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Microbial pathways to sustainable polycyclic aromatic hydrocarbon remediation

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ABSTRACT: Spent engine oil seriously disrupts microbial communities in both soil and water, leading to cascading effects on many ecosystem processes critical for the functioning of ecosystems, which include nutrient cycling, nitrogen fixation, carbon sequestration, and degradation of organic matter. This work examines the potential of *Alcaligenes aquatilis*, isolated from farmland adjoining the Mechanic Village in Uyo, Akwa Ibom State, Nigeria, for the degradation of Polycyclic Aromatic Hydrocarbons (PAHs). The biodegradation competence of *A. aquatilis* was measured indirectly through viable cell density, optical density, and pH variation during the incubation period (up to 15 days). Bioremediation efficiency was also assessed through the percentage removal of total PAHs from day 0 to day 60, estimated by gas chromatography - mass spectrometry. The results indicated that *A. aquatilis* degraded 98.79, 95.58, 97.57, and 98.41% of the total PAH fractions in 4 kg of garden soil contaminated with 5, 10, 15, and 20% spent engine oil, respectively. In addition, monitored natural attenuation recorded 96.35% degradation at 5% contamination. Statistical analysis by ANOVA showed a highly significant difference, $p<0.001$, between the initial and residual PAH concentrations. Based on these findings, bioremediation with *Alcaligenes aquatilis* is strongly recommended for the cleanup of spent engine oil–impacted soils in contaminated environments.

Keywords: Bioaugmentation, natural attenuation, polycyclic aromatic hydrocarbons, spent engine oil.

INTRODUCTION

Spent engine oil, also known as used engine oil, is a complex mixture of chemical compounds which have undergone degradation and contamination during the course of engine use (Nkantion *et al*., 2025). With the growing global consumption of petroleum products, soil pollution by lubricants is rapidly increasing. In developing countries like Nigeria, hydrocarbon contamination of both terrestrial and aquatic environments has remained one of the major concerns since petroleum was first adopted as fuel for servicing engines (Nkantion *et al*., 2025).

The harmful effects of petroleum hydrocarbons and spent engine oil on the environment and their hazards to humans, animals, and plants have been widely discussed in the literature. Indeed, oil-polluted soils are of great ecological concern due to their unsuitability for agricultural and recreational activities and due to their potential to pollute surface and groundwater bodies (Li *et al*., 2020). PAHs, which are one of the major contents of spent engine oil, are highly lipophilic organic compounds containing only carbon and hydrogen atoms arranged as two or more fused aromatic rings (Nowakowski *et al*., 2021). Spent engine oil contains PAHs that contribute to long-term hazards such as mutagenicity, teratogenicity, and carcinogenicity, whereas fresh engine oil contains lighter hydrocarbons associated with acute toxicity (Vieira *et al*., 2018; Awa and Hadibarata, 2020).

Microorganisms can be found everywhere, and they contain numerous enzyme systems, which can metabolise recalcitrant pollutants and allow growth and survival under varying environmental conditions. Bioremediation of petroleum-contaminated environments consists of the degradation or sequestration of the toxic hydrocarbons, heavy metals, and volatile organic compounds present in fossil fuels by using microorganisms or plants (Adoki and Orugbani, 2007; Velez *et al*., 2020; Viera *et al*., 2020). Studies have shown evidence that autochthonous microorganisms have several advantages over allochthonous species because these are adapted naturally to the environment under consideration and hence can thrive more effectively in the particular contaminated environment (Adegbola *et al*., 2014; Nkantion and Itah, 2024).

Even though microbes capable of converting and mineralising a given pollutant often exist at contaminated sites, their natural population is usually too low for effective remediation. Where this is the case, strategies to enhance microbial activity, such as bioaugmentation, are employed. Bioaugmentation involves the introduction of known hydrocarbon-degrading microorganisms or pre-grown indigenous degraders to polluted soils or waters to accelerate biodegradation, taking into account the metabolic capacities of the organisms being introduced (Nkantion *et al*., 2025).

MATERIALS AND METHODS

Sources of materials

The soil samples were collected from adjoining farmlands to the automobile workshops in mechanic village, Uyo, Akwa Ibom State, where there were traces of hydrocarbons for isolation of hydrocarbonoclastic microorganisms and the University of Uyo garden soil. Spent engine oil was obtained from the automobile workshop at the same mechanic village, Uyo, Akwa Ibom State, Nigeria.

Isolation of hydrocarbonoclastic bacteria from spent engine oil impacted soil enrichment and isolation of bacterial isolates

The enrichment culture technique was employed. Precisely 1 g of spent engine oil impacted soil sample from mechanic village was inoculated into eight different sets of conical flasks containing 50 mL of sterile mineral salt medium [K2HPO4- 1.8 g, NaCl- 0.1 g, KH2PO4- 1.2 g, NH4Cl- 4.0 g, MgSO4- 0.2 g, FeSO4- 0.001 g, per 1 liter (pH 7.0± 0.2)] enriched with 1% of spent engine oil as carbon source. The medium was incubated at 28°C in a shaker incubator (100 rpm) for 7 days. The samples were thereafter serially diluted using sterile water as diluents and plated on Nutrient Agar (NA) to obtain viable bacterial cells. Discrete colonies obtained were sub-cultured onto fresh NA plate using the streak method as described by Cheesbrough (2006) to obtain pure cultures. The best spent engine oil utilising bacterial isolates were characterised based on their cultural and morphological attributes as well as their responses to standard biochemical tests as described by Holt *et al*. (1994).

Screening for the hydrocarbon utilising capability of bacterial isolates

Spent engine oil utilising the potential of the bacterial isolates was carried out using the hydrocarbon overlay method. Precisely 15 g of agar- agar was added to the mineral salt medium, sterilised and allowed to set. The solidified plates were overlaid with 1 mL of spent engine oil, allowed for 15 to 30 minutes then the test isolates were streaked on the surface of the plate. All inoculated plates were incubated at room temperature for 10 to 15 days for periodic observation. Colonies that eventually developed, showing areas of clearing, were selected and rated. Utilisation was rated based on diameter and luxurious nature of the developed colonies, i.e., +, ++ or +++, indicating the magnitude of the oil degrading potentials as described by Ekundayo and Obire (1987).

In-vitro determination of the influence of bioaugmentation on the spent engine oil degradation by the bacterial isolates

Using the principles of Ekanem *et al.* (2019), exactly 1 mL of the spent engine oil was incorporated into a conical flask containing 100 mL of sterile mineral salt medium. This was then inoculated with 1 mL of active inocula from 24 hours old peptone water broth culture of *Alcaligenes aquatilis* (2.1×10^6). The conical flask without augmentation served as a control. The conical flasks were incubated aerobically in a rotator shaker at 100 rpm. All the conical flasks were monitored 72 hourly for 15 days to know the rate of spent engine oil degradation based on viable plate count, turbidity and pH indices. The viable plate count was determined by the pour plate technique, while the degree of the turbidity and changes in pH were determined using a Spectrophotometer (Model No: 3001 at wavelength of 550 nm) and pH meter (CpH 102), respectively.

Ex- situ analysis of the influence of bioaugmentation on the degradation of spent engine oil impacted soil by the isolates

Before being sieved with a 2 mm sieve, garden soil from the University of Uyo agricultural farm was meticulously and physically sorted to remove debris. Using a 20 kg table measuring scale, 4 kg of soil was weighed and placed into nine wooden boxes with perforations. Following that, 4 kg of soil in five perforated 30 by 30 cm wooden boxes were polluted with graded percentages of spent engine oil (5, 10, 15, and 20%), and were augmented with 20 mL broth culture of *Alcaligenes aquatilis* (2.0 x 10^6) broth cultures (Okpokwasili *et al*., 1988). The control soil was polluted but not augmented. The impact of the bioaugmentation on the total polycyclic aromatic hydrocarbons (PAHs) at day 0 to total residual polycyclic aromatic hydrocarbons in the soil were evaluated at the end of the degradation course (2 months of treatment) by gas chromatography- mass spectrometry (GC-MS) techniques.

Determination of heavy metal in spent engine oil impacted soil by PAHs analysis

A gas chromatograph (GC, Hewlett-Packard HP model, 6890 series) coupled to a mass spectrophotometer (Ms 5971, Hewlett-Packard) was used to analyse and quantify the PAH compounds after they were extracted using hexane: dichloromethane and further fractionated, as described by Olajire *et al*. (2005). Bioremediation rates of PAHs were calculated using the formula below:

$$\text{Removal Efficiency (\%)} = \frac{\text{Initial Conc.} - \text{Final Conc.}}{\text{Initial Concentration}} x\ 100$$

Statistical analysis

One-way ANOVA (Analysis of Variance) was used to compare the initial PAHs concentrations and Residual PAHs using Statistical Package for Social Sciences (SPSS) version 22 by IBM Corporation, USA. Significance was determined at $p<0.001$.

RESULTS AND DISCUSSION

The potential of autochthonous bacterial taxon isolated from mechanic village automobile workshops in Uyo to utilise spent engine are presented in Table 1. The results divulged the existence of bacterial isolates with strong spent engine utilisation capabilities. *Psedomonas aeuuginosa*, *Pseudomonas putida*, *Alcaligenes aquatilis* and *Bacillus subtilis* were the efficacious

Spent engine oil exponent of hydrocarbons mineralisation potentials by *Alcaligenes aquatilis* and *Bacillus subtilis* during *in vitro* augmentation of autochthonous microbial community

In addition, the spent engine oil utilising strains of *Alcaligenes aquatilis* and *Bacillus subtilis* were subjected to in-vitro analysis. The degradability indexes of hydrocarbons by the bacterial isolates were measured by the density of viable cells produced, the amount of light scattered by the cells in suspension and variation in potential of hydrogen levels in the mineral salt medium. The results of the degradability indexes are presented in Figures 1, 2 and 3. The result of the total heterotrophic bacteria burden disclosed an increase in microbial population beyond the regular time within the degradation process, ranging from 3.2x10^6 to 8.0 x10^6 CFU/mL. The magnitude of increase was evidently higher in culture medium augmented with *Alcaligenes aquatilis* compared to culture medium augmented with *Bacillus subtilis*. Optical density analysis of *Alcaligenes aquatilis* during degradation studies divulged an increase in turbidity with time from 0.38±0.01 to 1.55±0.02. An increase in the acidity was clearly defined in the test media as the potential of hydrogen of the test substrates decreased (7.6±0.0 to 3.6 ±0.0) throughout the process, figuring eminent catabolic reaction.

Total PAHs contents of the polluted soil remediated with *Alcaligenes aquatilis* untreated soil

Figure 4 and Table 2 show the PAHs profiles of pretreatment and post treatment of spent engine oil contaminated soils during the bioremediation process. The result revealed significant diminution in the residual recalcitrant component of the spent engine oil within 8 weeks of remediation, as 0.56, 3.06, 3.36 and 4.08 mg/kg, respectively, and the soil used as a control had the total PAHs of 1.69 mg/kg within eight weeks of bioremediation. Over 98.79, 95.58, 97.57, and 98.41%, of the total PAHs fractions of the spent engine oil contamination at 5, 10, 15 and 20% concentration were respectively degraded in 4 kg of garden soil augmented with *Alcaligenes aquatilis*. Monitored natural attenuation had a percentage degradation of 96.35% at 5% contamination with spent engine oil, as shown in Figure 5.

Numerous studies on petroleum hydrocarbons and spent engine oil have demonstrated how quickly they deteriorate the environment and pose a risk to people, animals and the surrounding plants. Due to their unsuitability for both agricultural and recreational purposes, as well as their potential to contaminate surface and ground water, oil-polluted soils pose a serious threat to the ecosystem (Schwab *et al*., 2009).

PAHs are hazardous and long-lasting substances, particularly the more complex and molecularly heavy ones. In general, they are seen as having long-term environmental relevance. Because of their high hydrophobicity and relative recalcitrance in soil, PAHs are becoming more difficult to decompose (Leyva *et al*., 2013). Seventeen PAHs compounds are on the U.S. Environmental Protection Agency's (US-EPA) priority pollutants list. The initial polycyclic aromatic hydrocarbon content (PAHs) of the 4 kg of garden soil contaminated with 5, 10, 15 and 20% used for this study were 42.23, 84.46, 168.98 and 337.84 mg/kg. The possibility for *Alcaligenes aquatilis* and *Bacillus subtilis* to break down hydrocarbons was assessed in this study using an indirect method (the optical density, pH, and total viable count).

Table 1. Spent engine oil utilisation potential of hydrocarbonoclastic bacteria.

Isolates after 7 days	Growth on spent engine after 7 days	Growth on spent engine after 14 days
A. aquatilis	+++	+++
B. subtilis	+++	+++
P. putida	+++	+++
P.aeruginosa	++	++
Corynebacterium sp.	+	++
Micrococcus sp	++	++

Keys: - = Lack of growth, + = 1-5 mm (debile growth), + = 6-10 mm (restrained growth), ++ = 11-15 mm (strong growth), +++ = 16- 20 mm (strongest growth).

Table 2. PAHs profile at different percentages of contamination with spent engine oil augmented with *Alcaligenes aquatilis,* including control.

Peak Name	Percentages of contamination					Concentration (Units)
	5% of SEO + *A. aquatilis*	10% SEO + *A. aquatilis*	15% SEO + *A. aquatilis*	20% SEO + *A. aquatilis*	Control (5%) without augmentation)	
Naphthalene	ND	ND	ND	ND	ND	Ppm
Acenaphthylene	0.10	0.33	0.30	ND	ND	Ppm
Acenapthene	ND	ND	0.40	ND	ND	Ppm
Fluorene	0.11	0.20	0.45	ND	ND	Ppm
O-Terphenyl	0.85	0.80	0.88	ND	ND	Ppm
Anthracene	ND	ND	0.30	0.00	ND	Ppm
Phenanthrene	0.20	0.40	ND	ND	ND	Ppm
Fluoranthene	0.23	0.10	ND	ND	ND	Ppm
Pyrene	0.02	0.20	ND	ND	0.00	Ppm
Benz(a)anthracene	0.03	0.10	0.23	0.49	0.00	Ppm
Chrysene	0.03	0.20	0.00	0.78	0.00	Ppm
Benzo(k)fluoranthene	0.00	0.10	ND	0.86	ND	Ppm
Benzo(b)fluoranthene	0.00	0.20	ND	0.40	ND	Ppm
Benzo(a)pyrene	ND	ND	ND	ND	ND	Ppm
Dibenz(g,h.i) anthracene	0.10	0.10	0.25	0.20	0.34	Ppm
Indeno(1,2,3-, d) perylene	0.01	0.03	0.30	1.00	0.12	Ppm
Benzo(g,h,i) perylene	0.01	0.30	0.25	0.35	0.10	Ppm
Total	1.69	3.06	3.36	4.08	0.56	Ppm

Footnote: SEO = Spent Engine Oil; ND = Not detected; Ppm = parts per million.

The results showed an increase in total viable counts and corresponding optical density during the degradation of spent engine oil. Itah and Essien (2005) state that an elevated microbial count provides convincing evidence of a diverse species abundance in the spent engine oil, most of which were found to be able to utilise spent engine oil. In aerobic experimentation, terminal oxidation of hydrocarbons is the most frequent metabolic pathway exploited by bacteria. After the terminal oxidation, alcohol is oxidised to equivalent aldehyde and fatty acid by the use of pyridine nucleotide linked dehydrogenases. Consequently, microbial degradation of hydrocarbons usually results in the formation of organic acids and other metabolic byproducts (Adebusoye *et al*., 2007; Abioye e*t al*., 2012).

The drop in pH of the testing flasks during the 15 days incubation period additionally demonstrated chemical changes of the hydrocarbon substrates that are required to have been triggered by enzymes produced by microbial cells. This suggests the incorporation of spent engine oil as the only energy and carbon sources utilised by the oil degraders, resulting in their growth with an additional production of acid. These acidic metabolic products could be responsible for the decrease in pH of the cultures.

The GC analysis shows that both bioaugmentation and natural attenuation decomposed the polycyclic aromatic

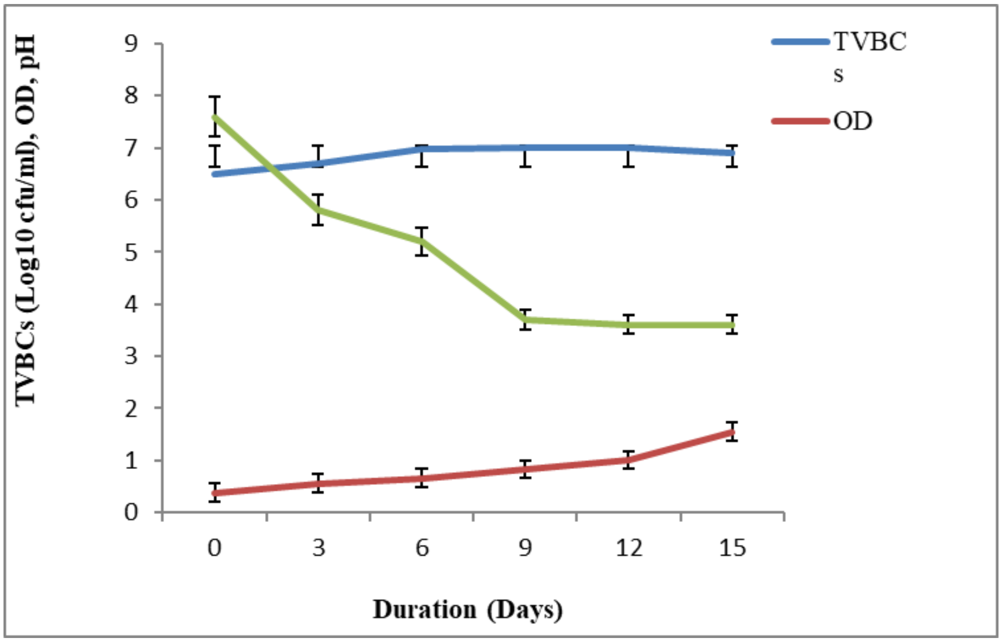

Figure 1. Variation in pH, optical density (550 nm) log of viable bacterial counts in mineral salt medium supplemented with spent engine oil and augmented with *Alcaligenes aquatilis* during 15 days of hydrocarbon degradation study (Source: Field data 2024).

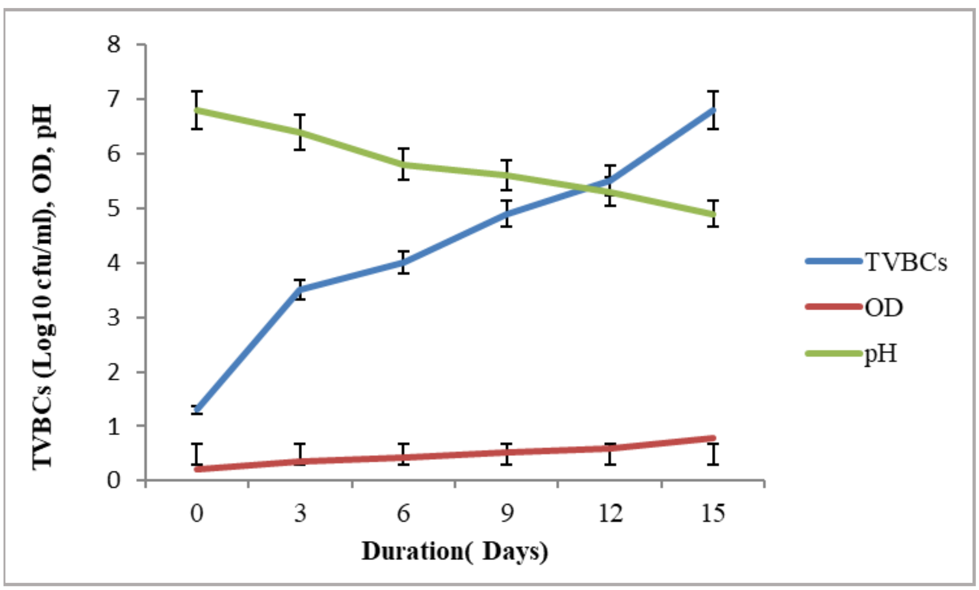

Figure 2. Variations in pH, Optical density (550 nm) log of viable bacterial counts in mineral salt medium supplemented with spent engine oil and augmented with *Bacillus subtilis* during 15 days of hydrocarbon degradation study. Source: Field data (2024).

hydrocarbons (PAHs) fractions beyond 95.58% (Vieira *et al*., 2018). Variation in the rate of degradation of spent engine oil impacted soil samples was observed with an increase in the percentage of the automobile waste oil added in the microcosm. This conforms with the study of Bartha and Atlas (1977), who stated that when the earthy surroundings are polluted with pollutants, the autochthonous microbial biomes may hold microbial populations of diverse taxonomic peculiarities with the capability of degrading the pollutants. According to Pilon (2005), natural attenuation can be employed to reinstate environments with depleted levels of contamination.

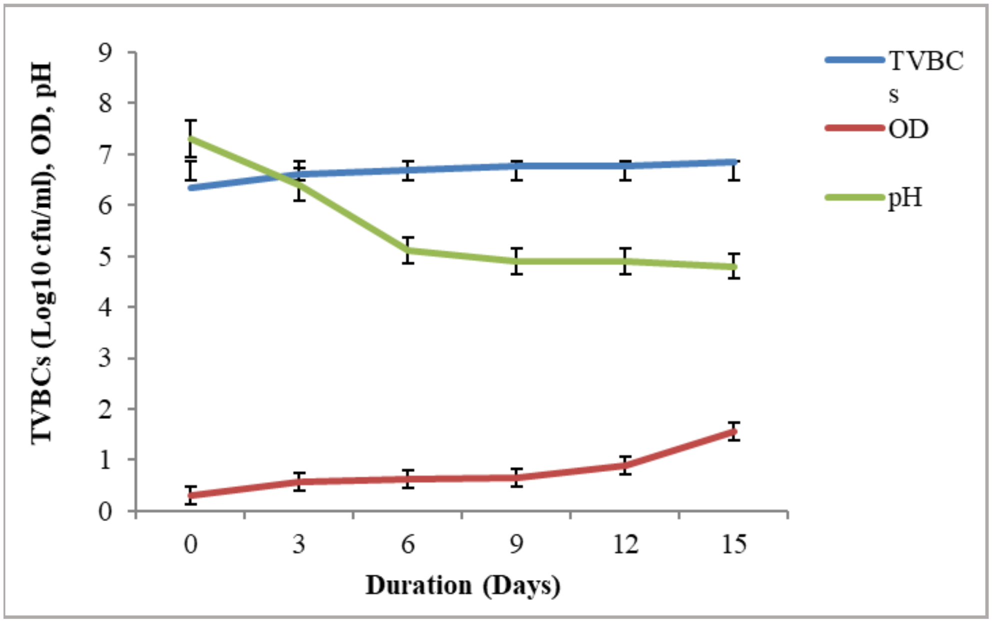

Figure 3. Changes in pH, optical density (550 nm) log of viable bacterial counts in mineral salt medium supplemented with spent engine oil and without augmentation during 15 days of hydrocarbon degradation course. Source: Field data, 2024).

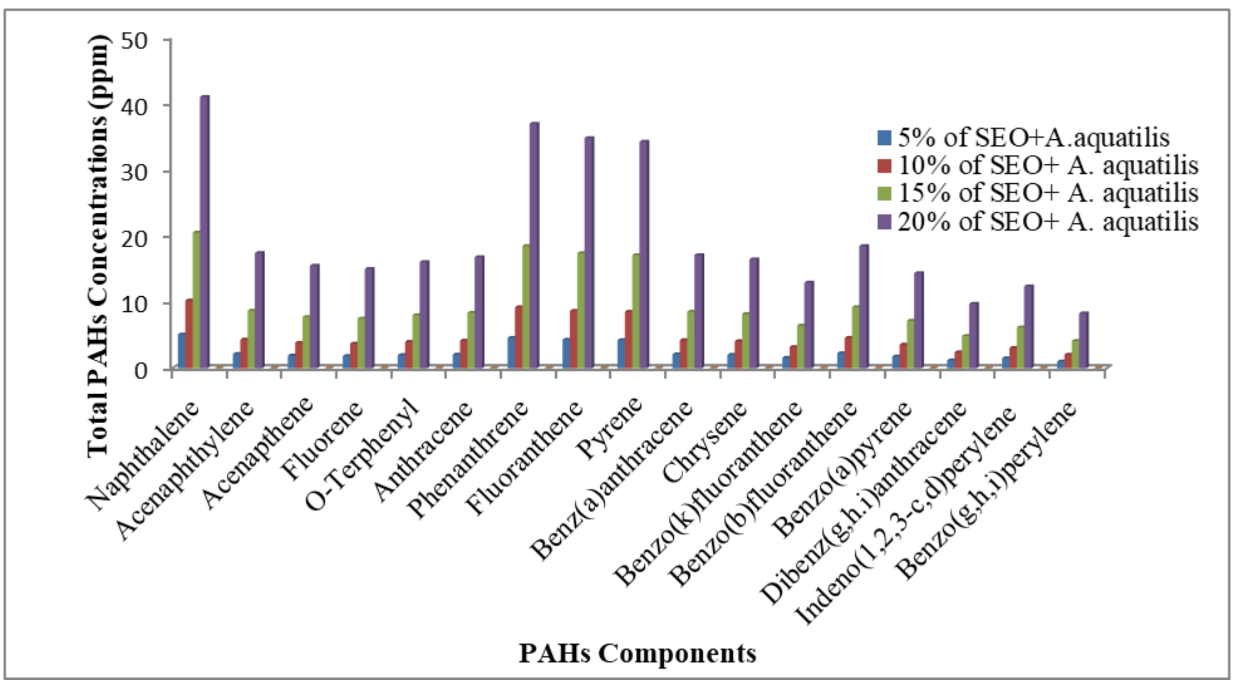

Figure 4. PAHs profile at graded percentage of contamination with spent engine oil augmented at zero day of contamination. **Footnote:** SEO= Spent engine oil (**Source:** Field data, 2024).

About 25 and 57% of land contaminated with petroleum products has been treated using natural attenuation (Nkantion *et al.*, 2025). Statistical analysis (ANOVA) showed a highly significant difference ($p<0.001$) in PAHs content between the mean initial and residual concentrations of PAHs. Therefore, in this study, 5% contamination of 4 kg garden soil with spent engine oil was not toxic to microbial communities, even without augmentation; rather, it serves as a carbon source, and they worked in synergy or consortium to ensure effective bioremediation.

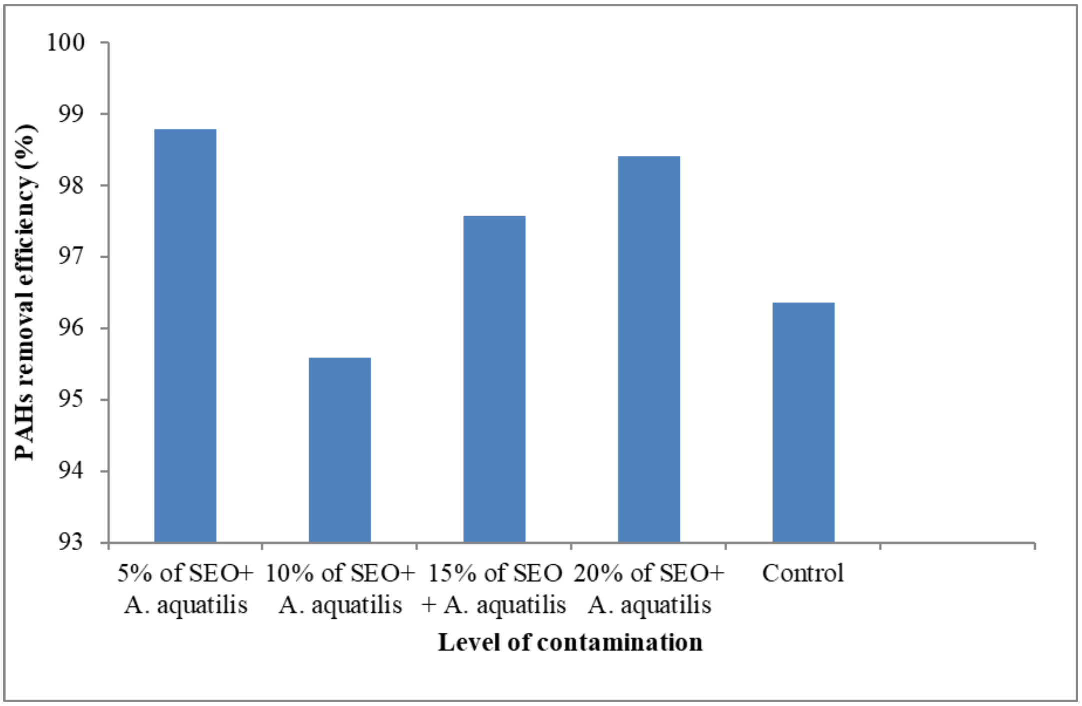

Figure 5. The rate of PAHs degradation obtained from 5%, 10%, 15% and 20% contamination of spent engine oil, augmented with *Alcaligenes aquatilis* and without augmentation (control) during bioremediation (Source: Field data, 2024).

Conclusion

Based on findings, contamination of the garden soil with spent engine oil has negative impact on the natural flora abundance as well as the soil's chemical and physical characteristics thus fertility state. The present finding indicates that polluted soil can be amended with broth culture of *Alcaligenes aquatilis* since it proved a better candidate for abatement program in spent engine oil impacted soil and improvement of soil fertility.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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