Stimulation and Bioremediation of Diesel- contaminated Soil Using Organic Wastes

Dadrasnia, A. * and Agamuthu, P.

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

Tel :+6012-2590518 *Fax*: +60 379674631

*(Corresponding author: are.dadrasnia@gmail.com)

ABSTRACT

Various aspects of wide-scale production, transportation, global use and disposal of petroleum products have made these a major source of contaminants in the environment. Bioremediation of oil contaminated site with amendment of organic material (waste) is a viable choice. This study evaluated the applicability of three organic wastes: tea leaf (TL), soy cake (SC) and potato skin (PS) as bioremediation enhancers for soils contaminated with 10% (w/w) diesel fuel, for a period of three months, under laboratory condition. The total hydrocarbon content was markedly reduced with the addition of amendments. At the end of 84 days, the highest percentage of oil biodegradation (76%) was recorded in soil amended with SC. Hydrocarbon utilizing bacteria (HUB) and diesel utilizing bacteria (DUB) were significantly abundant in oil polluted soil amended with organic wastes, compared to unamended polluted soil. As for toxicity, 90%, 80% and 60% seed germination was recorded in soils amended with SC, PS and TL, respectively, over this period. Two predominant bacterial species were isolated and identified (*Bacillus licheniformis* and *Ochrobactrum tritic)* as the prime enhancers of bioremediation. First-order kinetic model revealed that SC was the best of the three organic wastes used, with biodegradation rate constant of 0.103 day^{-1} and half-life of 6.67 days. High correlation was found among the amount of TPH degraded, the amount of CO_2 evolved, and the dehydrogenase activity. In conclusion, significant degradation of the diesel fuel was achieved by addition soy cake, which possibly provided an alternative source of N and P, to stimulate microbial activity. The results obtained demonstrated the potential of organic wastes for diesel bioremediation in the order SC > PS > TL.

Key words: Biodegradation, Diesel fuel, Hydrocarbon, Organic waste

1. Introduction

Every year, 1.7 and 8.8 million metric tons of oil are released into the world's water and soil every year (Abu and Dike 2008) due to leakage from underground and aboveground storage tanks, as well as other accidental releases (Gallego et al. 2001, Juteau et al. 2003, Juteau P 2003). In the recent years, a high number of polluting compounds has been released into the environment because of several industrial and/or agricultural activities. Due to their widespread use, diesel fuel and other petroleum distillates are among the most common environmental pollutants. Diesel fuel is a complex mixture of hydrocarbons with an average carbon number of C_8–C_{26} (Gallego et al, 2001).

Over the last decades, there has been an increasing interest in biological methodologies, collectively indicated as bioremediation that may help reduce the risk of organic pollutants in soil and effectively restore polluted sites. Bioremediation may restore contaminated soils through the broad biodegradative capabilities evolved by microorganisms towards undesirable organic compounds (Reid et al. 2000). Understanding bioremediation and its effectiveness is rapidly advancing, bringing available molecular approaches for examining the presence and expression of the key genes involved in microbial processes.

Using fertilizer provides nutrients (N and P) in order to increase the capability of the organisms to degrade hydrocarbon in the soil (Molina-Barahona et al. 2004). However, the use of fertilizer to remove oil spill may be expensive and leads to contamination of underground water. In this study we investigated the potential of tea leaf, soy cake and potato skin as a organic waste for enhanced biodegradation of diesel fuel in contaminated soil and, also to determine the optional organic waste that is cheap and available in our environment for stimulating oil degradation.

2. Methods:

2.1 collection of samples

Soil sample used was collected from the Nursery section of Asia–European Institute, University of Malaya, Kuala Lumpur in a sack and transported to the laboratory for analysis. Diesel fuel was purchased from petrol station in Petaling Jaya, Malaysia. Organic wastes used in this study were collected from different locations; tea leaf (TL) and potato skin (PS) were collected from

IGS canteen, University of Malaya and soy cake (SC) was prepared in the laboratory. Physicochemical property of soil and the organic wastes were determined using standard methods.

2.2 Microcosm Set-up Description

Soil (1.5 kg; sieved with 2-mm mesh size) was placed in plastic bags labeled A to D and polluted with 10% (w/w; Ijah and Antai, 2003a) diesel fuel (100,000 mg kg^{-1} soil) and left undisturbed for 2 days. After 2 days, 10% of each organic waste was individually introduced into each oil-polluted soil labeled A, B, and C, respectively, and thoroughly mixed. Vessel D with only soil and diesel fuel acted as control. Additional control treatment comprising of autoclaved soil containing 0.5% (w/w) NaN_3 was also set up, to determine non-biological loss of diesel oil from the soil. The soils were mixed daily to provide sufficient air and oxygen. The soil was moistened by the addition of distilled water every two days to adjust water holding capacity to 60% throughout the experimental period. The plastic bags were incubated at room temperature (30 ± 2^{o} C). All the treatments were set up in triplicates.

2.3 Sampling and analysis

Periodic sampling from each vessel was carried out at 14-day intervals for 84 days. Composite samples were obtained by mixing 5 g of soil collected from four different areas of the microcosm in order to determine pH, organic carbon, dehydrogenize enzyme, total petroleum hydrocarbon and isolation, enumeration and identification of bacteria.

Hydrocarbon utilizing bacteria (HUB) in the soil samples was enumerated using oil agar (OA) of Zajic and Supplission (Zajic and Supplisson 1972); (1.8 g K_2HPO_4, 1.2 g KH_2PO_4, 4.0 g NH_4Cl, 0.2 g $MgSo_4$.7H2O, 0.1 g NaCl, 0.01 g $FeSO_4.7H_2O$, 20 g agar, 2 ml diesel fuel, 1000 ml distilled water). The oil agar plates were incubated for 5 days at 30 ^{o}C before counting the colonies. Bacterial colonies were randomly picked and pure culture obtained by repeated sub-culturing on nutrient agar. The bacterial isolates were characterized based on their color and biochemical properties (Krieg et al., 1994).

2.4 Total Petroleum Hydrocarbon (TPH)Determination

The total extent of diesel fuel biodegradation in soil was determined by suspending 10 g of soil in 20 ml of n-hexane in a 250 ml capacity flask. After shaking for 1 h on an orbital shaker (Model N-Biotek), the solvent- oil mixture was filtered and completely evaporated by using rotary evaporation (Ijah and Ukpe 1992).

2.5 CO_2 production & Dehydrogenase activity

CO_2 production was determined by sampling the headspace of the bottle microorganism. Air samples (1 ml) were collected from each bottle at 7, 14, 21, 28, 35 and 42 days, and analyzed using gas chromatography (GC-8A) with a thermal conductivity detector (Miles and Doucette 2001). Dehydrogenase activity was determined by monitoring the rate of reduction of 2,3,5-triphenyltetrazolium chloride (INT) as a substrate (Rosa and Franz 2005).

2.6 Seed Germination Toxicity Test Of Remediated Soil

Toxicity of the remediated soil was assessed using germination test. Lettuce (*Lactuca sativa* L.) was used in this study owing to its sensitivity to hydrocarbon in soil(Vaajasaari et al. 2002) .The germination test was conducted over a five days test period. The number of seedlings that emerged from the surface of the sand was counted and percentage of seed germination calculated.

2.7 Kinetics of diesel removal and Half- Life

Total Petroleum Hydrocarbon (TPH) data was fitted to first-order kinetics model of Yeung et al. (1997),

$Y = ae^{-kt}$

where y is the residual hydrocarbon content in soil (g kg−1), a is the initial hydrocarbon content in soil (g kg−1), k is the biodegradation rate constant (day−1), and t is time (day). The model estimated the biodegradation rate and half-life of hydrocarbons in soil relative to treatments applied. Half-life was then calculated from the same model

Half life = ln(2)/k

This model was based on the assumption that the degradation rate of hydrocarbons positively correlated with the hydrocarbon pool size in soil (Yeung et al., 1997).

2.8 Gas chromatography-mass spectroscopic analysis

Samples of soil removed at the initial and final stages of the experiment were analyzed by GC/MS to determine the quantity and composition of the total hydrocarbons. GC/MS analyses of all samples were carried out in the Chemistry laboratory, University of Malaya. GC model was 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, increased to 200°C at a rate of 10°C min^{-1}, then to 250°C at 20°C min^{-1} (held for 5 min)(Padayachee and Lin 2011).

Statistical analysis of data was carried out using Analysis of Variance (ANOVA) with SPSS 19.

3. Result and discussion:

3.1 Physicochemical properties of soil and organic wastes

The physicochemical properties of the investigated soils and organic wastes used in bioremediation are presented in Table 1. It is clear that the soil had low N (0.8%) and P (0.6%) content compared to organic wastes. The soil used for bioremediation had C: N ratio of 16.4. This is a low ratio for effective biodegradation of oil in the soil, hence needed addition of organic wastes as a source of nutrients (Wilfred et al. 2002)

3.2 Biodegradation of diesel fuel in soil

The level of biodegradation of oil throughout this study is shown in Fig.1. The extent of fuel degradation in organic waste amended soil ranged between 52% to 76% after 84 days compared to control autoclaved soil with 9% oil degradation. This finding is similar to that of, Abioye et al., (2010) who reported that degradation of used-lubricating oil using brewery spent grain was more than 90% within the same period. However, the total extent of diesel fuel biodegradation was about 12% higher in soil amended with SC than that of PS, and about 23% higher than that of soil amended with TL. This might be due to high N and P content in SC (Table1), because these two elements are known as most important nutrients needed by hydrocarbon utilizing

bacteria to carry out effective and efficient biodegradative activities of xenobiotics in the soil environment(Dadrasnia and Agamuthu 2010, Ijah and Antai 2003, Kim 2005).

During this study there was a rapid decrease in total petroleum hydrocarbon in all the treatments amended with organic wastes, compared to unamended soil. Effective bioremediation of soil by organic wastes and compost has been reported (Adesodun and Mbagwu 2008; Joo et al. 2007).

3.3 Enumeration of the heterotrophic count (HPC)

Statistical analysis revealed that there is significant difference in the counts of hydrocarbon utilizing bacteria (HUB) between the amended soil and unamended soil ($P<0.05$). HUB recorded in SC treated soil ranged from 10×10^6 to 90×10^6 CFU/g, while HUB counts in PS and TL ranged between 8×10^6 to 55×10^6 CFU/g and 8×10^6 to 45×10^6 CFU/g, respectively. This finding agrees with the report of Odu (1972) who reported that the highest application of oil (39%) to Nigerian soil possessed the highest number of bacteria(Adesodun et al. 2010, Odu 1972).

3.4 Seed Germination Toxicity Test of Remediated Soil

The germination test was conducted over a five days test period (Vaajasaari et al., 2002). The result shows 90 %, 80 % and 60% of seed germination in soil treatment with TL, SC and PS, respectively. These results further proved the effectiveness of SC in enhancing biodegradation of hydrocarbon in oil contaminated soil. These results are similar to the findings of Oleszczuk (2008), who reported that compost reduced phytoxicity of diesel and wastewater sludge after 76 days (Oleszczuk 2008).

3.5 Correlation among Microbial activity, TPH and CO_2

Table 3, shows the correlation coefficients among TPH degraded, cumulative CO_2 evolved and dehydrogenase activity. Degradation of TPH was significantly related to microbial respiration as measured by CO_2 evolution ($r = 0.90$, $P \leq 0.01$). Significant positive correlation between the amount of CO_2 evolved and the dehydrogenase activity was also found ($r = 0.87$, $P \leq 0.01$) and there was also correlation between TPH and Dehydrogenase was ($r = 0.90$, $P \leq 0.01$). This result indicates that the amount of CO_2 evolved and dehydrogenase activity matched well with TPH degradation (Balba et al., 1998; Mehrasbi et al, 2003)

3.6 Kinetics of diesel removal and Half-life

Soil amended with SC had the highest biodegradation rate of 0.103 day $^{-1}$ and half life of 6.67 days; the biodegradation rate and half-life of PS and TL were 0.085 day $^{-1}$, half-life 8.15 days and 0.0063 day $^{-1}$, half-life 10.8 days, respectively (table 4) . Adesodun and Mbagwu (2008), who showed highest biodegradation rate in oil contaminated soil amended with pig wastes, had highest percentage of biodegradation throughout the study period (Adesodun and Mbagwu 2008).

3.7 GC-MS analysis

Significant reduction in diesel content (C_8-C_{26}) was observed in the biostimulation samples compared to the natural attenuation and the sterilized controls. The substances monitored were analyzed and identified from their mass spectra and retention times, as indicated by the chromatogram of the remaining diesel after biodegradation tests (Fig. 2). The hydrocarbons above C_{14} adsorb to the soil particles, 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). A decrease in the intensities of hydrocarbon in all supplemented and naturally attenuate was observed, compared with those in the sterilized samples. 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).

4. Conclusion

Our study demonstrates the stimulating effect of organic wastes amendments on bioremediation of diesel-contaminated sites. Bioremediation, with addition of amendments, is a viable choice for the remediation of oil contaminated soil. Biodegradation of diesel fuel was high (53–76%) in all the soil amended with different organic wastes compared to the unamended soil (27%). Kinetic model data in this study showed that the rate of degradation of diesel fuel in soil amended with SC was higher than all other treatments. HUB counts in all the soil amended with various organic wastes were higher compared to that of unamended control soil. This may be due to differences in microbial ecology of the soil or characteristics of the experimental soils. The

reason for higher counts of bacteria in amended soil may be the result of the presence of appreciable quantities of N and P in the organic wastes, especially high N content in SC which is a necessary nutrient for bacterial biodegradative activities.

Other advantages of inorganic nutrients in organic wastes as bioremediation agents include low cost, availability and ease of application. In conclusion, the remediation method adopted in this study is simple and inexpensive.Therefore, the results obtained demonstrated the potential of organic wastes for oil bioremediation in the order SC > PS > TL.

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Refrences:

Abioye, P., Abdul Aziz, A., & Agamuthu, P. (2010). Enhanced Biodegradation of Used -Engine Oil in Soil Amended with Organic Wastes. Water, Air, & Soil Pollution, 209(1), 173-179.

Abu GO, Dike PO. (2008). A attstudy of natural attenuation processes involved in a microcosm model of a crud oil impacted wetland sediment in the Niger delta. Bioresource Technology 9: 4761-4767.

Adesodun JK, Mbagwu JSC. (2008). Biodegradation of waste-lubricating petroleum oil in a tropical alfisol as mediated by animal droppings. Bioresource Technology 99: 5659-5665.

Adesodun JK, Atayese M, Agbaje T, Osadiaye B, Mafe O, Soretire A. 2010. Phytoremediation Potentials of Sunflowers for Metals in Soils Contaminated with Zinc and Lead Nitrates. Water, Air, & Soil Pollution 207: 195-201.

Balba MT, Al-Awadhi N, Al-Daher R. 1998. Bioremediation of oil-contaminated soil: microbiological methods for feasibility assessment and field evaluation. Journal of Microbiological Methods 32: 155-164.

Dadrasnia A, Agamuthu P. 2010. Enhanced Degradation of Diesel-Contaminated Soil using Organic Wastes. Malaysian Journalof Science 29: 225-230.

Dalhammar MEáASáG. 1998. Biological degradation of diesel fuel in water and soil monitored with solid-phase micro-extraction and GC-MS. Appl Microbiol Biotechnol 50: 129-134.

Gallego JLR, Loredo J, Llamas JF, Vázquez F, Sánchez J. 2001. Bioremediation of diesel-contaminated soils: Evaluation of potential <i>in situ</i> techniques by study of bacterial degradation. Biodegradation 12: 325-335.

Huang X-D, El-Alawi Y, Gurska J, Glick BR, Greenberg BM. 2005. A multi-process phytoremediation system for decontamination of persistent total petroleum hydrocarbons (TPHs) from soils. Microchemical Journal 81: 139-147.

Ijah UJJ, Ukpe LI. 1992. Biodegradation of crude oil by bacillus strains 28A and 61B isolated from oil spilled soil. Waste Management 12: 55-60.

Ijah UJJ, Antai SP. 2003. Removal of Nigerian light crude oil in soil over a 12-month period. International Biodeterioration & Biodegradation 51: 93-99.

Joo H-S, Shoda M, Phae C-G. 2007. Degradation of diesel oil in soil using a food waste composting process. Biodegradation 18: 597-605.

Juteau P, ., Bisaillon G, Lepine F, Ratheau V, Beaudet R, Villemur R. 2003. Improving the biotreatment of hydrocarbon-contaminated soils by the addition of activated sludge taken from the wastewater treatment facilities of an oil refinery. Biodegradation 14: 31-40.

Juteau P BG, Lepine F, Ratheau V, Beaudet R, Villemur R. 2003. Improving the biotreatment of hydrocarbon-contaminated soils by the addition of activated sludge taken from the wastewater treatment facilities of an oil refinery. Biodegradation 14: 31-40.

Kim S CD, Sim DS, Oh Y. 2005. Evaluation of bioremediation effectiveness on crude oil-contaminated sand. Chemosphere 59: 845 - 852.

Mehrasbi M, Haghighi B, M Shariat C, Naseri S, Naddafi K. 2003. Biodegradation of petroleum hydrocarbons in soil as affected by heating and forced aeration Iranian J Publ Health, 32: 28-32.

Miles RA, Doucette WJ. 2001. Assessing the aerobic biodegradability of 14 hydrocarbons in two soils using a simples microcosm/respiration method. Chemosphere 45: 1085-1090.

Molina-Barahona L, Rodríguez-Vázquez R, Hernández-Velasco M, Vega-Jarquín C, Zapata-Pérez O, Mendoza-Cantú A, Albores A. 2004. Diesel removal from contaminated soils by biostimulation and supplementation with crop residues. Applied Soil Ecology 27: 165-175.

MR Mehrasbi1 BH, M Shariat 1, S Naseri 1, K Naddafi. 2003. Biodegradation of petroleum hydrocarbons in soil as affected by heating and forced aeration Iranian J Publ Health, 32: 28-32.

Odu CTI. 1972. Microbiology of soil contaminated with petroleum hydrocarbons. 1. Extent of contamination and some soil microbial properties after contamination. Journal of Institute of Petroleum 58: 201-208.

Oleszczuk P. 2008. hytotoxicity of municipal sewage sludge composts related to physicochemical properties, PAHs and heavy metals. Ecotoxicology and Environmental Safety 69: 496–505.

Padayachee D, Lin J. 2011. The effect of fertilizer amendment on diesel biodegradation in contaminated soils. African Journal of Microbiology Research 5: 1729-1739.

Rosa M, Franz S. 2005. Manual of soil analysis-Monitoring and assessing soil bioremediation soil biology. 5: 309-320.

Vaajasaari K, Joutti A, Schultz E, Selonen S, Westerholm H. 2002. Comparison of terrestrial and aquatic bioassays for oil-contaminated soil toxicity. Journal of Soil and SedimentS 4: 194 – 202.

Wilfred FM, Ro¨ling L, Michael G, Milner D, Martin J, Kenneth L, Fabien D, Richard J, Swannell P, Ian M. 2002. Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation. Applied And Environmental Microbiology, 68: 5537–5548.

Zajic JE, Supplisson B. 1972. Emulsification and degradation of “Bunker C” fuel oil by microorganisms. Biotechnology and Bioengineering 14: 331-343.

Table 1: Physicochemical Properties of Soil and Organic Wastes Used for Bioremediation

		Organic Wastes		
Parameters	Soil	TL	SC	PS
Total nitrogen (%)	0.8 ± 0.1	1.02± 0.08	1.3± 0.1	1.10±0.04
Phosphorus (%)	0.6± 0.5	0.7± 0.6	0.9±0.9	0.7±0.1
Moisture content (%)	10.2±0.8	34.3±0.5	75.9±1.6	62.1 ±2.0
Organic C (%)	13.1± 1.3	55.6±1.2	72.2± 0.9	66.3±1.1
pH	7.0 ± 1.5	6.5±1.2	6.8±1.2	6.9±0.5

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

Table 2 : Seed germination toxicity test (%)

Treatments					
A	B	C	D	E	F
60±3.0	90±5.0	80±6	40±6	20±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

Table 3. Matrix of correlation coefficients for the parameters used in this research

	TPH degraded	Cumulative CO_2	Dehydrogenase activity
TPH degraded	1	0.901**	0.905**
CO_2		1	0.87**
Dehydrogenase activity			1

** Correlation is significant at the 0.01 level.

Table 4. Biodegradation rate and half-life of hydrocarbon in diesel-polluted soil

Treatment	Biodegradation constant (k) day^{-1}	Half- life (days)
A	0.0063 c	10.85
B	0.103 a	6.67
C	0.085 b	8.15
D	0.026 b	26.1
E	0.0066 c	104.6

A= soil+oil+TL, B= soil+oil+SC, C= soil+oil+PS D= soil+oil E = autoclaved soil+oil+NaN_3
Values followed by "a" shows significant difference at $p < 0.05$ level respectively.

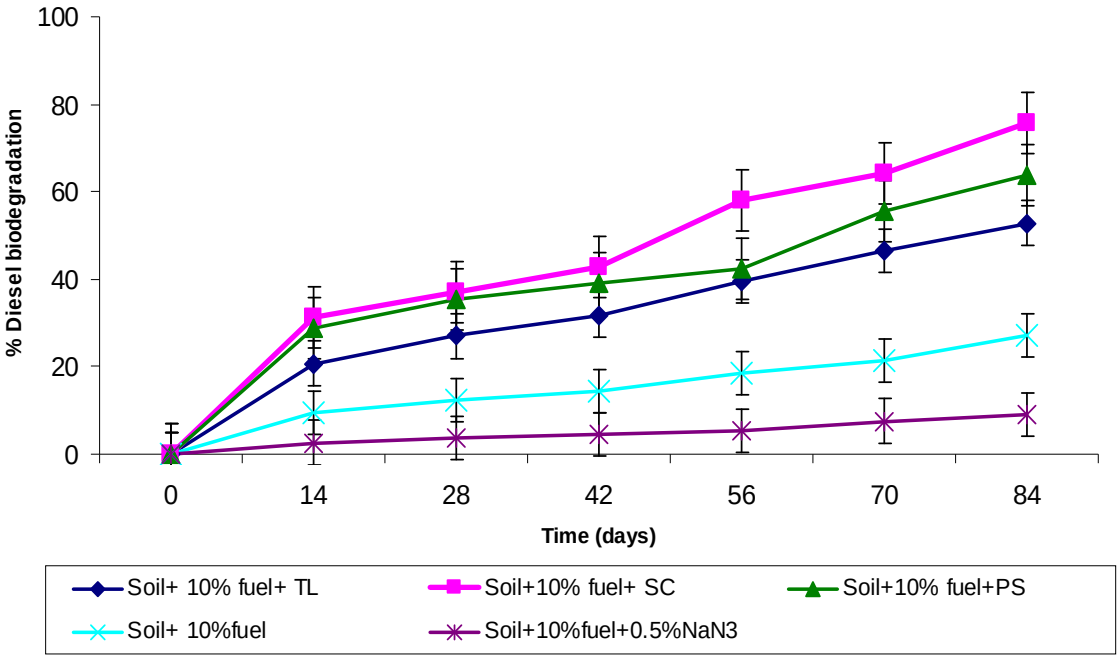

Figure1. Percentage biodegradation of 10% diesel fuel in contaminated soil.

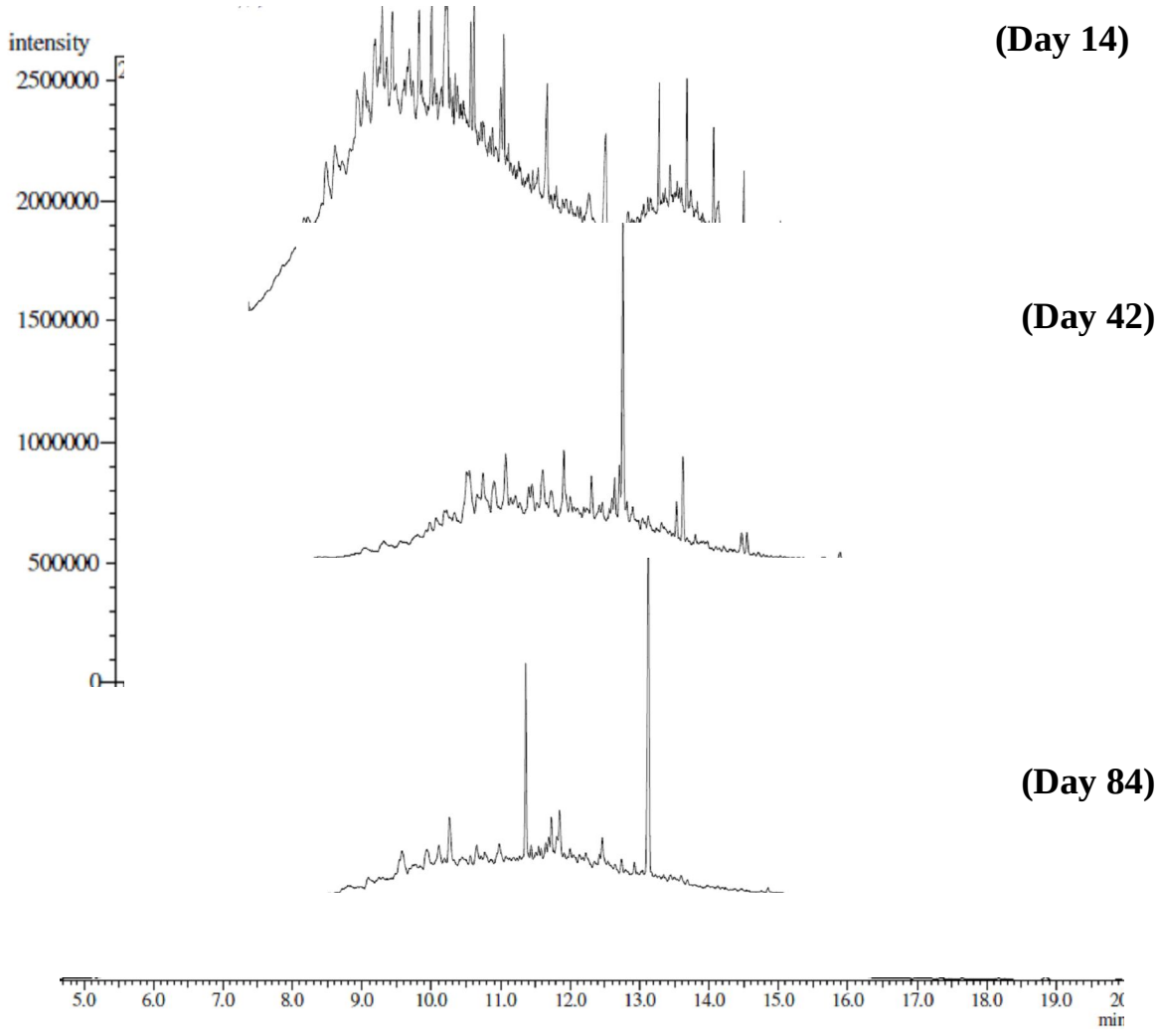

Figure2**.** Chromatogram of residual diesel fuel in soil at day 14, 42 and 84.