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# POTENTIAL OF BIOWASTES TO REMEDIATE DIESEL FUEL CONTAMINATED SOIL

**AGAMUTHU P.**
**DADRASNIA A. ***

*Institute of Biological Sciences*
*Faculty of Science, University of Malaya*
*Kuala Lumpur, 50603, Malaysia*

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[1,*]to whom all correspondence should be addressed:
e-mail: are.dadrasnia@gmail.com

**ABSTRACT**
The unintended release of hydrocarbons into the environment can negatively impact human and animal health, and could further change the characteristics of soils. The aim of the present work was to investigate the rate of biodegradation at 10 and 20% diesel fuel in contaminated soil amended with 10% of three different organic wastes (tea leaf, soy cake, and potato skin) for a period of 126-days. 82 and 25% oil loss was recorded in soil amended with soy cake at 10% and 20 % oil pollution, respectively. Diesel fuel utilizing bacteria counts were high in all organic wastes amended treatments, ranging from $150\times10^6$ to $176\times10^6$ CFU $g^{-1}$ of soil, compared with the unamended control soil which gave $23\times10^6$ CFU $g^{-1}$.
Dehydrogenase activity in soil was markedly enhanced by the application of organic wastes. Diesel oil composition monitored by GC/MS indicated complete degradation of n-$C_9$ – $C_{12}$. First-order kinetic model showed that among the three organic wastes used, soy cake had the highest biodegradation rate constant of 0.153 $day^{-1}$ at 10% oil pollution, while biodegradation rate was 0.033 $day^{-1}$ at 20% oil pollution. The results showed there is potential for soy cake, potato skin and tea leaf to enhance biodegradation of diesel in contaminated soil.

**KEYWORDS:** Bioremediation, Diesel fuel, Hydrocarbon, Organic waste.

## INTRODUCTION
Contamination as a result of petroleum leakages in both underground and above ground tanks via industrial and transportation processes, are hazardous to human, animals and plants (Adesodun and Mbagwu, 2008; Bamforth and Singleton, 2005; Hamdi *et al.*, 2007). Global production of crude oil is estimated at more than twelve million metric tons annually (EIA, 2011). It has been reported that 1.7 to 8.8 million metric tons of petroleum hydrocarbon escapes into the soil and world's water bodies annually. Methods for treating petroleum contaminations are through; chemical, physical and biological processes. Both physical and chemical methods of treatments are expensive, compared to biological treatments (Bioremediation); the use of microorganisms to remove hydrocarbon pollutants from the environment (Collin, 2001). This method is recognized to be efficient, cost effective and environmentally sound treatment. Bioremediation of contaminants can be accomplished by two methods; bioaugmentation and/ or biostimulation. In some previous studies, bioaugmentation (addition of commercial microbial cultures), compared with biostimulation (adding nutrients), did not significantly enhance rates of oil biodegradation (Bento *et al.*, 2005; Selina and Ian, 2005). Numerous research projects have addressed the successful applications of biostimulation in the remediation of hydrocarbon-contaminated soil and enhanced degradation (Abioye *et al.*, 2009; Adesodun and Mbagwu, 2008; Akinde and Obire, 2008; Bento *et al.*, 2005; Manuela *et al.*, 2012). Therefore, the combined actions of biostimulation through compost addition and bioaugmentation provided a good option for removal of total petroleum hydrocarbon (TPH) from diesel-contaminated soil (Taccari *et al.*, 2012). Many factors can be affected by the rate of TPH, such as lack of essential nutrients, especially nitrogen and phosphorus. Some nutrient samples like inorganic fertilizer's, compost, biowastes, and manure have been used in biostimulation studies (Adesodun and Mbagwu, 2008; Dadrasnia and Agamuthu, 2010; Namkoong *et al.*, 2002; Pankrantz, 2001). Antai (1989)

reported high rate of biodegradation of hydrocarbon's in crude oil contaminated soil with 20% and 10% compared with those contaminated with 40% and 30% oil within a period of one year. Positive effects of nitrogen amendment on petroleum hydrocarbon degradation and microbial activity have been demonstrated (Antai and Mgbomo, 1989). This study is aimed at evaluating three different organic wastes (biowastes); Tea leaf (TL), Potato skin (PS) and Soy cake (SC) as supplements for bioremediation, to enhance the biodegradation of diesel oil polluted soil. The objectives include the determination of the rate of biodegradation of the diesel in the contaminated soil and the half-life of the diesel oil degradation using kinetic model. Isolation and screening of some potential microorganisms on the diesel oil degradation, in both contaminated and uncontaminated soil was also carried out.

## MATERIAL AND METHOD

The soil used in this study, (silty loam), was obtained from the garden section of Asia-Europe Institute, University of Malaya, Kuala Lumpur. It was air dried and sieved through a 2-mm mesh sieve. The organic wastes used in this study were collected from different locations; tea leaf (TL) and potato skin (PS) was collected from the IGS (Institute of graduate studies) canteen, University of Malaya, while the soy cake (SC) was made in the laboratory. Physicochemical properties of organic wastes and soil employed were determined using standard methods. Triplicate determinations were made.

### Bioremediation Setup

Concentration range of the oil used for the treatment is within 5-25 % as reported by earlier researchers (Ijah and Antai, 2003a). 1.5 kg of fresh soil was placed in plastic poly bags, labeled A to E and contaminated with 10 and 20 % (w/w) diesel fuel (100,000 and 200,000 mg $kg^{-1}$). After 2 days, 10% each of organic wastes (TL, SC and PS) were added into each of the oil-polluted soil. These soil were mixed daily to provide sufficient aeration and moistened by the addition of water every other day to adjust the water holding capacity at 60% throughout the experimental period. The soil bags were incubated at room temperature (30 ± $2^{o}$ C). The controls with only soil and diesel fuel, additional same day "twice autoclaved" control treatment (E) at (121◦C and 15 psi for 1 h) with the addition of 0.5% (w/w) $NaN_3$ to determine the non-biological loss on diesel oil from the soil. All the treatments were set up in triplicates.

### Sampling

The contaminated soils were sampled once in every two weeks for a period of 126 days. To determination the total petroleum hydrocarbon in soil. Aerobic heterotrophic bacteria (AHB) and diesel utilizing bacteria (DUB) were isolated, counted and identified.

### Enumeration of Bacteria and Identification

0.1 mLof serially diluted soil samples were plated out on nutrient agar medium (Oxide) for isolation of aerobic, heterotrophic bacteria with 50 µg $mL^{-1}$ fungazol to suppress the growth of fungi. Plates were incubated at 32 $^{o}$C for 24, hours after which the colonies were counted. Diesel fuel utilizing bacteria in the soil samples were enumerated using oil agar (OA) (Zajic and Supplisson, 1972). The bacterial isolates were characterized using Biolog Microstation method (Ruan *et al.*, 2005).

### Measurement of diesel fuel in soil

The extent of diesel biodegradation in soil was determined by gravemtric method using rotary evaporation. Percentage of degradation of diesel fuel was calculated using the following formula (Ijah and Ukpe, 1992):

$$\% \text{ biodegradation} = [\text{TPH control} - \text{TPH treatment} / \text{TPH control}] \times 100, \quad (1)$$

where TPH is total petroleum hydrocarbon.

### $CO_2$ production and dehydrogenase activity

$CO_2$ production was determined by sampling the headspace of the sealed Wheaton bottle containing sample of soil, oil and the organic supplement. Using gas chromatography (GC-8A) with a thermal conductivity detector (Miles and Doucette, 2001). Temprature of the GC were; detector 110 $^{0}$C, injector 110 $^{0}$C and column at 130 $^{0}$C. Dehydrogenase activity was determined by monitoring the

rate of reduction of 2,3,5-triphenyltetrazolium chloride (INT) as a substrate (Margesin and Schinner, 2005).

**Seed germination toxicity test**

Ten viable seeds of lettuce (*Lactuca sativa L.*) were placed evenly on each Petri dish and covered with dry sand for 7 days. The numbers of seedlings that emerged from the surface of the sand was counted and recorded (Banks and Schultz, 2005). Germination index of lettuce seed on the remediated soil was calculated using the formula of (Millioli *et al.*, 2009).

$$\text{Germination index (\%)} = \frac{(\%\ SG)\ x(\%\ GR)}{100} \quad (2)$$

% SG = (% EG/%CG) × 100

% GR = (GERm/GERCm) ×100

where % SG = seed germination, % GR = growth of the root, % EG = germination on contaminated soil, % CG = germination on control soil, GERm = elongation of root on contaminated soil, GERCm = elongation of root on control soil.

**GC-MS analysis**

Analysis of the residual hydrocarbon in the soil was determined using GC (2010 A) coupled to a mass spectrophotometer QP2010 Plus. Helium carrier gas flow was 1.27 mL $min^{-1}$. The column oven was initially held at 100 °C for 2 min then increased to 200 °C at a rate of 10 °C $min^{-1}$,and further to 250 °C at 20 °C $min^{-1}$ (held for 5 min) (Padayachee and Lin, 2011).

**Kinetics of diesel removal and Half- Life**

First- order kinetics model used is expressed by the following equation (Chu and Chan, 2003):

$$C_t = C_i \exp(-k\,t) \quad (3)$$

where $C_t$ (mg $g^{-1}$), is the diesel fuel concentration in soil at instant t, $C_i$ (mg $g^{-1}$) is the initial concentration of soil, k is the rate constants of the first order expressed in (day $^{-1}$) and t is the time (Padayachee and Lin). The model estimated the biodegradation rate and half- life of hydrocarbons in soil relative to treatments applied.

$$\text{Half life} = \ln 2/k \quad (4)$$

The data were analyzed for significant differences ($p < 0.05$) between treatments using one-way analyses of variance (ANOVA) with SPSS 18.

## RESULTS AND DISCUSSION

**Physicochemical properties of soil and organic wastes**

The physicochemical properties of the investigated soil (silty loam) and biowastes used in this biodegradation study are presented in Table 1. It is clear that the soil had a low N (0.8%) and P (0.6%) content compared to the organic wastes. The soil used for bioremediation had C: N ratio of 16.4. Röling *et al.* (2002) reported that stimulated biodegradation of hydrocarbons in soil amended with 2.5g of N per kilogram gives C:N ratio greater than 300. This is a low ratio for effective biodegradation of oil in the soil, hence needed addition of organic wastes as a source of nutrient. Abioye *et al.*, (2009a) recorded low percentage of organic carbon in the soil (0.8) compared to yellow melon shell and white melon shell amended with 1.06 and 1.08% of organic C, respectively. SC had the highest N and P content among the three organic wastes used; this is one of the most important limiting nutrients for effective bioremediation (Kim, 2005; Okoh, 2006). The moisture content SC (75.9%) was higher than those of PS (62.1%) and TL (34.3%); this might enable SC to harbor some important microorganisms that will contribute positively to the biodegradation of oil in the soil.

*Table 1.* Physicochemical properties of soil and organic wastes used for bioremediation

| Parameters | Soil | TL | SC | PS |
|---|---|---|---|---|
| Nitrogen (%) | 0.8 ± 0.1 | 1.02± 0.08 | 1.3± 0.1 | 1.1± 0.04 |
| Phosphorus (%) | 0.6± 0.5 | 0.79± 0.68 | 0.9±0.9 | 0.7± 0.1 |
| Moisture content (%) | 10.2±0.8 | 34.3±0.5 | 75.9±1.6 | 62.1 ± 2.03 |
| Organic C (%) | 1.14± 1.3 | 0.89±1.2 | 1.26± 0.9 | 1.15 ±1.1 |
| pH | 7.03 ± 1.5 | 6.5±1.2 | 6.8±1.2 | 6.9 ± 0.5 |

TL: Tea Leaf, SC: Soy Cake, PS: Potato Skin

**Biodegradation of diesel fuel in soil**

The level of biodegradation of oil throughout this study is shown in Figures 1 and 2. The results demonstrated the high biodegradation rate of the diesel fuel (between 33% and 82%) at the end of 126 days in soil contaminated with 10% diesel fuel. The percentage of biodegradation with 20% diesel fuel showed between 5% to 26%. Abioye *et al.* (2010) reported that degradation of used lubricating oil using brewery spent grain was more than 90% within the same period. There was a rapid decrease in TPH of all the treated soils amended with organic wastes compared with unamended control soil. At the end of 56 days, there was 39%, 42% and 58% TPH reduction in soil amended with TL, PS and SC in soil amended with 10% oil respectively, compared to the control soil with 18% oil degradation (Figure 2). The reason for this relatively high and progressive biodegradation in all the soil contaminated with 10% diesel oil might be due to low concentration of oil compared with 20% oil in the soil which could pose serious challenge to the metabolic activities of the soil microorganisms. Also, it could also be due to the presence of organic waste amendments which likely provided nutrients to the microbial population present in the contaminated soil, thereby enabling them to degrade almost completely the oil contaminant.

Bartha (1986), reported that when oil is applied to soil at rates of 0.5 to 10% based on weight, extensive biodegradation of the oil components occurred within the first three months. Result indicated 9% and 4% of degradation in autoclaved control at 10 and 20% oil, respectively.

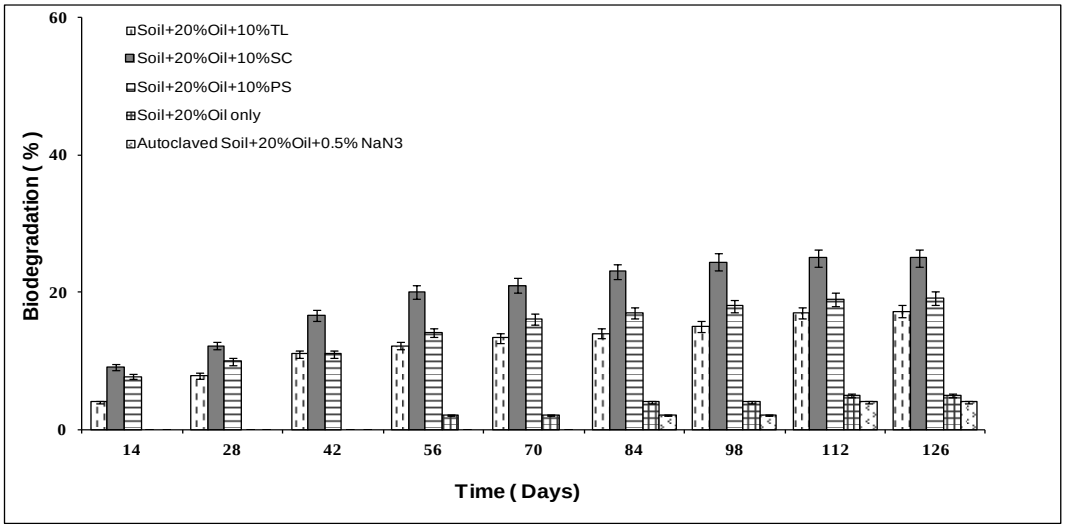



*Figure 1***.** Biodegradation of soil amended with 10 % organic waste and 20% diesel fuel. Error bars indicate standard errors(n = 3)

It might be due to non-biological factors such as evaporation or photodegradation. This was based on poisoned control soil i.e. autoclaved contaminated soil treated with 0.5% sodium azide. This was contrary to the findings of Palmroth *et al.* (2002), who recorded as high as 70% diesel fuel loss within 28 days of study. The differences in these results might be because poisoned control in this study was autoclaved soil mixed with 0.5% sodium azide, whereas, Palmroth *et al.* (2002), used only 0.5% sodium azide without autoclaving the soil, thus the sodium azide possibly could not completely sterilize the soil.

In soil contaminated with 20% oil, there were 26%, 20%, and 17% TPH degradation in soil amended with SC, PS, and TL, respectively. The reason for the low oil degradation be attributed to the toxicity of the oil on the microbial flora of the soil, due to high concentration of oil which propably had a negative effects on the biodegradative activities of the microbial population. However, there are significantly differences among organic wastes and control during the degradation period. Results indicate that the degradation of oil in soil amended with SC was significant different with soil amended compared to PS and TL ($p < 0.05$). Similar results were obtained by Adesodun and Mbagwu (2008), who reported significant differences between the soils amended with cow dung and poultry manure in soil polluted with crude oil.

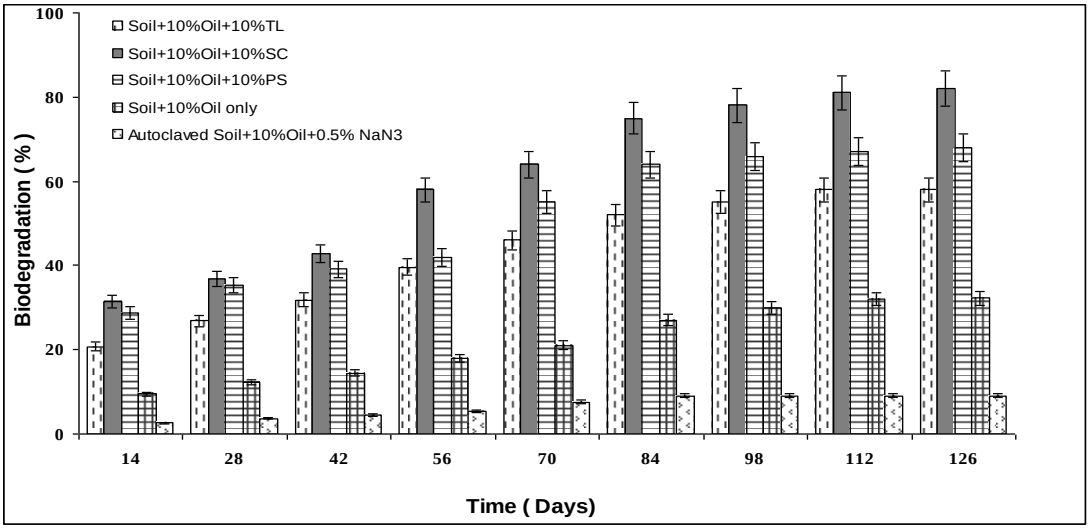



*Figure 2.* Biodegradation of soil amended with 10 % organic waste and 10% diesel fuel. Error bar indicate standard errors (n = 3)

The highest loss of TPH was recorded in SC amended soil during the 126 days, followed by PS and TL. This may be due to the presence and bioavailability of nutrient elements like N (1.3%) and P (0.9%) in SC (Table 1). Nitrogen is known as one of the limiting nutrient necessary for biodegradation of organic pollutants in soil (Barahona *et al.*, 2005). It is also supported by the findings of Joo *et al.* (2007), who observed that the addition of food waste compost to contaminated soil, leads to increase in the rate of removal of diesel fuel in the soil. However, the rate of oil breakdown in soil amended with TL was lower than other organic wastes, which may be due to low pH recorded in soil treated with TL throughout this study. Low pH is known to affect the rate of oil breakdown by bacteria in oil contaminated soil (Okoh, 2006). The decrease in the degradation rates were associated with increased oil concentrations (Ijah and Antai, 2003b).

**Microbial population**

Active aerobic heterotrophic bacterial colonies (AHB) were recorded in SC treated soil, ranging from $11\times10^7$ to $141\times10^7$ CFU $g^{-1}$, while AHB counts in PS and TL ranged between $8\times10^7$ and $115\times10^7$ CFU $g^{-1}$ and $10\times10^7$ to $122\times10^7$ CFU $g^{-1}$ of polluted soil with 10% oil, respectively (Figure 3). Unamended soil (control) gave a range of $1\times10^7$ to $20\times10^7$ CFU $g^{-1}$ of soil, which is in line to the findings of (Ijah and Antai, 2003b). It was reported by Hinchee (1995), that when the population of microorganisms capable of degrading the target contaminant is less than $10^5$ colony-forming units (CFU $g^{-1}$ of soil), bioremediation will not occur at a significant rate. However, at the end of the experiment, soil amended with 20% diesel fuel SC recorded higher number of colony ($70\times10^7$ CFU $g^{-1}$) compared to other treatments and the control treatment. Statistical analysis showed that there was significant difference between treatments and control at ($p < 0.05$). DUB isolated ( *Bacillus licheniformis, Bacillus circulans, Ochrobactrum tritici and Staphylococcus acidaminiphila*) from the oil contaminated soil were identified by using Biolog methods.

These bacteria species had been identified in organic compound degradation by different researchers (Abioye *et al.*, 2009; Bento *et al.*, 2005; Margesin *et al.*, 2007).

**Germination toxicity test and Seed Germination Index**

The results of germination toxicity test with lettuce for soil amended with 10% and 20% diesel fuel are shown in Tables 2 and 3. Lettuce is sensitive to toxic chemicals (mostly petroleum contaminants) and it is an important agricultural crop, which led to its wide use for toxicity tests (Banks and Schultz, 2005; Oleszczuk, 2008). The results indicated 90%, 70%, and 60% germination in soil contaminated with 10% oil amended with SC, PS, and TL, respectively.

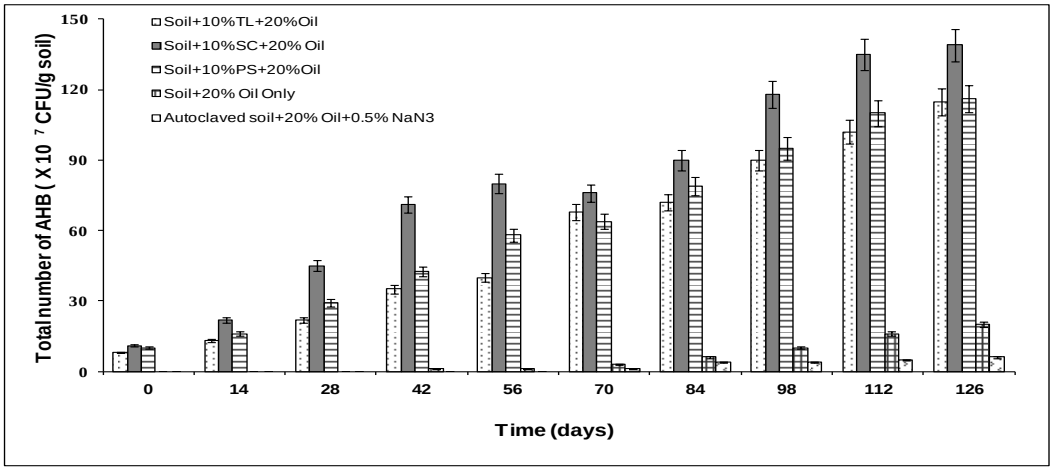



*Figure 3.* Aerobic heterotrophic bacteria (AHB) population in 10% diesel fuel polluted soil. Vertical bars indicate SE (*n* = 3)

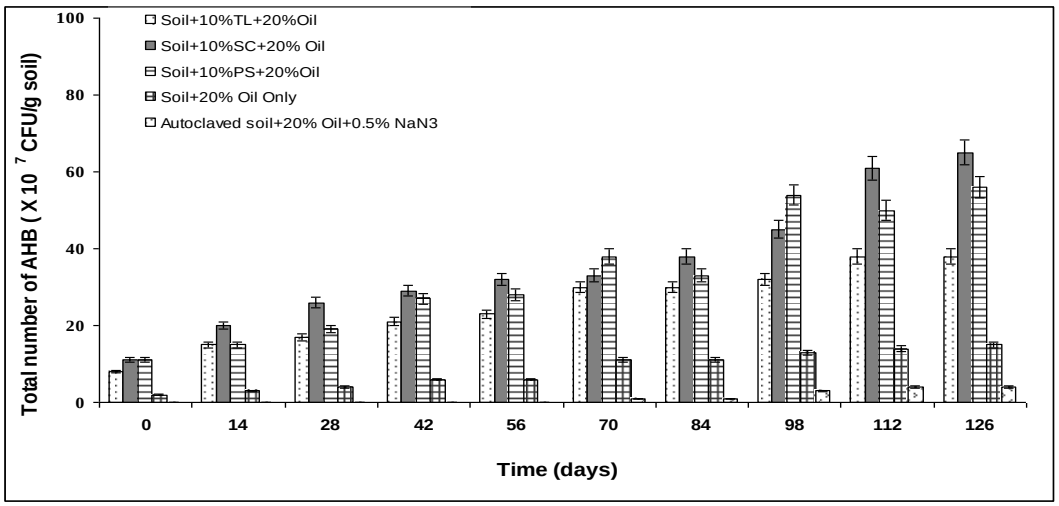



*Figure 4.* Aerobic heterotrophic bacteria (AHB) population in 20% diesel fuel polluted soil. Vertical bars indicate SE (*n* = 3)

*Table 2.* Seed germination (%) toxicity test with 10 % organic waste amendments

| Percentage of oil pollution | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | A | B | C | D | E | F |
| 10 | 60 ± 8 | 90 ± 10 | 70 ± 7 | 30 ± 5 | 0 | 100 |
| 20 | 20 ± 6 | 40 ± 6 | 20 ± 5 | 10 ± 5 | 0 | 100 |

A = Soil + Oil+ TL, B = Soil + Oil + SC, C = Soil + Oil + PS, D = Soil + Oil, E = Autoclaved soil + Oil + $NaN_3$, F = Uncontaminated soil

However, 40%, 20%, and 20% seed germination were recorded in soil contaminated with 20% oil and amended with SC, PS, and TL, respectively. 100% germination was recorded in uncontaminated control soil, while 0% was recorded in poisoned controlled soil in both diesel oil

concentrations. The result shows positive correlation between loss of oil in the remediated soil and seed germination. It was also obvious that remediation of soil contaminated with high concentration of petroleum hydrocarbons needed a longer period of time. Bank and Schultz (2005) and Millioli *et al.* (2009) recorded a decrease in the number of germinated seeds with increased quantities of petroleum concentration in the soil. Germination index (GI) of soil treated with SC recorded the highest value (63.33 and 10) in soil amended with 10 and 20 % oil (Table 3). However, the GI of unamended polluted soil and that of amended soil contaminated with 20% diesel oil was very low, signifying low biodegradation of oil in this treatment. Hydrocarbons may affect root surface, preventing or reducing gas and water exchange and nutrient absorption. They may also enter the seeds and alter the metabolic reactions and kill the embryo. Hydrocarbons damage cell membranes and reduces the metabolic transport and respiration rate (Ogboghodo *et al.*, 2004; Oleszczuk, 2008).

*Table 3.* Seed germination (%) index test with 10 % organic waste amendments

| Percentage of oil pollution | Treatments | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| 10 | 34.33 | 63.33 | 40.00 | 12.34 | 0 |
| 20 | 2.3 | 10.00 | 6.67 | 1.06 | 0 |

A = Soil + Oil+ TL, B = Soil + Oil + SC, C = Soil + Oil + PS, D = Soil + Oil, E = Autoclaved soil + Oil + $NaN_3$

**Microbial activity and soil respiration**

Dehydrogenase enzyme activity has been used to monitor microbial activity as an index for the total oxidative activity (Alef, 1995). At the second sampling (42-d), dehydrogenase activity of the contaminated soil with SC showed a value significantly (2.2 -fold) higher than that of uncontaminated soil at 10% oil concentration. This may be an evidence that high intensity of microbial activity took place at the initial stage of degradation, which could have efficiently utilized organic carbon of TPH. Therefore, dehydrogenase activity may be used as a parameter representing microbial activity. At the end of the experiment all of the amendments showed a decrease in microbial activity, this final decrease indicated the lack of optimum growth conditions for microorganisms. Increase in microbial activity in this research indicates that the high population of organisms in those treatments which were amended with organic wastes is metabolically more active and may contribute to the biodegradation process of TPH, compared to unamended treatments (Lee *et al.*, 2008). These results is similar to the findings of Margenis *et al.* (2000) who reported an initial phase characterized by an increase in dehydrogenase activity in hydrocarbon contaminated soils. CO2 evolution has been used as an index representing microbial activity, because CO2 is a by-product of organic compound degradation. The cumulative amount of CO2 evolved shows more distinct effect of organic amendments on TPH degradation. The metabolic activity (respiration) of microbes increased significantly. Dramatic increase in CO2 evolution at early stage was probably due to the rapid degradation of TPH at the same period. In treatments (TL, SC and PS), where hydrocarbons were added to soil, except for the sterilized contaminated soil (SCS), it was possible to observe significantly higher values for CO2. Tables 4 and 5 showed the correlation coefficients for TPH degraded, cumulative CO2 evolved and dehydrogenase activity. Degradation of TPH was significantly related to microbial respiration as measured by CO2 evolution ($r = 0.91$, $P \leq 0.01$) at 10% oil pollution. High positive correlation was also found between TPH degraded and dehydrogenase activity.

*Table 4.* Matrix of correlation coefficients for the parameters used in this research at 10% diesel fuel

| | TPH degraded | Cumulative $CO_2$ | Dehydrogenase activity |
|---|---|---|---|
| TPH degraded | 1.00 | 0.91** | 0.89** |
| $CO_2$ | | 1.00 | 0.91** |
| Dehydrogenase activity | | | 1.00 |

** Correlation is significant at the 0.01 level

*Table 5***.** Matrix of correlation coefficients for the parameters used in this research at 20% diesel fuel

| | TPH degraded | Cumulative $CO_2$ | Dehydrogenase activity |
|---|---|---|---|
| TPH degraded | 1.00 | 0.92** | 0.91** |
| $CO_2$ | | 1.00 | 0.89** |
| Dehydrogenase activity | | | 1.00 |

** Correlation is significant at the 0.01 level

**GC-MS analysis**

The residual oil was analyzed and identified from their mass spectra and retention times, as indicated by the chromatogram of the remaining diesel after biodegradation tests. Hydrocarbons above $C_{14}$ adsorbed to the soil particle, which makes them less volatile, and they do not give a detectable concentration in the gas phase when sampling times are as short as those used in this experiment (Dalhammar, 1998). Significant reduction in diesel content ($C_8$-$C_{26}$) was observed in the biostimulation samples compared to the natural attenuation and the sterilized controls. A decrease in the intensities of hydrocarbon in all supplemented treatments and natural attenuation were observed, compared with those in the sterilized samples (Figure 5). The peaks of long-chain petroleum hydrocarbons were relatively higher than those of short chain hydrocarbons. Similar results were shown by (Huang *et al.*, 2005).

**Kinetics of diesel removal and Half-life**

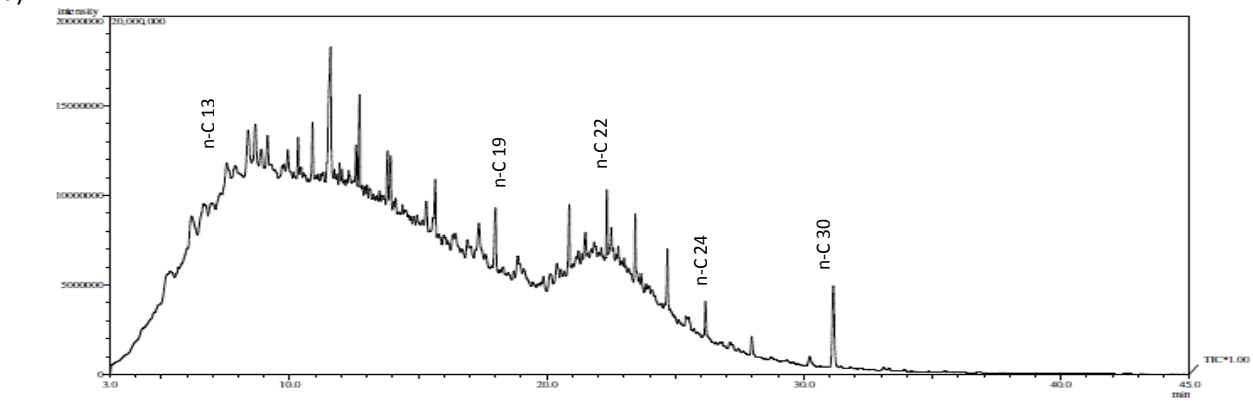
First- order kinetics model proposed by Chu and Chan (2003), was used for determination of biodegradation of used oil in the various treatments. Table 6, shows the biodegradation rate constant (k) and half-life ($t_{1/2}$) for the different treatments. Soil amended with SC had the highest biodegradation rate of 0.153day $^{-1}$ and half life 4.53 days at 10% concentration of diesel oil; the biodegradation rate and half-life of PS and TL were 0.115 day $^{-1}$, half-life 6.02 days and 0.076 day $^{-1}$, half-life 9.12 days at 10 % concentration oil, respectively. The biodegradation rate of unamended control and autoclaved soil were observed to be 0.037 day$^{-1}$ and 0.01 day $^{-1}$. It has been reported by Namkoong *et al.* (2002) that higher biodegradation rate constant and low half-life in diesel contaminated soil were observed in 1:0.3 sewage sludge compared to those amended with 1:0.1 under the same conditions. Adesodun and Mbagwu (2008), reported that biodegradation rates in oil contaminated soil, amended with pig wastes, showed highest percentage of biodegradation throughout the study period.

*Table 6*. Biodegradation rate and half-life of hydrocarbon for oil-polluted soil

| Treatment | Biodegradat constant (k) day$^{-1}$ | Half- life ( days) $t_{1/2}$ | Coefficients of determination ($r^2$) |
|---|---|---|---|
| Soil+10%Oil+TL | 0.076 | 9.12 | 0.92 |
| Soil+10%Oil+SC | 0.153 | 4.53 | 0.97 |
| Soil+10%Oil+PS | 0.113 | 6.02 | 0.90 |
| Soil+ 10% Oil | 0.037 | 18.74 | 0.94 |
| Autoclaved soil + 10% | 0.010 | 64.78 | 0.89 |
| Soil+20%oil+TL | 0.019 | 35.36 | 0.90 |
| Soil+20%Oil+SC | 0.033 | 21.00 | 0.88 |
| Soil+20%Oil+PS | 0.025 | 27.50 | 0.85 |
| Soil+ 20% Oil | 0.001 | 70.73 | 0.91 |
| Autoclaved soil + 20% | 0.001 | 630.13 | 0.98 |

* Shows significant difference at the $p < 0.05$ level respectively.

a)

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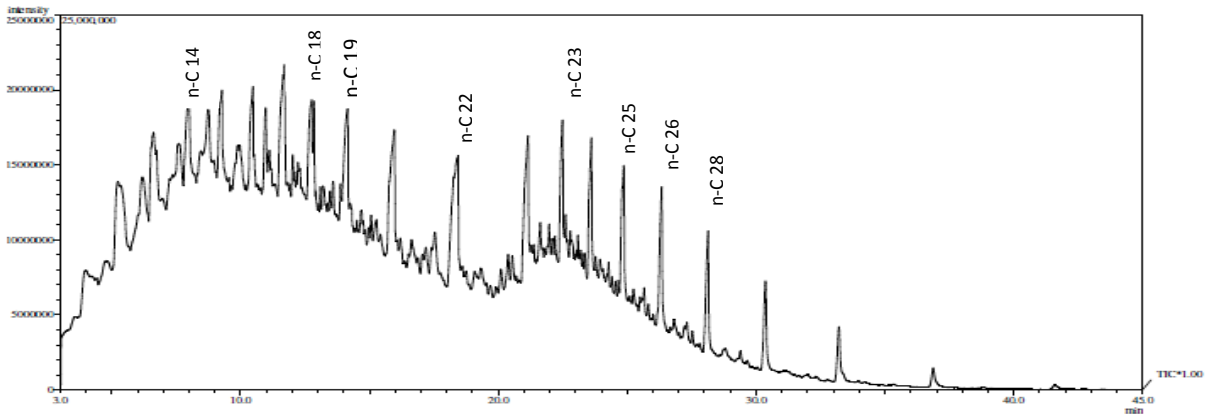


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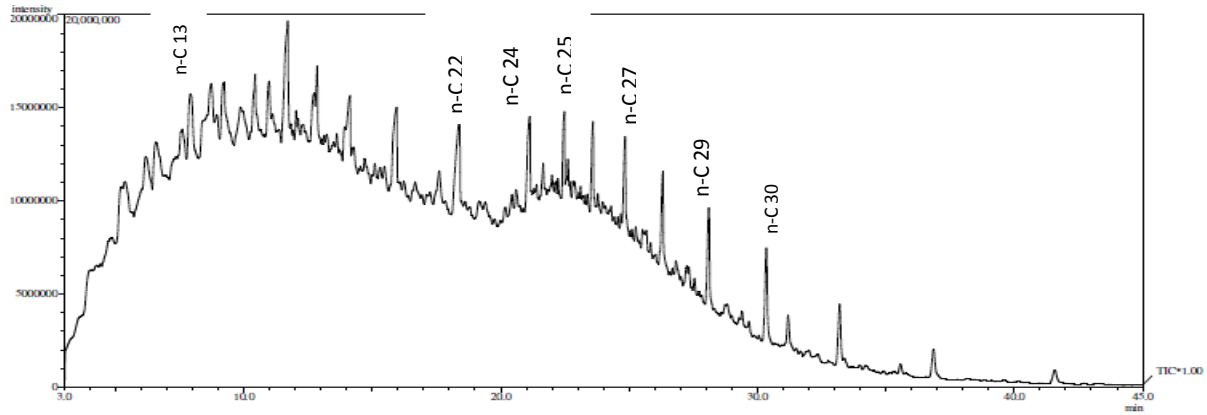


*Figure 5.* Chromatogram of residual diesel fuel in soil amended with SC at the end of experiment a) 10% oil concentration, b) 20% oil concentration and c) control at 0 day.

## CONCLUSION

The results of this study demonstrated the potential of organic wastes amendments to remediate hydrocarbons contaminated soil. In this study, a significant reduction in diesel fuel was achieved by adding soy cake, which is a waste from soy bean processing, possibly because it was more effective than other amendments in providing an alternative source of N and P, to stimulate microbial activity. High correlation ($r = 0.91, 0.89$) was found among the amount of TPH degraded, the amount of $CO_2$ evolved, and dehydrogenase activity at 10% oil. The study therefore proves the viability of using SC amendment in remediating hydrocarbon-contaminated soil. This affords an alternative method in

removing oil contaminants from soil. Kinetic model data in this study showed that the rate of degradation of diesel oil in soil amended with SC was higher than all other treatments. Overall, the differential performance of these organic amendments followed SC > PS > TL.

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