average temperatures of (22.0 and 23.2 °C) in each location respectively. A single large core was collected for each soil type from the A horizon, not including the surface litter layer, kept in hermetic bags, and transported to the laboratory for immediate soil analysis. The soil was sieved using a 5 mm diameter stainless sieve. The characteristics which were determined using standard techniques are listed in Table 1. Used lubricating oil was collected from a gasoline and car service station close to the Federal University of Viçosa, Brazil. Amnite P1300 consisted of special bacterial strains (Amnite P1300) specially made to degrade used lubricating oil was obtained from Cleveland Biotech Ltd., UK.

Soil analysis

The soil types from the A horizon, without the surface litter layer, were kept in sack and transported to the laboratory for analysis. The soils were sieved using a 5 mm diameter stainless sieve. The physicochemical characteristics of the soils that were made in triplicates in the laboratories were presented in our previous work (Adeyemo, 2019); these show the selected properties of the non-contaminated soil samples used for the bioremediation studies. The textural classes of the soil particle size were performed using hydrometer method (Sheldrichk and Hangwang, 1993). Total nitrogen content of the soil was determined using the micro-Kjeldahl method (Bremner, 1996), the available phosphorus was determined by colometry after Mehlich 1 extraction, while organic carbon content by the procedure of Walkley and Black using the dichromate wet oxidation method (Nelson and Sommers, 1992). The pH was determined using a 1:2.5 ratio by weight with distilled water (w/v) after 30-min equilibration using a pH meter and electrode calibrated with pH 4.0 and 7.0 standards (Defelipo and Ribeiro, 1981). Determinations were made in triplicate.

Determination of petroleum hydrocarbons in soils

Total Petroleum Hydrocarbons (TPHs) were extracted according to EPA method 3546 (US EPA, 2007) using the Microwave Automated Reaction System from CEM (Matthews, NC). Briefly, sodium sulfate (Na2SO4) was purified by drying overnight in an oven at 150 °C and quickly transferred into desiccators. Five grams (5 g) of homogenized contaminated soil was weighed out, mixed with 5 g dry anhydrous Na2SO4 ground to less than 1 mm particle sizes, and extracted in a 25 mL GreenChem vessel containing 1:1 hexane: acetone mixture according to manufacturer's protocol, and were kept at 100 °C for 20 minutes. The n-hexane and acetone mixture were filtered through Whatman No 1 filter paper to separate the extract from the soil particles and transferred into 100 mL amber vials through a separatory funnel which was sequentially rinsed with equal volume of solvent mixture. The solvent was evaporated to partial dryness with a rotary evaporator (Fizatom Rotavapor 801), transferred into 2 mL vials, and then dried completely using nitrogen gas. Dried samples were dissolved in 600 µL dichloromethane for gas chromatography analysis. The residual oil was analyzed on a Shimadzu GC-17A chromatograph equipped with a Flame-Ionization Detector (FID) by using fused silica capillary column DB-5 (30 x 0.25 mm), and AOC-17 Shimadzu auto-injector complying with Environmental Protection Agency (EPA) standard method 8015 (US EPA 2007). The flow rate of the helium carrier gas was 1.81 mL/min with a linear

velocity of 38.49 cm/s. The initial temperature was programmed at 40 °C and held for 15 min. The temperature was then increased to 280 °C at a rate of 10 °C /min. The final temperature was held for 31 min. The injector was set in the split mode, the split ratio was set to 1:10; the injection volume was 1 µL and the injector and the detector temperature for GC were maintained at 260 and 280 °C respectively and the oven temperature was programmed to rise from 40 to 280 °C in 10 °C/min increments and to hold at 280 °C for 31 min. The dry weight of the soil samples was determined following baking of 10 g of wet soil at > 80 °C for at least 48 hours. Before analyzing the sample extract, a mixture of standards including n-alkanes (n-decane n-C10, n-dodecane n-C12, n-tetradecane n-C14, n-hexadecane n-C16, n-octadecane n-C18, n-eicosane n-C20, n-docosane n-C22, n-tetracosane n-C24, n-hexacosane n-C26, n-octacosane n-C28 and pure standards containing n-triacontane n-C30, n-dotriacontane n-C32, n-tetratriacontane n-C34, and n-hexatriacontane n-C36, and a mixture of polycyclic aromatic hydrocarbon consisting of acenaphthene, acenaphthylene, anthracene, benzo (a) anthracene, benzo (a) pyrene, benzo (b) fluoranthene, benzo (g, h, i) perylene, benzo (k) fluoranthene, chrysene, dibenz (a, h) anthracene, fluoranthene, fluorine, indeno (1, 2, 3-cd) pyrene, naphthalene, phenanthrene, pyrene, 1-methylnaphthalene and 2-methylnaphthalene, Supelco) were used for calibration. Five-point calibration curves using peak areas were obtained and the response factors were used to determine the concentrations of various hydrocarbons in the sample extract. The total petroleum hydrocarbons were identified and quantified by comparing the peak area of samples with that of the standard of the TPH mixture regarding the curve derived from standards. The percentage of degradation was calculated by the following expression in Equation 2:

$$\%\ biodegradation = \left[\frac{TPH\ Control - TPH\ treatment}{TPH\ Control}\right] x\ 100$$

Statistical data analysis

Data collected were subjected to statistical analysis using a general linear model of analysis of variance (ANOVA). Significant differences in the treatment means were compared using the Tukey test at P > 0.05 (Statistical Software 8.0: Stat. Soft, 2007).

Results and Discussion

Table 1: Selected physical and chemical characteristics of the noncontaminated soil samples

Parameters	Soil 1 (S_1)	Soil 2 (S2)
pH (H_20)	5.20	4.92
Total Nitrogen (%)	0.43	0.11
Avail. P (mg/dm^3)	1.00	0.40
Organic C (%)	3.50	0.81
C:N ratio	8.14	7.56
Moisture Content (%)	33.80	11.30
Sand (dag/kg)	11.00	68.00
Silt (dag/kg)	9.00	4.00
Clay (dag/kg)	80.00	28.00
Texture	Clayey	Clay loamy sand
Soil Type	Red Intosol	Red yellowish latosol

Biodegradation of Aliphatic hydrocarbon fractions in used lubricating oil. Biodegradation of hydrocarbon fractions present in the used

lubricating oil was determined at fifteen-day intervals for 90 days using GC/FID. The hydrocarbon fractions were divided into three fractions which are: C10 – C14, C15 – C28, and C29 – C36 (Alberdi *et al.*, 2001).

i. Biodegradation of C10 – C14 fractions in used lubricating oil

Oil-contaminated soil with 3 % amended with Amnite P1300) and augmented with $(NH_4)_2SO_4$ and K_2HPO_4 recorded complete biodegradation of C10 – C14 aliphatic hydrocarbon fractions below the detection limit within ninety days in the clayey soil (S1) compared to unamended and the control. In the sandy soil (S2) however, only the treatment with Amnite P1300 recorded complete degradation below the detection limit. There was no complete degradation of the fractions (C10 – C14) in nutrients amended, unamended and sterile contaminated soils throughout the ninety days periods. Soil contaminated with 4.5 % used lubricating oil did not record any oil biodegradation below detection limit in all the treatments within the ninety days of the experiment, but there were appreciable degradation in the soils contaminated with the used lubricating oil amended with Amnite P1300, followed by nutrient amendment and unamended soils, the control soil had the least biodegradation throughout the period of the experiment as shown in Figure 1. The sterile polluted soils at 90 days has residual C10 – C14 fractions of 437.43 and 747.00 mg/kg in clayey soil at 3.0 and 4.5 % level of contamination, and 464 and 817 mg/kg in sandy soil at 3 and 4.5 % level. The rapid biodegradation of C10 – C14 fractions has been reported to be among the most rapidly biodegraded components of oil, although they are also susceptible to removal by extensive water washing. Empirically, the first sign of biodegradation are usually n-alkane in the C10 to C13 range, which probably reflects an optimal carbon number with increasing enthalpy of reaction and decreasing water solubility as the alkane carbon number increases (Palmer, 1993; Masterson, et al., 2001). The results, like those of C10 – C14 contaminated at 3.0 % revealed the effectiveness of Amnite P1300 to effect complete degradation of C10 – C14 fractions in this level of contamination in both soils. This still pointed out its ability to degrade the short-chain hydrocarbon fractions better than other treatments applied during the 90 days of the

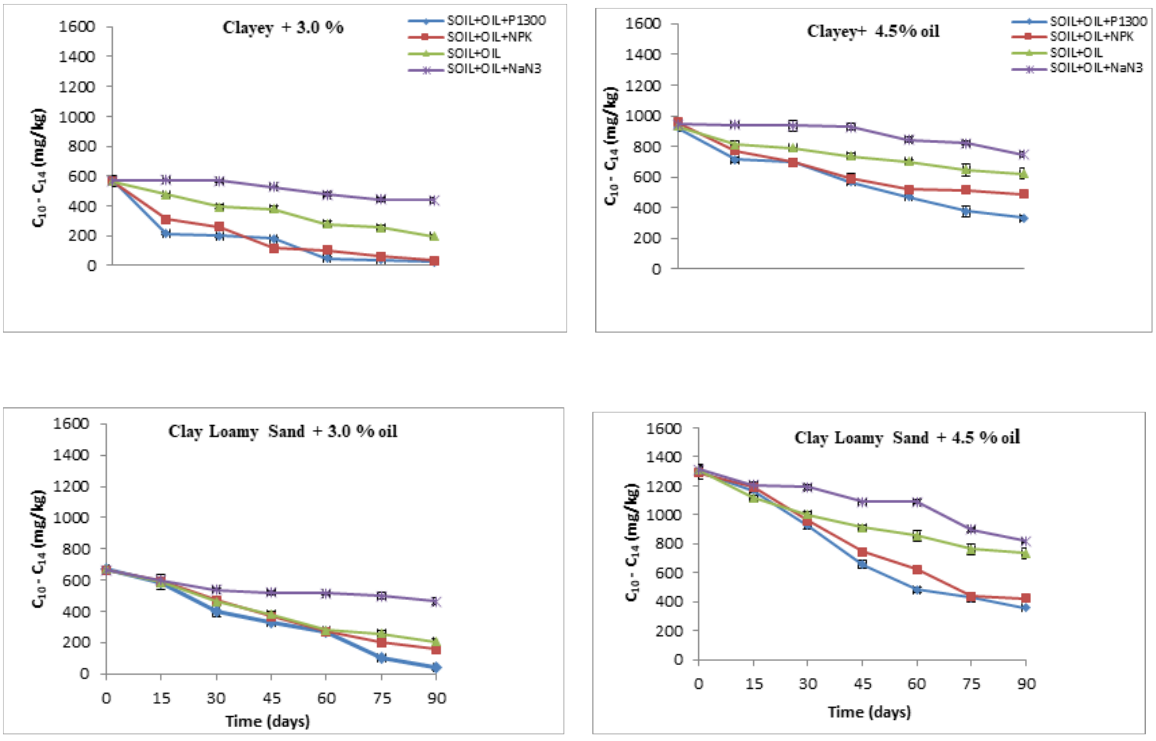

Figure 1: Concentration (mg/kg) of aliphatic hydrocarbon fractions (C10 – C14) in different soil types (S1) and (S2) contaminated with 3.0 % and 4.5 % used lubricating oil

experimental period. The results are similar to that of Chang et al., (2010) who reported a substantial degradation of C10-C16 hydrocarbon fraction in aged petroleum hydrocarbon-contaminated soil.

ii. Biodegradation of C15 – C28 fractions in used lubricating oil

The results show that the hydrocarbon fractions C15 – C28 were not degraded below the detection limit in all the treatments within the experimental periods; however, the degree of biodegradation varies greatly based on the percentage of oil pollution and the amendments. The reason for incomplete biodegradation of these hydrocarbon fractions below the detection limit might be due to their complex structure, and this has proven to have which always posed some significant difficulty to hydrocarbon-utilizing bacteria in their complete biodegradation (Xu et al., 2018). In soils contaminated with 3.0 % used lubricating oil,

Amnite P1300 amended soil recorded the highest biodegradation in C15 – C28 hydrocarbon fractions from the initial concentration of (16,245 and 16,348 mg/kg) to (3,154 and 3,658 mg/kg) in clayey and sandy soil respectively after 90 days of the experiment. Studies with soil contaminated with 4.5 % oil pollution also revealed that Amnite1300 amended soil has the best treatment where the oil fractions were reduced from the initial concentration of (22,954 and 23,254 mg/kg) to (9,448 and 10,748 mg/kg) in clayey and sandy soil respectively after 90 days of the experiment as shown in Figure 2. The unamended contaminated and control soils recorded very low biodegradation of the C15 – C28 fractions throughout the 90 days in both soils at different levels of contamination with used lubricating oil. The increase in the biodegradation of C15 – C28 fractions in soil amended with Amnite P1300 might be due to the ability of the bacterial products conditioned to degrade heavy hydrocarbons.

iii. Biodegradation of C29 – C36 fractions in used lubricating oil

The results of the study revealed that these fractions of petroleum hydrocarbons were not properly degraded in all the treatments. The incomplete degradation of these hydrocarbon fractions has been reported by different authors that they are not easily degraded by microorganisms in the soil because they are hydrophobic solids at physiological temperatures (Pandolfo et al., 2023). In soil contaminated with 3 % used lubricating oil, soil amended with Amnite P1300 recorded a reduction in C29 – C36 in the concentration from

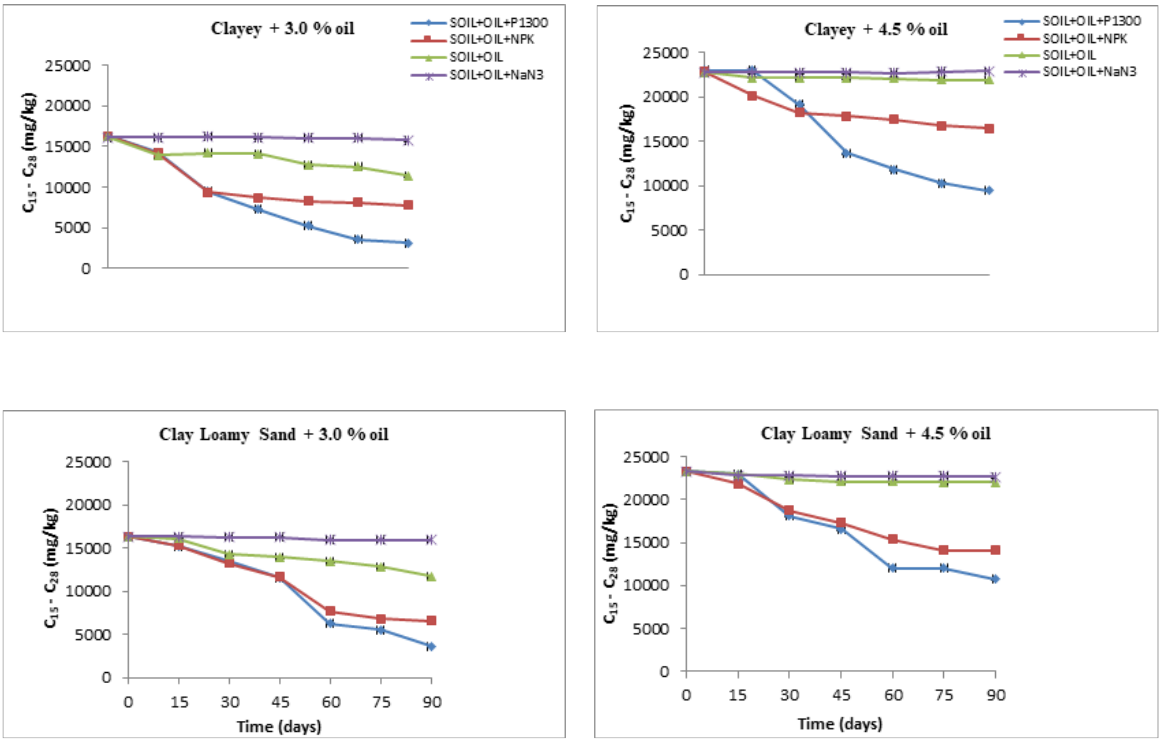

Figure 2: Concentration (mg/kg) of aliphatic hydrocarbon fractions (C15 – C28) in different soil types (S1) and (S2) contaminated with 3.0 % and 4.5 % used lubricating oil

9441 and 9684 mg/kg to 4566 and 3981 mg/kg in clayey and sandy soil, respectively, after 90 days of

the experimental study, whereas in the control contaminated soil, the biodegradation of the hydrocarbon fractions was minimal (reduction from 9321 and 9743 mg/kg to 8816 and 9291 mg/kg in clayey and sandy soil, respectively, after 90 days of the experimental study as shown in figure 3. The same pattern was also recorded in the 4.5 % contaminated level with the highest reduction recorded in the Aminte P1300 amended soil, though lower compared with the degradation recorded in the 3.0 % contaminated level in the hydrocarbon fractions C29 – C36. The reason for the low biodegradation of these hydrocarbon fractions might also be attributed to the fact that during the biodegradation of hydrocarbons in soil or sediments, low molecular weight fractions are known to be degraded first by microorganisms before degrading the higher molecular weight petroleum fractions (Begoña et al., 2024). Therefore, in this study possibly the low molecular weight fractions were first degraded by indigenous microorganisms before the higher molecular weight, thus, accounting for the low biodegradation of the higher molecular fractions in the range of C29 to C36.

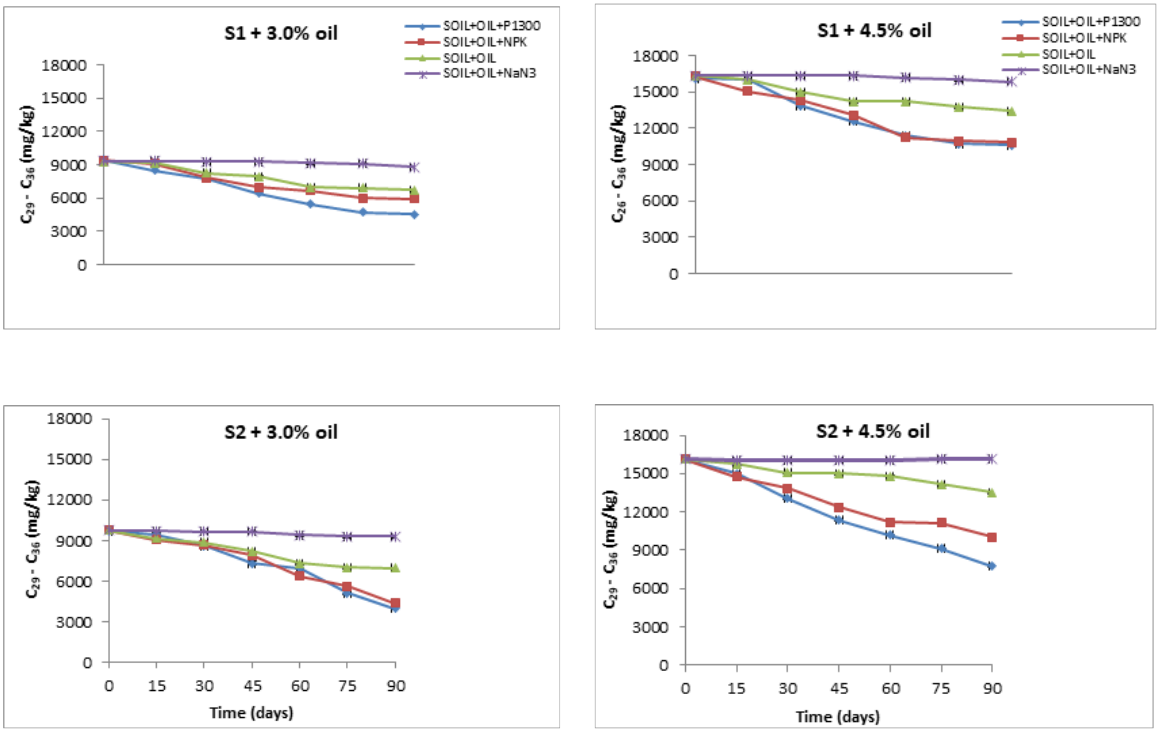

Figure 3: Concentration (mg/kg) of aliphatic hydrocarbon fractions (C29 – C36) in different soil types (S1) and (S2) contaminated with 3.0 % and 4.5 % used lubricating oil

Biodegradation of Polycyclic Aromatic Hydrocarbons in used lubricating oil

The results from this study demonstrate the degradation of eight PAHs present in the used lubricating oils during the biodegradation studies, using a microbial consortium present in Amnite P1300 as an amendment, addition of nutrients, unmended and the control soil with sodium azide to degrade soils contaminated with used lubricating oil. These PAHs have been identified by the US Environmental Protection Agency (EPA) as priority pollutants. Figures (4 to 7) show the results (concentration in mg/kg) of different residual PAHs present in the used lubricating oil in each treatment during the bioremediation studies. Xiaohui (2017) detected sixteen PAHs with 2- to 6-rings in used lubricating oils. In other similar studies, twenty-five PAHs with 2- to 6-rings were recorded by Xiaohua (2017), and as many as eighty-four were reported by Hussein a Mona (2016). The following eight PAHs with 2- to 4-rings (Naphthalene (Nap), Acenaphthylene (Can), Acenaphthene (Anth), Fluorene (Flu), Phenanthrene (Phr), Anthracene (Ant), Fluoranthene (Flt) and Pyrene (Pyr) were the main PAHs detected in our studies during the bioremediation experiment in soils contaminated with used lubricating oil. These contrasting results demonstrated the difficulty of detecting individual PAHs in used lubricating oils whose matrices become complex after high-temperature combustion.

The quantity and the composition of PAHs in the laboratory incubations were determined at the start of the experiment, and every 30 days thereafter till

the end of the incubation. After 90 days of incubation, Naphthalene, Acenaphthylene, and Acenaphthene were depleted in all the treatments and the abiotic control samples in both soils and contamination levels below the detection limit of 0.5 mg/kg, indicating abiotic removal, probably due to volatilization. It has been observed that PAHs resistance to oxidation, reduction, and vapourization increases with increasing molecular weight, whereas the aqueous solubility of these compounds decreases (Haneen et al., 2022). The results of biodegradation of different PAHs of higher molecular weights within the period of the study revealed the degradation of fluorene below the detection limit of 0.5 mg/kg in Amnite P1300 (T1), Nutrients amended (T2) and unamended (T3), in both soils and contamination levels, whereas, limited degradation was recorded in the control soils (T4) in both contamination levels within the period of the experiment. Complete degradation of phenanthrene and anthracene was only achieved in T1 and T2, while the two PAHs were not completely degraded in T3 and T4 in both soils and contamination levels.

Complete degradation of fluoranthene and pyrene below the detection limit was also revealed in T1, the effect of the addition of the nutrients was negligible in the degradation of these two PAHs. However, it was clear that two – to four-ring aromatic compounds in the used lubricating oil had been substantially degraded in Amnite P1300 amended soils at both contamination levels, while other treatments did not record complete degradation of fluoranthene and pyrene after 90 days of the experimental study. The reason for the complete degradation of PAHs recorded in soil amended with Amnite P1300 might be due to the soil texture improvement from a possible increase in oxygen transfer as a result of the increase in the bacterial consortium present in the contaminated soil. Bekele et al., (2022) asserted that individual microorganisms can metabolize only a limited range of hydrocarbon substrates, so assemblages of mixed populations with broad enzymatic capacities are required to increase the rate and extent of PAH biodegradation. Also, the loss of PAHs recorded in the sterile polluted soil might be due to different processes such as volatilization, adsorption, photolysis, or chemical degradation which are known to contribute to PAHs degradation in contaminated soil Mohamed et al., 2018).

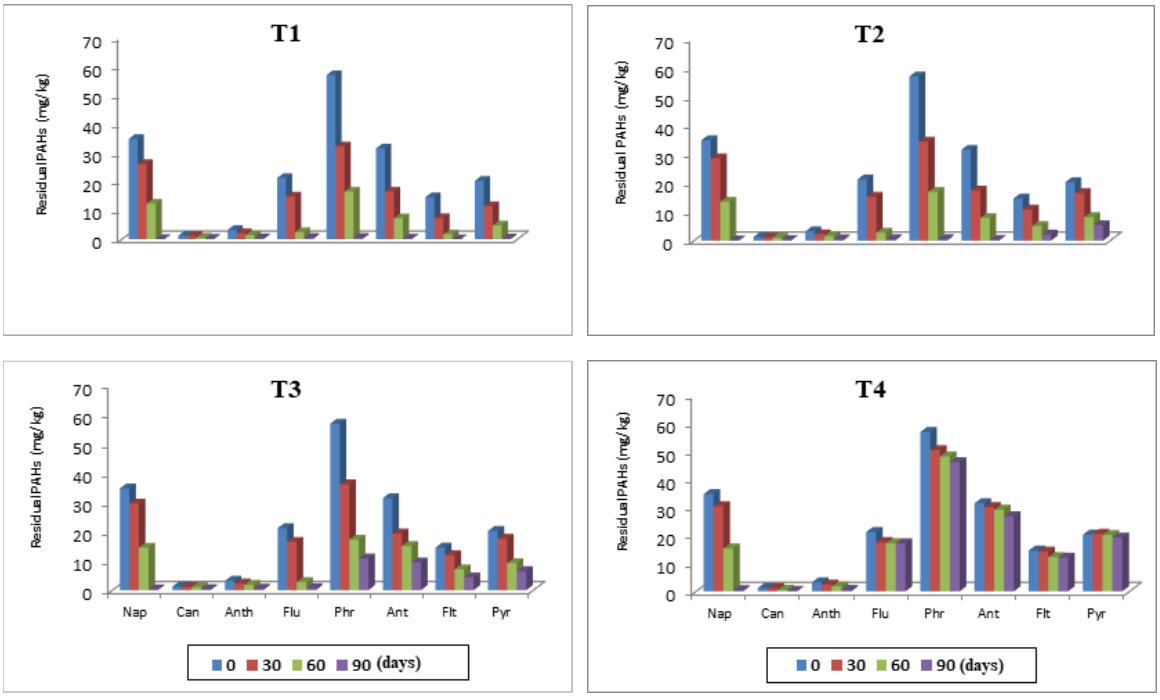

Figure 4: Concentration (mg/kg) of Polycyclic Aromatic Hydrocarbons (PAHs) in Soil 1 (S1) contaminated with 3.0 % used lubricating oil. PAHs present are: Nap - Naphtalene; Can – Acenaphthylene; Anth – Acenaphthene; Flu – Fluorene; Phr – Phenanthrene; Ant – Anthracene; Flt - Fluoranthene and Pyr – Pyrene

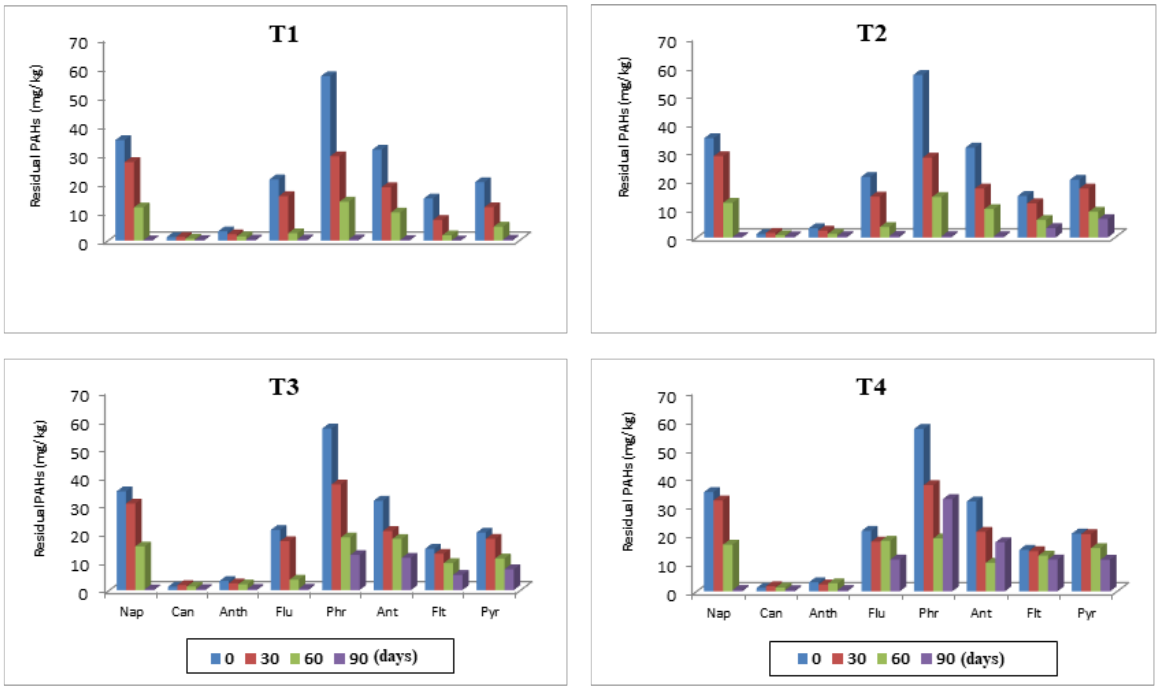

Figure 5: Concentration (mg/kg) of Polycyclic Aromatic Hydrocarbons (PAHs) in Soil 1 (S1) contaminated with 4.5 % used lubricating oil. PAHs present are: Nap - Naphthalene; Can – Acenaphthylene; Anth – Acenaphthene; Flu – Fluorene; Phr – Phenanthrene; Ant – Anthracene; Flt - Fluoranthene and Pyr – Pyrene

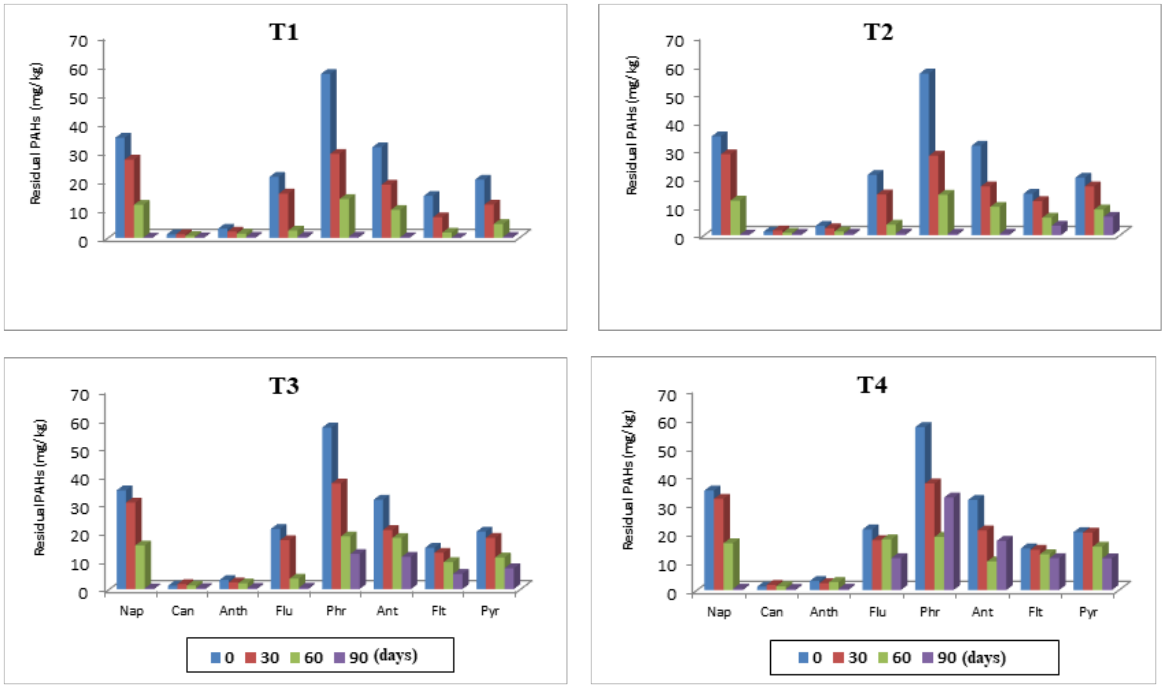

Figure 6: Concentration (mg/kg) of Polycyclic Aromatic Hydrocarbons (PAHs) in Soil2 (S2) contaminated with 3.0 % used lubricating oil. PAHs present are: Nap - Naphthalene; Can – Acenaphthylene; Anth – Acenaphthene; Flu – Fluorene; Phr – Phenanthrene; Ant – Anthracene; Flt- Fluoranthene and Pyr – Pyrene

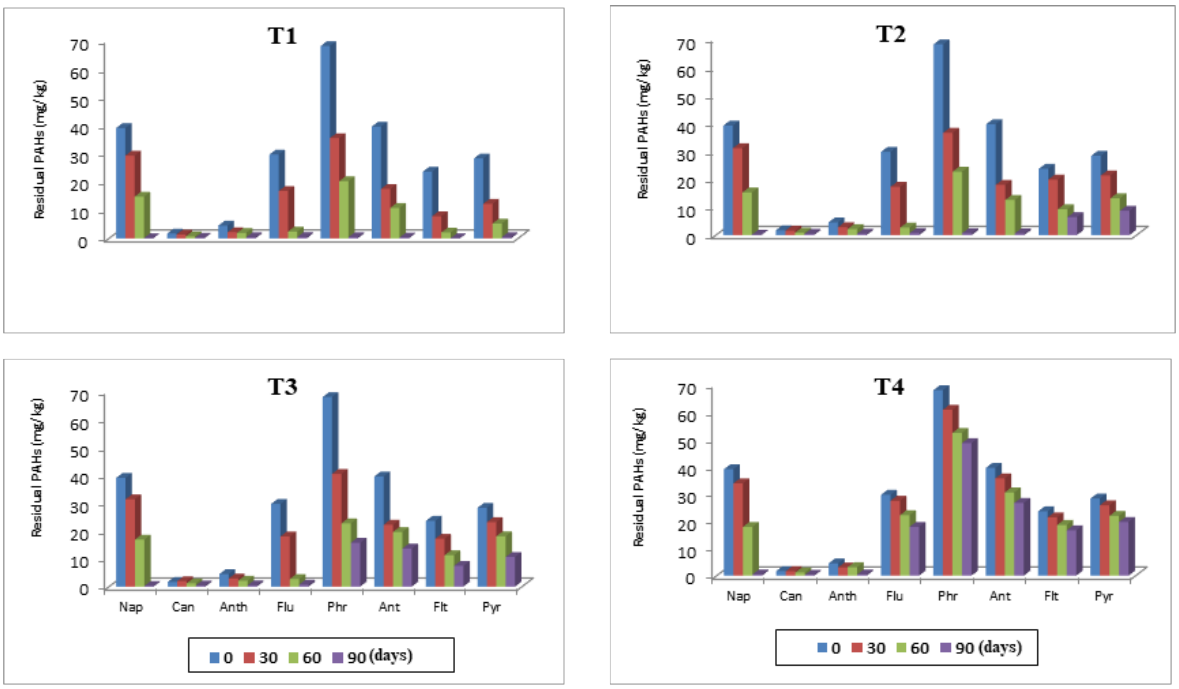

Figure 7: Concentration (mg/kg) of Polycyclic Aromatic Hydrocarbons (PAHs) in Soil 2 (S2) contaminated with 4.5 % used lubricating oil. PAHs present are: Nap - Naphthalene; Can – Acenaphthylene; Anth – Acenaphthene; Flu – Fluorene; Phr – Phenanthrene; Ant – Anthracene; Flt- Fluoranthene and Pyr – Pyrene

Conclusions

The study clearly showed the efficacy of using mixed microbial consortium in the degradation of used engine oil components. This work suggests the use of the mixed microbial consortium for bioremediation of used lubricating oil-contaminated soils (clayed and sandy). The results of this study, therefore, indicated that Amnite P1300 is a product that could facilitate the biodegradation and reduction of hydrocarbon components in soil contaminated with used engine oil. However, there is a recalcitrant fraction of the hydrocarbon constituents that remain in the soil after the evaluated period. The shorter-chain aliphatic hydrocarbon constituents are more degradable than the longer-chain aliphatic hydrocarbons in the soil types tested. Upscaling with more trial tests is therefore needed to increase the confidence of the results and the conclusions that can be drawn from this study to generalise the findings for large field investigation.

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