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Enhanced Biodegradation of Spent Engine Oil Contaminated Soil using Organic Wastes

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Abstract

Physical and chemical methods of remediating contaminated soils are less environment-friendly compared to the biodegradation method. This study investigated the ability of selected organic wastes to enhance biodegradation of Spent Engine Oil (SEO) contaminated soil. One kilogram of uncontaminated soil was thoroughly mixed with 10% (w/v) SEO in seven treatments with two replicates. Spent Fruit Residues (SFR), Cassava Peel (CP) and a combination of Bean Husk and *Chromolaena odorata* (BHC) were added at 10% and 20% (w/w), with an untreated control. Total Heterotrophic Bacterial Count (THBC), Total Fungal Count (TFC), Total Hydrocarbon Degrading Bacterial Count (THDBC) and Total Hydrocarbon Degrading Fungal Count (THDFC) of the contaminated and uncontaminated soils were determined using standard microbiological methods. Isolates were screened for SEO utilization using 2,6-dichlorophenol indophenol indicator. Hydrocarbon contents of the soils were determined using Gas Chromatography-Mass Spectrometry (GC-MS). The data obtained were subjected to statistical analysis. The THBC ranged from $1.3x10^6$ to $2.9x10^6$ CFU/g, TFC ranged from $5.4x10^4$ to $2.0x10^5$ CFU/g, THDBC ranged from $0.5x10^3$ to $1.9x10^4$ CFU/g while THDFC ranged from $2.0x10^3$ to $1.0x10^4$ CFU/g. The isolated bacteria were *Pseudomonas* spp., *Bacillus* spp., *Klebsiella* spp., *Proteus mirabilis*, *Burkholderia cepacia*, *Micrococcus luteus*, *Providencia rettgeri*, *Enterococcus faecalis*, *Streptococcus bovis* and *Enterobacter cloacae* while the isolated fungi were *Candida* spp., *Aspergillus niger*, *Saccharomyces cerevisae*, *Penicillium chrysogenum* and *Trichophyton* sp. *Pseudomonas aeruginosa* and *Aspergillus niger* utilized the oil better than other isolates with absorbance of 0.26 and 0.49 at 600 nm, respectively. The GC-MS revealed that SFR 20% (w/w) treatment had the highest percentage degradation of 70.5%. This study confirms that spent fruit residues can enhance biodegradation of spent engine oil contaminated soil.

Keywords: Biodegradation; Organic wastes; Spent engine oil; Contaminated soil

Introduction

Any lubricating oil that has served its major purpose in engines and is withdrawn from the intended area of application is referred to as spent engine oil (SEO), being considered unfit for the initial intended purpose [1]. The relatively large amounts of hydrocarbons in spent engine oil include highly toxic Polycyclic Aromatic Hydrocarbons (PAHs) [2]. Also, most heavy metals such as vanadium, lead, aluminium, nickel, iron, chromium and zinc which were below detection in fresh engine oil have been reported at higher concentrations in SEO [3]. These metals may be retained in soils in the form of oxides, hydroxides, carbonates or exchangeable cations bound to soil organic matter. It was noted by Agbogidi and Ejemete (2005) [4] that oil has deleterious effects on the biological, chemical and physical properties of the soil, depending on dose, soil type and other factors.

In Nigeria and some developing countries, about 20 million gallons of waste engine oil are generated annually from mechanical workshops, and discharged carelessly into the environment [5]. A number of innovative physical and chemical technologies are available to remediate and reclaim soil contaminated with hydrocarbon pollutants especially in developed countries [6]. However, these methods are expensive, laborious and may only lead to partial and incomplete remediation. For this reason increasing research attention has been directed towards new environmentally-friendly soil remediation strategies and technologies. In the past few years, biological method (biodegradation) has proved to be versatile, economical and efficient for remediation of soils contaminated with petroleum products, pesticides and other Industrial pollutants [7].

Soil bioremediation can be promoted or activated through stimulation of the indigenous microflora, by introducing nutrients into the soil (biostimulation) or through inoculation of an enriched microbial consortium into the soil (bioaugmentation) [8]. Lack of essential nutrients such as nitrogen and phosphorus is one of the major factors affecting biodegradation of hydrocarbon by microorganisms in soil and water environments. The uses of nitrogenous fertilezers to stimulate microbial activity and/or hydrocarbon degradation have been widely demonstrated with positive results [9]. However, in developing countries, inorganic or chemical fertilizers are costly as well as insufficient for agriculture, let alone for remediating contaminated environments.

These constraints are driving a search for cheaper and more environmentally-friendly options. One such option is the use of organic wastes that could act as bulking agents and also supply nitrogen, phosphorus and hydrocarbon-degrading microorganisms to enhance the remediation process [10]. In addition, it provides an efficient, eco-friendly, cost effective solid waste management option [11-12]. This study investigated the ability of spent fruit residues, cassava peel and a combination of bean husk and *Chromolaena odorata* to enhance biodegradation of SEO in contaminated soil.

Materials and Methods

1) Sample collection and preparation

A soil sample was collected from uncultivated soil at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta at a depth of 0-15 cm using a sterile soil auger. The sample was air-dried at room temperature, then sieved with a 2 mm mesh size. SEO was collected from mechanic village camp, Abeokuta. Cassava peel was collected from *Gari* producers while spent fruit residues were collected from fruit shops at Osiele, Abeokuta. Bean husks were collected from bean sellers while leaves of *Chromolaena odorata* were collected from bushes around Isolu, Abeokuta. The cassava peel was soaked in clean water for one week to eliminate any residual cyanide that may be present. The water was then decanted and the cassava

peel was air-dried and ground. The spent fruit residues were composted in a plastic container for one week. The bean husks were mixed with shredded leaves of *Chromolaena odorata* in a 50:50 ratio.

2) Experimental design

One kilogram of the prepared soil sample was weighed in 7 different 5 litre-containers and 100 ml SEO was added at a concentration of 10% (w/v). The contents of the containers were thoroughly mixed and left undisturbed for two days. Spent Fruit Residues (SFR), Cassava Peel (CP) and the combination of Bean Husks and *Chromolaena odorata* (BHC), respectively, were added into the containers at two concentrations of 10% (w/w) and 20% (w/w), while untreated soil served as the control. The experiment was conducted in two replicates. The soil was occasionally mixed manually. Samples were taken from each container every week for six weeks.

3) Microbial counts

Total Heterotrophic Bacterial Count (THBC) was determined using the method of Ayansina et al. (2014) [13]. 1 ml each of serially diluted samples were inoculated on sterile Plate Count Agar (Lab M, UK) using the pour plate method and incubated at 37 ºC for 24 h. Total Fungal Count (TFC) was also determined using the method of [13]. 1 ml each of serially diluted samples were inoculated on sterile Saburoud Dextrose Agar (Lab M, UK) incorporated with 0.1 % (v/v) streptomycin using pour plate method and then incubated at 28 ºC for 48-72 h. Total Hydrocarbon Degrading Bacterial Count (THDBC) was determined as described by Balogun and Fagade (2010) [14]. 1 ml each of serially diluted samples were inoculated on Mineral Salt Medium (MSM) incorporated with 2% agar and 1% sterile SEO and then incubated at 28 ºC for 5 days. Total Hydrocarbon Degrading Fungal Count (THDFC) was determined using the method described by Iheanacho et al. (2014) [15]. 1 ml each of serially diluted samples were inoculated on Bushnell Haas Medium (BHM) incorporated with 2% agar and 1% sterile SEO and then incubated at 28 ºC for 5 days.

4) Characterization and identification of the isolates

An Analytical Profile Index (API) 20E kit (Biomérieux, France) was used for the identification of the Gram-negative bacteria while Gram-positive bacteria were identified using standard biochemical tests with reference to Bergey's manual. The fungal isolates were identified based on cultural and morphological characterization with reference to de Hoog et al. (2000) [16] and Ellis et al. (2007) [17].

5) Screening of bacterial isolates for hydrocarbon utilization

The method described by Bidoia et al. (2010) [18] was used. The bacteria isolated from the treated soils were screened. Pre-cultured bacterial isolates (18-24 hours old) were inoculated into 7.5 ml of MSM incorporated with 50 µL sterile SEO. Then, 400 µL of 2, 6-dichlorophenol indophenol indicator was added and incubated at 28 °C for 5 days. The control was incubated without inoculation. The absorbance of the medium at 600 nm was measured at intervals of 24 h using a digital colorimeter (Jenway 6051, UK).

6) Screening of fungal isolates for hydrocarbon utilization

The method described by Iheanacho et al. (2014) [15] was used. Pure culture of each of the isolates were inoculated into 50 ml BHM incorporated with 1% sterile SEO and 1% DCPIP. The control was not inoculated. Incubation was done at 28 °C in an orbital shaker incubator (Gallenkamp, UK) for 10 days. The absorbance of the medium at 600 nm was measured at inter-

vals of 48 h using a digital colorimeter (Jenway 6051, UK).

7) Physico-chemical analyses of the soil sample and organic wastes

The moisture content of the samples was determined using the method of Oyeyiola (2004) [19]. Total carbon and total nitrogen contents were determined as described by Riegel et al. (2002) [20]. The soil texture was determined using the method of Oyeyiola (2004) [19].

8) Extraction of SEO from contaminated soil

The method of Sojinu et al. (2011) [21] was used. 50 ml dichloromethane (DCM) was added to 10 g contaminated soil in a clean bottle and the mixture was placed on a mechanical shaker for 2 h. The contents were allowed to separate into phases and the organic phase was then drawn off and concentrated. Cleanup was done using a glass column packed with activated silica and alumina. Glass wool was introduced into the column to prevent elution of silica and alumina through the tap of the burette. Anhydrous $NaSO_4$ was introduced into the column as a drying agent. The extract was eluted with 70 mL DCM and n-hexane (3:7 by volume) and was further concentrated to approximately 1 mL and transferred to a 2 mL vial for gas chromatography-mass spectrometry (GC-MS) analysis.

9) Determination of biodegradation efficiency

The percentage degradation of the hydrocarbons in the contaminated soil was determined from the GC-MS results using the formula described by Mohan et al. (2006) [22].

$$B.E\ (\%) = 100 - [(As \times 100) / Aac]$$

Where;
B.E = Biodegradation efficiency
As = Total area of peaks in each sample
Aac = Total area of peaks in the appropriated abiotic control

Results and Discussion

1) Microbial counts

The Total Heterotrophic Bacterial Count (THBC) showed that Spent Fruit Residues (SFR) had the highest count of 2.9 x 10^6 CFU/g while Cassava Peel (CP) had the lowest count of 1.3 x 10^6 CFU/g. The Total Hydrocarbon Degrading Bacterial Count (THDBC) ranged from 1.9 x 10^4 CFU/g for SFR to 5.0 x 10^2 CFU/g for CP. The uncontaminated soil sample had the highest Total Fungal Count (TFC) of 2.0 x 10^5 CFU/g while the Bean Husk+*C. odorata* (BHC) treatment had the lowest count of 5.4 x 10^3 CFU/g. Total Hydrocarbon Degrading Fungal Count (THDFC) ranged from 1.0 x 10^4 CFU/g to 2.0 x 10^3 CFU/g BHC (Table 1). The variation in the total count of hydrocarbon-degrading bacteria and fungi in all treatments over the six week period of biodegradation is shown in Figures 1 and 2. THDBC levels ranged from 5.0 x 10^2 to 9.8 x 10^5 CFU/g, while THDFC ranged from zero to 3.6 x 10^4 CFU/g.

Hydrocarbon degrading bacteria and fungi were isolated from the uncontaminated soil as well as the organic wastes used in this study. A similar report by Agarry and Latinwo (2015) [10] reported that brewery waste effluents contained certain hydrocarbon-degrading microorganisms. Okoh [23] earlier stated that hydrocarbon degraders may be readily isolated from environments without any previous contamination. The decrease in the microbial count observed at the first week of this study confirmed the toxic effects of SEO on indigenous soil microflora. This is consistent with the findings of Nwoko et al. (2007) who revealed that after a major oil spill on soil or water body, microbial populations were drastically reduced as a result of the toxic impacts of the oil [24]. The microorganisms later tended to recover as they adapted to the environment. However, this study showed an increasing population of hydrocarbon degraders in the treated soils from the first week to the fourth week but gradually reduced at the

fifth and sixth weeks. This was probably due to depletion in nutrients (nitrogen and phosphorus) contained in the organic wastes added; this trend is in agreement with Agarry and Latinwo (2015) who reported that total hydrocarbon degrading bacterial counts in diesel contaminated soils amended with brewery waste effluents followed an increasing trend from the first to twenty-eighth day [10].

2) Physico-chemical properties

Soil analysis revealed that the physical and chemical properties supported optimum biodegradation of the SEO (Table 2). This is in consonance with Vidali (2001) who reported an optimal pH range of 6-8, moisture content of 40-60%, C:N ratio of 10:1, hydrocarbon content of 5-10% and soil texture with low clay or silt as an optimum environmental conditions for microbial activity during bioremediation of petroleum hydrocarbon [25].

Table 1 Microbial counts of the uncontaminated soil and organic wastes

Sample	THBC ($\times10^5$ CFU/g)	TFC ($\times10^4$ CFU/g)	THDBC ($\times10^3$ CFU/g)	THDFC ($\times10^3$ CFU/g)
Uncontaminated soil	24.5±2.9	20.5±2.5	15.0±1.2	10.6±1.2
Spent fruit residues	29.7±3.2	14.9±1.7	19.2±1.9	7.3±0.8
Cassava peel	13.5±1.5	10.8±1.2	0.5±0.1	2.5±0.4
Bean husks + *C. odorata*	16.2±1.3	5.4±0.6	1.5±0.2	2.0±0.3

Note:

THBC is Total heterotrophic bacterial count

TFC is Total fungal count

THDBC is Total hydrocarbon degrading bacteria

THDFC is Total hydrocarbon degrading fungi

Values are reported as means of triplicate readings ± standard deviation

Table 2 Physical and chemical properties of the uncontaminated soil and organic wastes

Property	Values			
	Uncontaminated soil	Spent fruit residues	Cassava peels	Beans husk + *C. odorata*
pH	6.3	5.9	6.8	7.6
Moisture content (%)	61	-	-	-
Carbon:nitrogen (C:N) ratio	9:1	11:1	27:1	39:1
Available phosphorus (mg/kg)	7.68	9.21	13.11	6.12
Exchangeable acidity (cmol/kg)	0.3	-	-	-
Exchangeable bases:				
Na^+ (cmol/kg)	0.35	-	-	-
K^+ (cmol/kg)	0.62	-	-	-
Ca^{2+} (cmol/kg)	13.3	-	-	-
Mg^{2+} (cmol/kg)	19.6	-	-	-
Soil texture	Sandy-loam	-	-	-

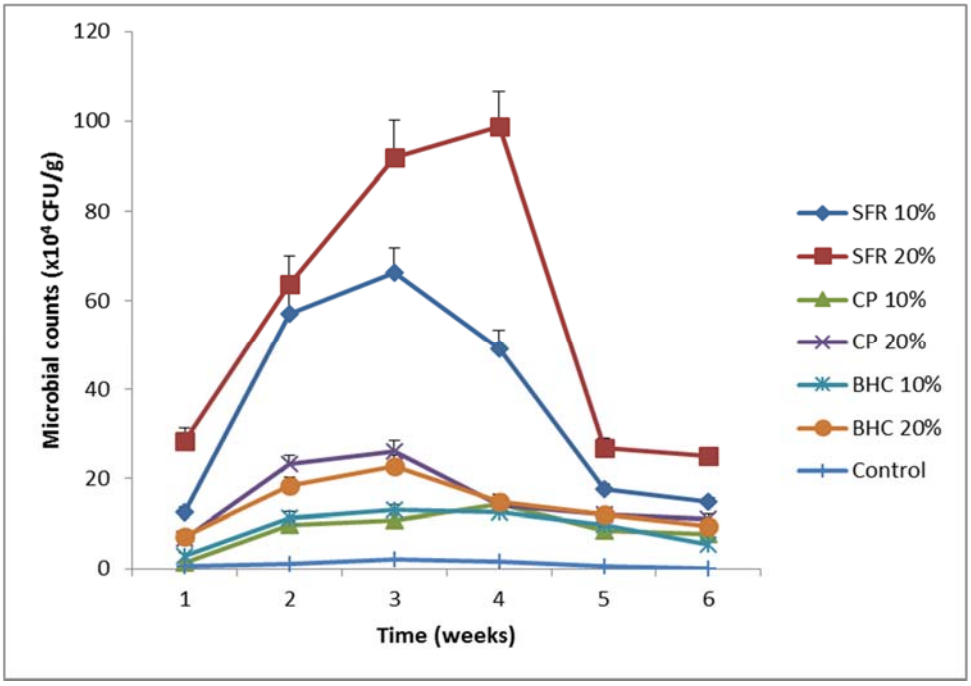

Figure 1 Variation in the bacterial counts of the treatments with time

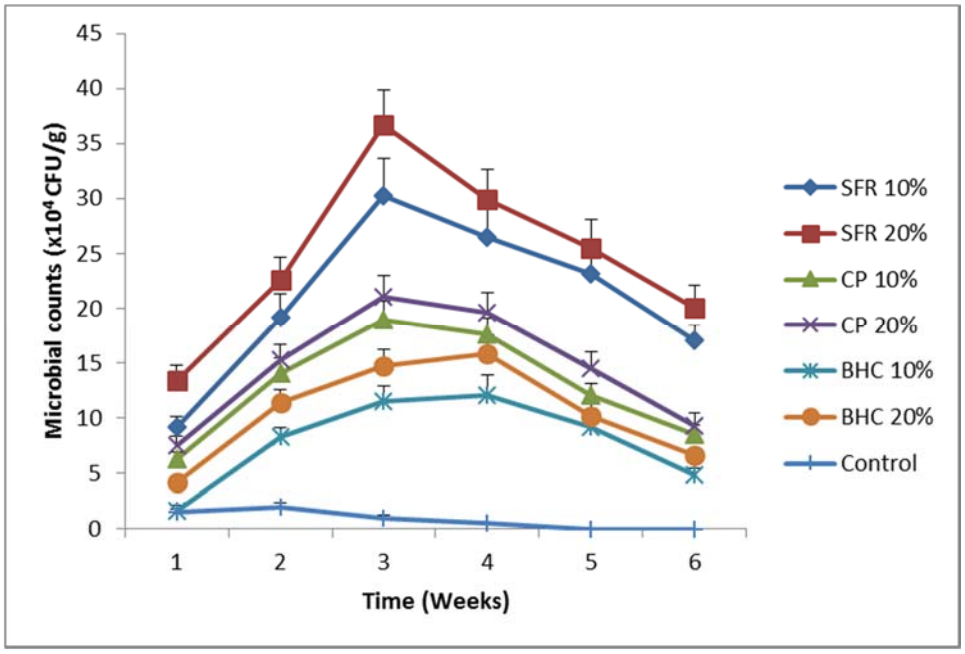

Figure 2 Variation in the fungal counts of the treatments with time

Note:

SFR is Spent fruit residues
CP is Cassava peel
BHC is Bean husks + *C. odorata*

Seventeen bacterial isolates were identified; six Gram-positive and 11 Gram-negative. The bacterial genera and their incidence rate are as follows: *Pseudomonas* spp (29.4%), *Bacillus* spp (17.6%), *Klebsiella* spp (11.7%), *Proteus mirabilis* (5.9%), *Burkholderia cepacia* (5.9%), *Micrococcus luteus* (5.9%), *Providentia rettgeri* (5.9%), *Enterococcus faecalis* (5.9%), *Streptococcus bovis* (5.9%) and *Enterobacter cloacae* (5.9%). Six fungal isolates were identified; three moulds and three yeasts. The fungal genera and their incidence rate are as follows: *Candida* spp (33.3%), *Aspergillus niger* (22.7%), *Saccharomyces cerevisae* (16.7%), *Penicillium chrysogenum* (16.7%) and *Trichophyton* sp. Among the bacterial isolates, only *Pseudomonas* and *Bacillus* were able to succeed other genera at sixth week in all the treatments while *Candida* and *Aspergillus* were the succeeding genera among the fungal genera.

Bacteria isolated in this study including species of *Pseudomonas*, *Bacillus*, *Burkholderia*, *Providentia*, *Klebsiella* and *Micrococcus* have been implicated as prominent hydrocarbon degraders [26-30]. More so, the isolated fungi including species of *Candia*, *Aspergillus*, *Saccharomyces*, *Penicillium* and *Trichophyton* have been well documented as hydrocarbon degrading fungi [31-33]. Among the isolated bacteria however, *Pseudomonas* and *Bacillus* species had the highest occurrence in this study. Similarly, [30] reported that *Pseudomonas* species were dominant among oil-degrading bacteria isolated from bitumen-contaminated soil. This might be due to the wider range of metabolic activities of *Pseudomonas* species while *Bacillus* species form spores that withstand unfavorable environmental conditions [34]. In addition, *Pseudomonas* species have been noted for their biochemical (metabolic) versatility with the ability to grow on diverse substrates and chemicals [35]. Also, the high occurrence of *Candida* species observed in this study has been earlier reported by Ilori et al. (2011) [36] who studied biodegradation of Nigerian crude oil (*Escravos Light*) by yeast strains [36].

3) Screening of isolates for SEO utilization

All isolates were able to utilize the SEO to varying extents. Among the bacterial isolates, *P. aeruginosa* was able to utilize SEO better than all other isolates as shown by its low absorbance value of 0.26, while *E. cloacae* had the highest absorbance value of 0.63, indicating the lowest SEO utilization (Table 3). On the other hand, *A. niger* was able to utilize SEO better than other fungal isolates as shown by its absorbance value of 0.49, while *Trichophyton* sp. had the least utilization with an absorbance value of 0.59 (Table 4).

This study showed that *Pseudomonas aeruginosa* utilized SEO more effectively than other screened bacteria. The emergence of this bacterium has been previously reported by Obayori et al. (2014) who stated that *P. aeruginosa LP*5 degraded 90 percent of used engine oil in a liquid culture medium within 21 days [37]. Similarly, [38] also reported that *P. aeruginosa* isolated from a hydrocarbon-polluted site was able to utilize 81 percent of used motor oil within 4 weeks. On the other hand, *Aspergillus niger* utilized the SEO better than other isolated fungi. This is in agreement with George-Okafor et al. (2014) who reported that *A. niger* and *A. versicolar* degraded 98 percent of selected policyclic aromatic hydrocarbons in liquid culture medium [32]. This is also consistent with the findings of April et al. (2000) who reported *A. niger* as one of the 64 species of hydrocarbon-degrading filamentous fungi [31].

Table 3 Oxidation of 2, 6-dichlorophenol indophenol by the bacterial isolates

Isolate	Absorbance at 600 nm				
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Bacillus subtilis	0.47	0.42	0.36	0.31	0.28
Pseudomonas alcaligenes	0.48	0.42	0.38	0.33	0.29
Enterobacter cloacae	0.88	0.80	0.77	0.71	0.63
Pseudomonas luteola	0.58	0.51	0.44	0.36	0.31
Klebsiella aerogenes	0.82	0.75	0.64	0.58	0.53
Klebsiella oxytoca	0.71	0.66	0.61	0.54	0.48
Micrococcus luteus	0.79	0.72	0.68	0.59	0.51
Bacillus cereus	0.51	0.43	0.38	0.33	0.29
Pseudomonas aeruginosa	0.48	0.41	0.33	0.29	0.26
Burkholderia cepacia	0.73	0.65	0.58	0.47	0.39
Bacillus megaterium	0.66	0.60	0.54	0.45	0.33
Streptococcus sp.	0.85	0.71	0.66	0.61	0.58
Proteus mirabilis	0.64	0.59	0.50	0.41	0.38
Pseudomonas mendocina	0.55	0.50	0.43	0.36	0.30
Pseudomonas putrefaciens	0.69	0.55	0.49	0.40	0.33
Providentia rettgeri	0.77	0.69	0.61	0.54	0.48
Enterococcus faecalis	0.85	0.79	0.71	0.64	0.60
Control	0.90	0.90	0.89	0.86	0.86

Table 4 Oxidation of 2, 6-dichlorophenol indophenol by the fungal isolates

Isolate	Absorbance at 600 nm				
	Day 2	**Day 4**	**Day 6**	**Day 8**	**Day 10**
Candida tropicalis	0.66	0.64	0.59	0.51	0.47
Candida lipolytica	0.78	0.71	0.64	0.59	0.50
Saccharomyces cerevisae	0.65	0.63	0.59	0.54	0.49
Aspergillus niger	0.63	0.61	0.55	0.52	0.46
Penicillium chrysogenum	0.81	0.79	0.71	0.66	0.57
Trichophyton sp.	0.77	0.71	0.68	0.61	0.59
Control	0.93	0.93	0.91	0.90	0.89

4) GC-MS results of the biodegraded soil

The GC-MS results revealed the presence of aliphatic hydrocarbons ranging from C_{10}-C_{28} and aromatic hydrocarbons including the polycyclic aromatic hydrocarbons in the SEO. Figure 3 shows the percentage degradation of SEO obtained after biodegradation. The percentage degradation obtained after six weeks showed that soils treated with spent fruit residues recorded the highest percentage degradation of SEO among the organic wastes. This might be due to the fact that spent fruit residues (SFR) contain nutrient elements needed by the hydrocarbon degrading microbes, and also because SFR harbour certain microorganisms with hydrocarbon degrading abilities. Similar findings have been documented by Bahadure et al. (2013) who reported that residues of fruits have a high biostimulation potential for naturally contaminated soil. It was also observed that the percentage degradation recorded for 10% (w/w) of the organic wastes were lower than those for 20 % (w/w) concentrations [39]. This implies that the higher the concentration of the organic waste, the greater the expected percentage degradation. Contrarily, Das and Chandran (2011)

stated that although nutrient elements are essential for successful biodegradation of hydrocarbon, excessive nutrient concentrations can also inhibit biodegradation activity [40]. Oudot et al. (1998) reported the negative effects of high NPK levels on biodegradation of hydrocarbons, especially the aromatics [41].

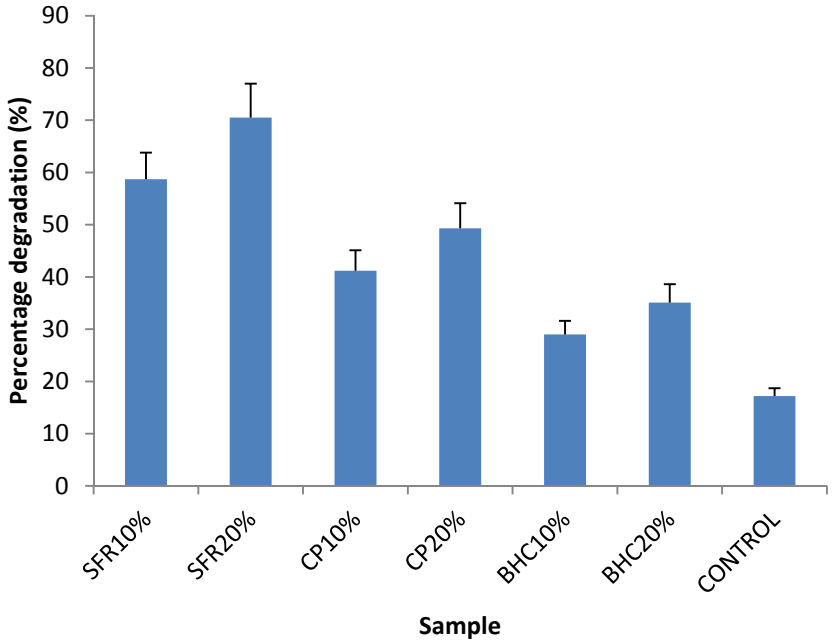

Figure 3 Percentage SEO removal in all treatments after six weeks

Conclusion

Proper application of organic wastes can effectively enhance biodegradation of soil contaminated with SEO as revealed in this study. Organic wastes contain essential nutrient elements needed for microbial growth as well as significant populations of hydrocarbon-utilizing microbes. This practice is beneficial as it removes and manages organic wastes, thereby reducing environmental pollution**.**

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