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ENHANCED DEGRADATION OF HYDROCARBONS IN SPENT ENGINE OIL CONTAMINATED SOIL BY *Pseudomonas aeruginosa*

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ABSTRACT
As the local consumption of engine oil in Nigeria increases due to the upsurge in the number of vehicles and other machines that make use of this lubricant, pollution from spent engine oil has become a major environmental problem in Nigeria. In this study, spent engine oil contaminated soil was treated with oil adapted *Pseudomonas aeruginosa* with a view to accelerate bioremediation of soils polluted with spent engine oil. Spent engine oil contaminated soil treated with oil adapted *Pseudomonas aeruginosa* culture served as inoculated treatment (IT), while the non-inoculated control (NIC) was a set-up similar to IT but not treated with *Pseudomonas aeruginosa*. Oil degradation was monitored following standard microbiological and chemical methods. Percentage of oil degraded determined gravimetrically revealed that 97.35% of the spent engine oil was degraded within 10 weeks in the IT which received additional oil adapted *Pseudomonas aeruginosa*, whereas only 44.1% of the oil was degraded in the NIC within the same period. From gas chromatogram (GC) analysis of residual oil content, reduction in peaks of hydrocarbons was much more pronounced in the IT. Analysis of data obtained from this study revealed that the differences between the mean residual oil concentration (ROC) of the IT and that of the NIC differed significantly at 5% probability level, indicating that the magnitude of oil degraded was much more remarkable in the IT compared with the NIC, and this could be attributed to the additional activities of the bacterial culture added. Therefore, inoculation of oil contaminated soil with oil adapted *Pseudomonas aeruginosa* may be a suitable alternative method for rapid rehabilitation of the polluted soil.

KEYWORDS: Used engine oil, soil pollution, physico-chemical parameters, *Pseudomonas aeruginosa*, bioremediation.

INTRODUCTION

Spent engine oil is a brown-to-black liquid produced when new mineral-based crankcase oil is subjected to high temperature and high mechanical strain. It is a mixture of different chemicals, including low and high molecular weight (C_{15}-C_{20}) aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, lubricative additives, decomposition products, heavy metal contaminants such as aluminium, chromium, tin, lead, manganese, nickel, and silicon that come from engine parts as they wear down (Achuba, and Peretiemo-Clarke, 2008; Wang *et al*., 2000; ATSDR, 1997). As the world's population increases, there is a concomitant increase in the demand for petroleum and petroleum products, which obviously constitutes a source of environmental pollution (Akoachere *et al.*, 2008; Raven *et al.*, 1993). Oil pollution is a major environmental concern in many countries. This has led to a concerted effort in studying the feasibility of detoxifying oil contaminants using oil-degrading microorganisms (Akoachere *et al.*, 2008; Clementina and Omoanghe 2008). The discharge of spent engine oil into gutters, watercourses, open vacant plots and farm-land are common practices among automachine operators. These practices increase the incidence of oil contamination of agricultural soils, and rendered the soils unfit for agricultural and recreational activities as well as potential sources for surface and ground water contamination (Igwo-Ezikpe *et al*., 2009; Achuba, and Peretiemo-Clarke, 2008; Nwoko *et al*., 2007). Pollution control protocols involving physico-chemical methods have often increased the problem rather than eliminated it. Biodegradation is an attractive method for the remediation of contaminated sites because of its economic viability and environmental soundness (Dinkla *et al*., 2001; Walker and Crawford, 1997). The use of microbes in pollution abatement either through natural selection or recombinant DNA technology is receiving increasing interest as this is cheap and most effective (Daane *et al*., 2001). In bioremediation, the contaminated site is exposed to an 'army' of microorganisms which gobble up the poison and leave behind harmless substances such as carbondioxide and water. However, the extent of biodegradation and the rate at which it occurs depend on the interactions between the environment, number and type of microorganisms present as well as the chemical structure of the contaminant (Akoachere *et al.*, 2008; Walker and Crawford, 1997; Atlas, 1981). An understanding of the microbial processes occurring in contaminated soil may suggest bioremediation strategies that could be effective in reducing hydrocarbon pollutants concentrations below toxic levels. Different naturally occurring species of *Pseudomonas* are known to contain relevant genes for the

degradation of different hydrocarbons (Prescott *et al*., 2005; Jawetz *et al*., 1991). Upon this background, *Pseudomonas aeruginosa* strain known to have hydrocarbon degradative ability was developed and used to enhance rehabilitation of spent engine oil contaminated soil in this study.

MATERIALS AND METHODS

The *Pseudomonas aeruginosa* used in this study was a strain previously isolated from crude oil polluted site in Nigeria (Nwachukwu, 1998). In order to be useful as a bioremediation agent, the ability of the strain to utilize a high concentration of spent engine oil (15% v/v) as a substrate was improved upon by a process described as step-up complex hydrocarbon utilization technique (Nwachukwu *et al*., 1999). Minimal salt broth (MSB) containing increasing concentrations of sterile spent engine oil as the sole carbon and energy source was used (Nwachukwu *et al*., 1999; Raymond *et al*., 1976).

Bioremediation programmes

Four kilogram (4 kg) each of thoroughly mixed non-sterile agricultural soil samples randomly collected from different spots on a plot in Ota were dispensed in open pans, 44cm x 44cm x 13cm (internal dimensions), and separately contaminated with 600 gm of sterile spent engine to give 15% (wt/wt) pollution level. The soil samples in three replicates were inoculated with 300 ml minimal salt broth containing *Pseudomonas aeruginosa* (approximately 1.34 ± 0.08 x10^8 cfu/ml). These designs were regarded as the inoculated treatment (IT). Similarly, control samples containing all the materials present in IT but without *Pseudomonas aeruginosa* inoculation were set up and regarded as the non-inoculated control (NIC). The set-ups were watered weekly with 300 ml sterile distilled water and kept at room temperature in the laboratory throughout the investigation period (10 week). Three samples of 10 gm each, from each replicate, were taken aseptically on day zero and at weekly intervals for analyses.

The mean changes in population densities of heterotrophic microorganisms were determined by standard plate counts with both selective and general purpose media (Nwachukwu, 2000c; Nwachukwu and Ugoji, 1995). Oil-degrading microorganisms were enumerated on minimal salt agar using motor oil as carbon and energy source as previously reported (Amund *et al*., 1994). Pure culture isolates of oil degrading microorganisms obtained by enrichment culture technique followed by replica plating were identified based on colonial morphology and biochemical characteristics according to the methods in Bergey's Manual of Determinative Bacteriology for bacteria (Buchanam and Gibbons, 1984) and the method by Smith (1969) for fungi.

Oil-weight loss following microbial degradation was assessed by gravimentric method after extraction with n-hexane: dichloromethane (1:1) solvent system (Le Dreau *et al*., 1997). Values obtained were expressed as percentages of the amount of oil in sample at time zero. For GC analysis, the residual oil was extracted with dichloromethane (1:1) and concentrated to1.0 ml before it was run under the following conditions: capillary column, 0V-101; carrier gas, nitrogen; temperature programme, 70 - 320^{o}C at 2^{o}C per minute; quantity injected; 1.0 µl. A 1.0 µl of standard crude oil was first injected into the GC machine to create a standard hydrocarbon window which was then used to detect the hydrocarbon present in the sample. Similarly, 1.0 µl of the concentrated oil sample was injected and the hydrocarbons detected (Le Dreau *et al*., 1997; Wang *et al*., 1994). The mean temperatures of the samples were determined using a mercury thermometer while the pH of the samples were determined using portable pH meter with combined glass and calomel electrodes as previously reported (Nwachukwu, 2000c). Phosphate, sulphate and nitrate concentrations were determined spectrophotometrically using the method of APHA (1998). The results reported were the arithmetic mean of the samples collected for both IT and NIC during each sampling session.

Statistical Analysis:

The data obtained over the 10 weeks study period were analyzed using SPSS 17.0 and Microsoft Excel 2010.

RESULTS AND DISCUSSION

To avoid long persistence of petroleum pollutant in the environment which was probably due to low numbers of hydrocarbon utilizers as well as the toxicity of oil hydrocarbons on natural flora (Nwachukwu, 2000a; Nwachukwu, 2000c; Amund and Igiri, 1990), we accelerated the rehabilitation of spent engine oil contaminated soil by introducing culture of *Pseudomonas aeruginosa* known to have degradative ability to supplement hydrocarbon utilizers naturally present. Moreover, the ability of this bacterial culture to adapt and breakdown spent engine oil was improved upon by including sterile spent engine oil in minimal salt broth used for its development. The developed broth culture inoculated into the IT contained approximately 1.34 ± 0.08 x 10^8 cfu/ml *Pseudomonas aeruginosa*, unlike the reference control broth inoculated into the NIC which had no microbial load. The mean population densities of the different microbial groups were enumerated during the study period (Table1). The initial drop in total heterotrophic microorganisms in both the IT and the NIC confirms the toxic impacts of spent engine oil, and probably proved that some of the microorganisms naturally present in the soil cannot survive in oil polluted environment which is in accordance with the reports by Mbah *et al*. (2009), Achuba and Peretiemo-Clarke (2008), Akoachere *et al*. (2008), Mandri and Lin (2007), Nwoko *et al*. (2007), Nwachukwu (2000c) and Atlas (1981). The results obtained showed a more rapid increase in the mean population densities of hydrocarbon utilizers present in the IT compared with the NIC (Table 1), and this could be attributed to the addition of bacterial culture to the IT. For example, the percentage total hydrocarbon utilizers (% THCU) calculated for both the IT and the NIC relative to the total heterotrophic microorganisms (THM), differed significantly at 5% probability level which corresponds with the previous report by Nwachukwu (2000b). The results summarized in Figure 1, revealed that the magnitude of oil degraded determined gravimetrically was much more pronounced in the IT than in the NIC and differed significantly at 5% probability level, indicating that the treatment given had impacted the biodegradation of the oil pollutant.

TABLE 1: Mean population densities of different microbial groups enumerated in treatment and control

Sampling time (weeks)	Mean population densities (cfu/gm ± SD) × 10^4)			
	Treatment		Control	
	Total HM	Total HCU; %	Total HM	Total HCU; %
0 (a)	246.64±0.02	17.16±0.02;6.96	229.26±0.22	1.16±0.02;0.51
(b)	229.47±0.30	1.18±0.01;0.51	229.47±0.30	1.18±0.02;0.51
1	95.12±0.11	18.23±0.11;19.17	72.06±0.03	1.17±0.03;1.62
2	99.48±0.22	20.49±0.02;20.60	72.58±0.02	1.23±0.02;1.69
3	104.67±0.30	22.56±0.30;21.55	72.95±0.11	1.46±0.11;2.00
4	107.89±0.11	24.08±0.01;22.32	73.22±0.02	1.59±0.03;2.17
5	111.95±0.21	26.22±0.03;23.42	73.78±0.03	1.81±0.02;2.45
6	114.86±0.03	27.91±0.22;24.30	73.95±0.02	1.99±0.22;2.69
7	118.31±0.02	29.46±0.01;24.90	74.10±0.11	2.01±0.03;2.71
8	120.34±0.02	31.76±0.11;26.39	74.51±0.02	2.25±0.03;3.02
9	123.78±0.03	33.85±0.21;27.35	74.86±0.11	2.51±0.01;3.35
10	127.13±0.02	35.97±0.02;28.29	75.34±0.02	2.77±0.02;3.68

SD – standard deviation, a – immediately after pollution and inoculation with developed broth.
b – Before pollution, HM – heterotrophic microorganisms,
HCU – hydrocarbon utilizers.

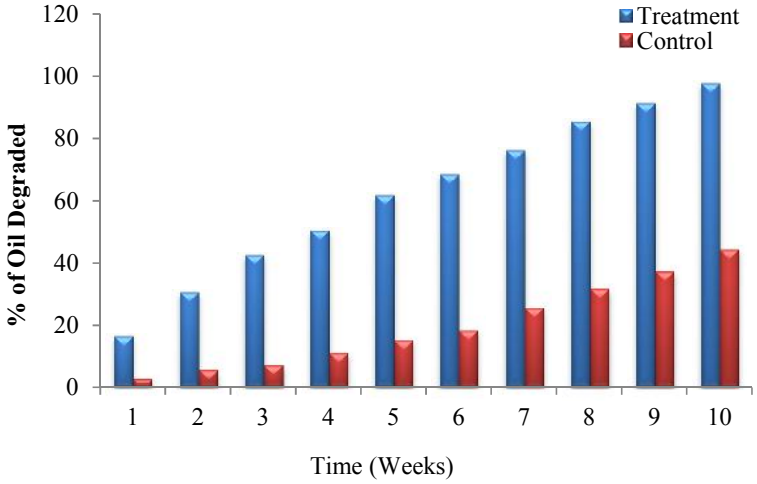
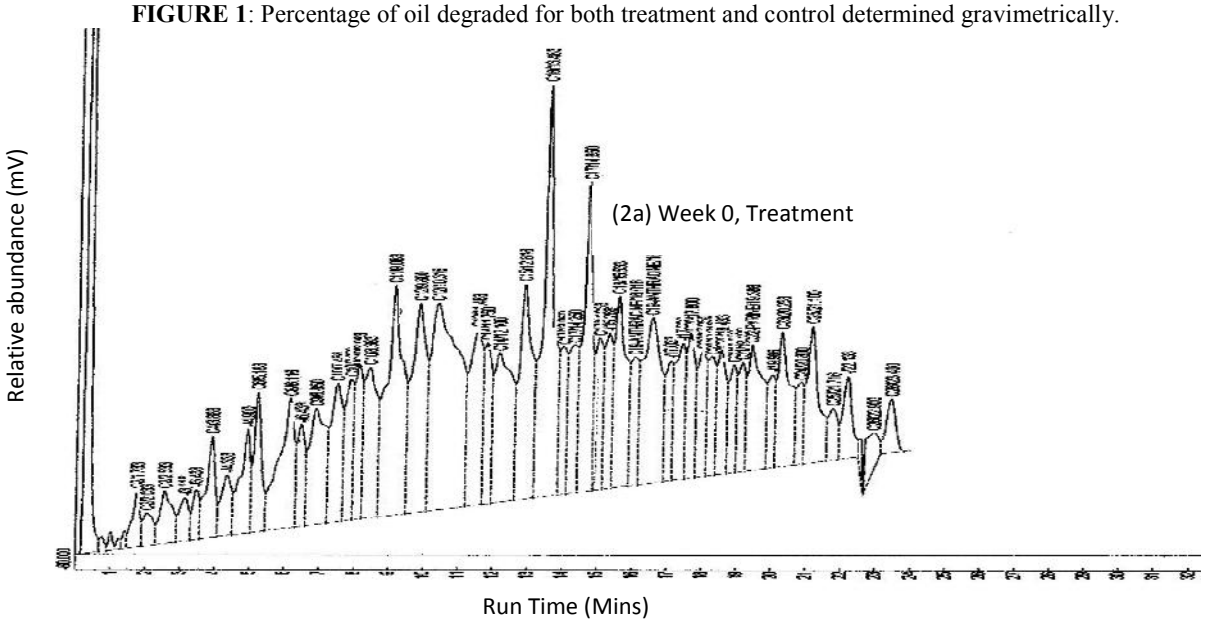


FIGURE 1: Percentage of oil degraded for both treatment and control determined gravimetrically.



Figures 2 (2a and 2b), 3 (3a and 3b) and 4 (4a and 4b) are the gas chromatograms of ROC for both the IT and the NIC at weeks 0, 5 and 10 respectively. The GC profile of ROC showed that reduction in peaks of hydrocarbons was much more remarkable in IT compared with NIC. Most importantly, these gas chromatographic characteristics correspond to criteria used to quantify petroleum degradation due to microbial activities (Le Dreau *et al*., 1997; Wang *et al*., 1994). Therefore, considering the fact that the initial THM and THCU were approximately equal in both IT and NIC before inoculation with the bacterial culture, the difference in most of these chromatographic characteristics in the IT when compared with the NIC could be attributed to the activities of the additional bacterial culture. At week 5, when the other hydrocarbon utilizers naturally present in the NIC were probably still adapting to the oil environments, more than 50% of the oil concentration had disappeared in the IT inoculated with the bacterial culture previously trained and adapted to degrade spent engine oil during its development in minimal salt broth.

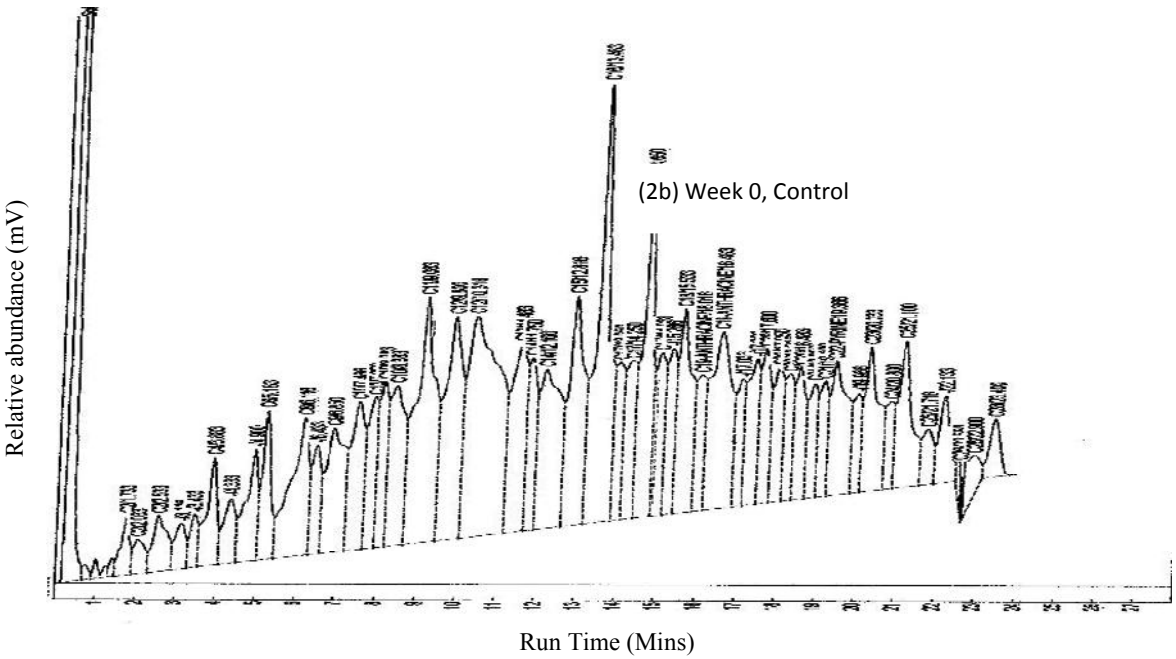
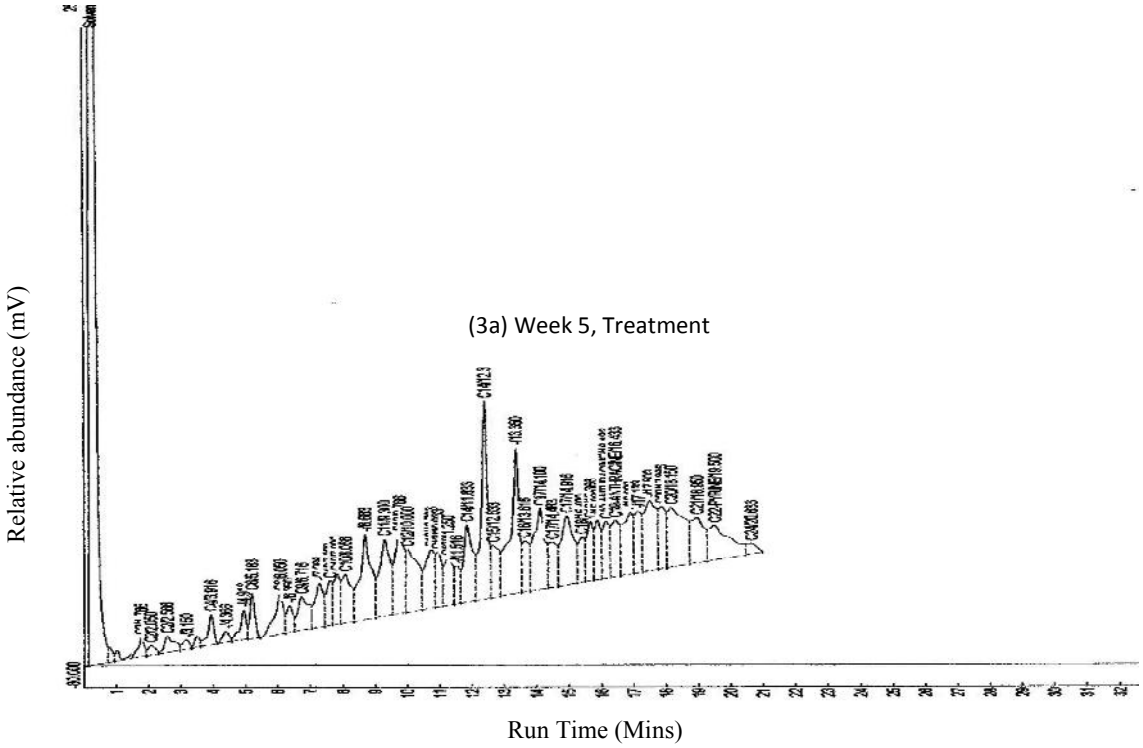


FIGURE 2: Gas chromatogram of residual oil content for treatment (2a) and control (2b) at week 0



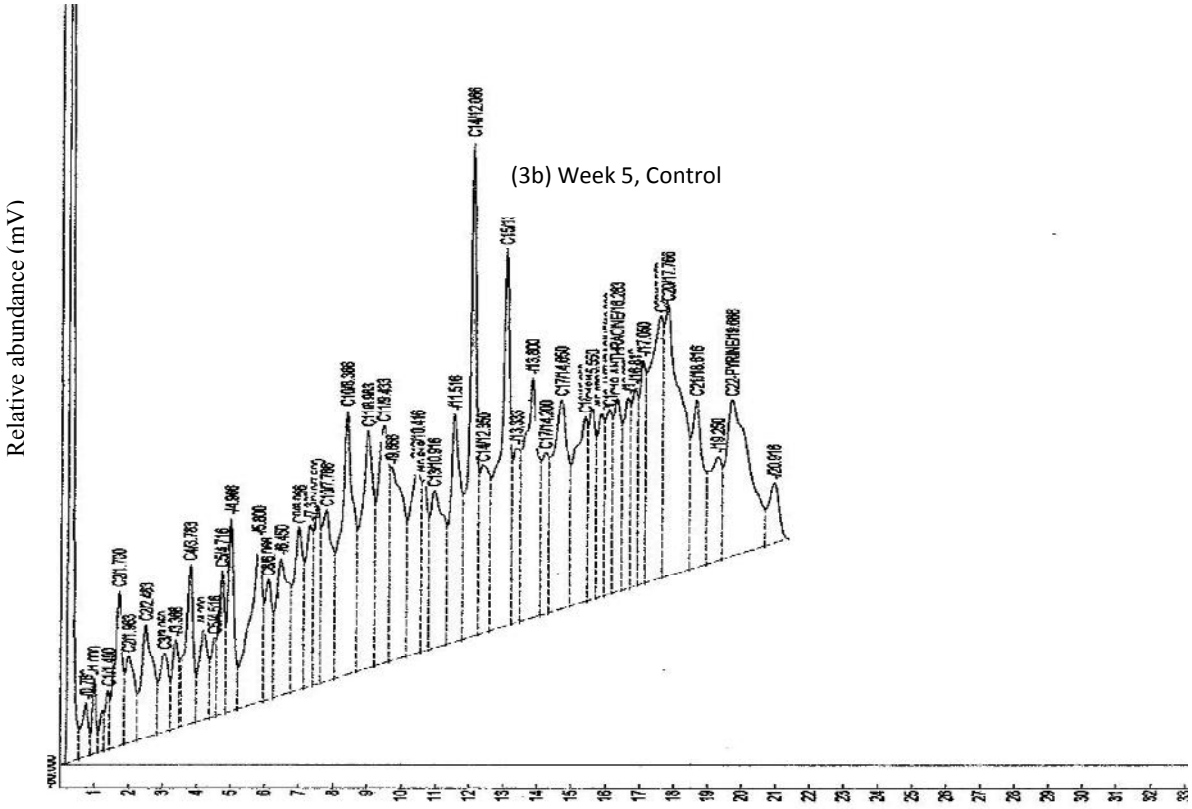
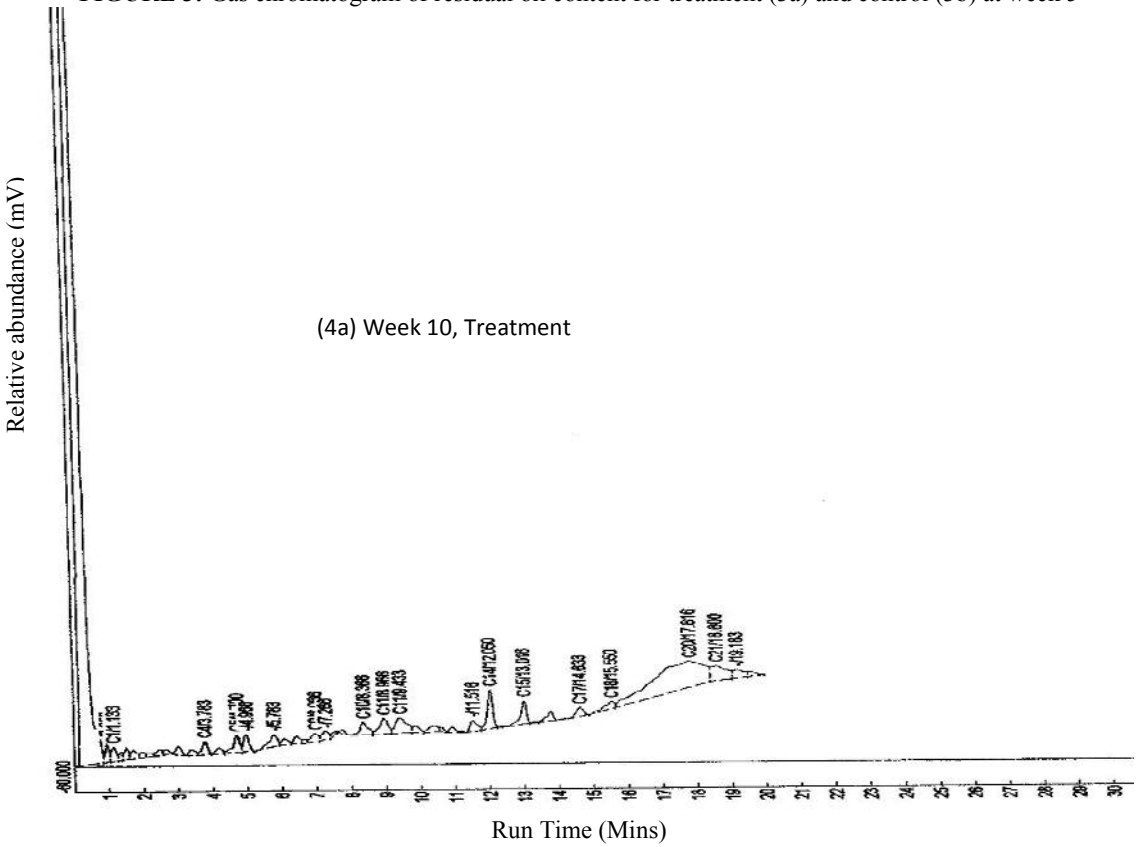


FIGURE 3: Gas chromatogram of residual oil content for treatment (3a) and control (3b) at week 5



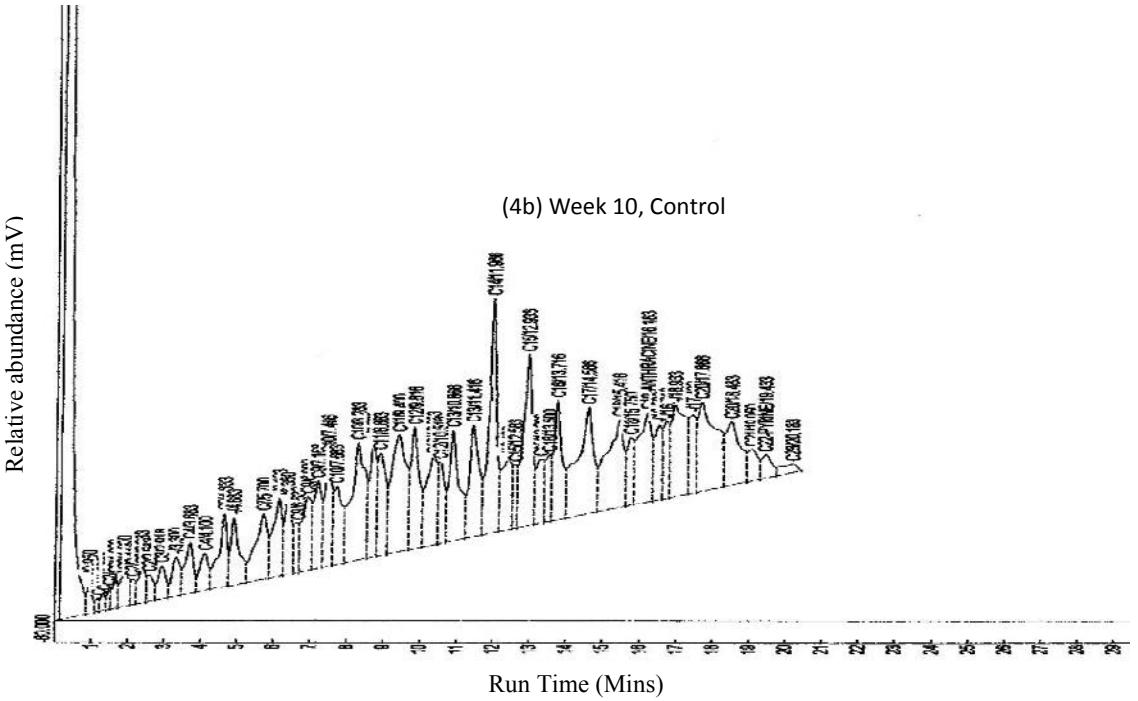


FIGURE 4: Gas chromatogram of residual oil content for treatment (4a) and control (4b) at week 10

Figure 5 indicates the trend of changes in nitrate, phosphate and sulphate concentrations for both IT and NIC. The concentrations detected for phosphate, nitrate and sulphate were fluctuating and this could probably be attributed to the presence of other compounds used as additives in the original engine oil, which corroborates the reports by Dominguez-Rosado and Pichtel (2004), Odjegba and Sadiq (2002), Wang *et al*. (2000), ATSDR (1997) and Vasquez-Duhalt (1989). In Figure 6, the mean changes in temperature and pH for both IT and NIC were presented. The predominant microorganisms isolated from the control sample that grew well on minimal salt agar containing sterile spent engine oil supplied in vapour phase as the sole carbon and energy source were *Pseudomonas* species, *Acinetobacter* species, *Corynebacterium* species (bacteria); *Aspergillus* species, *Candida* species and *Rhodotorula* species (Fungi). These microorganisms were therefore identified as the hydrocarbon utilizers (HCU).

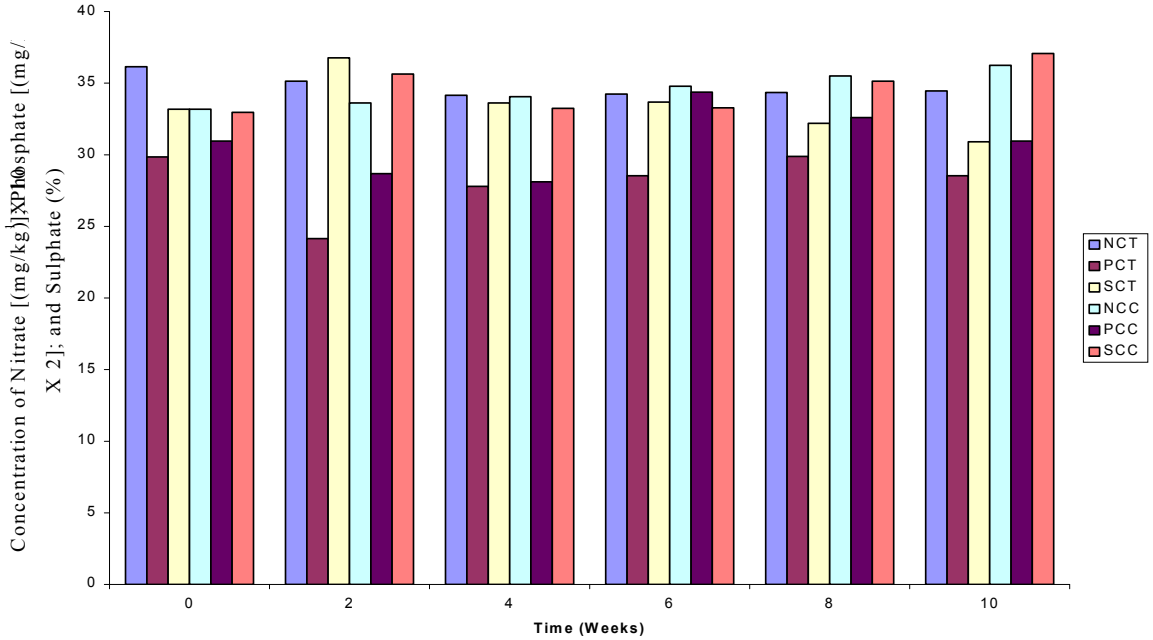


FIGURE 5: Mean Changes in Nitrate, Phosphate and Sulphate Concentrations for Treatment and Control. Where NCT, PCT and SCT are concentrations of nitrate, phosphate and sulphate for treatment respectively, while NCC, PCC and SCC are concentrations of nitrate, phosphate and sulphate for control respectively.

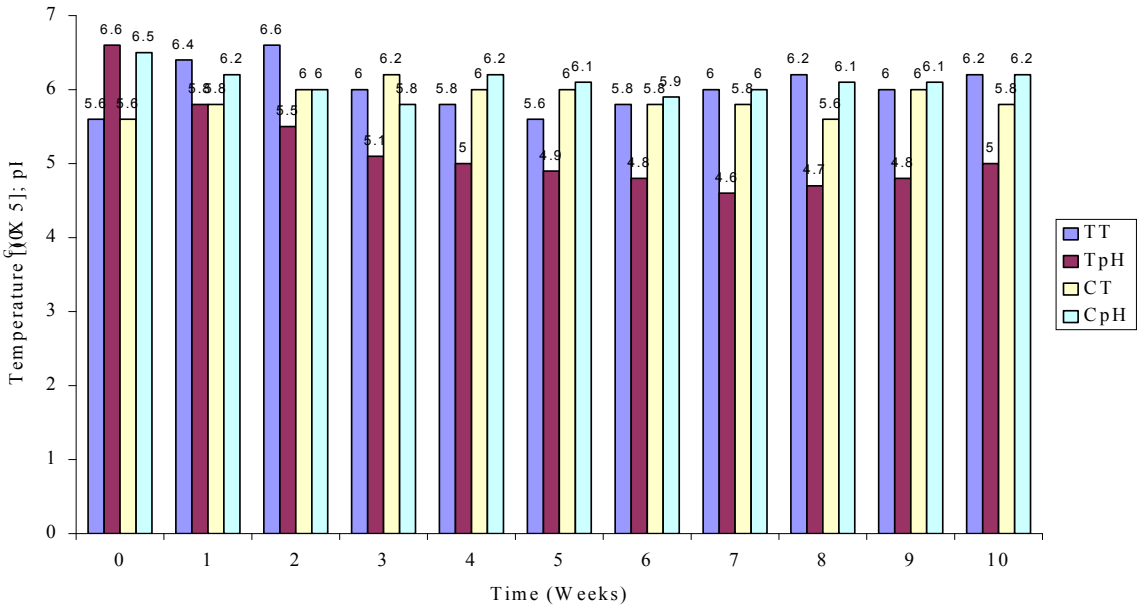


FIGURE 6: Mean Changes in Temperature and pH for Treatment and Control. Where TT and TpH are treatment temperature and pH respectively, while CT and CpH are control temperature and pH respectively.

Obviously the pollution of the soil with spent engine oil changed the soil environment resulting in the death of many biotic factors, hence the initial drop in the total heterotrophic microorganisms. However, hydrocarbon utilizing microorganisms present in these oil impacted soils quickly adapted to the oil environment and probably utilized the oil as a substrate for growth, and this may be the reason for the reduction in the ROC. Therefore, *Pseudomonas aeruginosa* strain, known to have degradative ability could probably be developed and used as an alternative inoculum to enhance rehabilitation of spent engine oil contaminated soil environment. However, motor and generator mechanics should be educated on the danger of indiscriminate dumping of spent engine oil to reduce further pollution of human environments.

REFERENCES

Achuba, F. I. and Peretiemo-Clarke, B. O. (2008) Effect of spent engine oil on soil catalase and dehydrogenase activities. *Int. Agrophy*. 22,1-4.

Akoachere, J. T. K., Akenji, T. N., Yongabi, F. N., Nkwelang, G. and Ndip, R. N. (2008) Lubricating oil-degrading bacteria in soils from filling stations and auto-machanic workshops in Buea, Cameroon: occurrence and characteristics of isolates. *African. J. Biotechnol.* 7, 1700-1706.

Amund, O. O., Omole, C. A., Esiobu, N. and Ugoji, E. O. (1994) Effects of waste engine oil spillage on soil physico-chemical and microbiological properties. *J. Sci. Res. Dev*. 1, 61-64.

Amund, O. O. and Igiri, C. U. (1990) Biodegradation of petroleum hydrocarbons under tropical estuarine conditions. *World J. Microbiol. Biotechnol*. 6, 225-262.

APHA. (1998) Standard methods for the examination of water and waste water. 20th edition. APHA – AWWA – WPCF. Washington DC. pp. (4 – 114) – (4 – 179).

Atlas, R. M. (1981) Microbial degradation of petroleum hydrocarbons: An environmental perspective. *Microbiol. Rev*. 45, 180-209.

ATSDR **(**1997) Toxicology Profile for used mineral-based crankcase oil. Department of Health and human Services, Public Health Service Press, Atlanta, GA, USA.

Buchanam, R. M. and Gibbons, N. E. Eds (1984) Bergey's Manual of Determinative Bacteriology. 8th Edn. The Williams and Wilkins Company, Baltimore.

Clementina, O. A. and Omoanghe, S. I. (2008) Bioremediation of engine oil polluted soil by the tropical white rot fungus, *Lentinus squarrosulus* Mont. (Singer). *Pak. J. Biol. Sci.* 11(12), 1634-1637.

Daane, L., Harjono, I., Zylstra, G. J. and Haggblom, M. M. (2001) Isolation and Characterization of polycyclic aromatic hydrocarbon-degrading bacteria associated with the rhizophere of soil marsh plants. *Appl. Environ. Microbiol*. 67, 2683-2691.

Dinkla, I. J. T., Garbo, E. M. and Janssen, D. B. (2001) Effects of iron limitation on the degradation of toluene by *Pseudomonas* strains carrying TOL (pWWO) plasmid. *Appl. Environ. Microbiol*. 67, 3406-3412.

Dominguez-Rosado, R. E. and Pichtel, J. (2004) Phytoremediation of soil contaminated with used motor oil. 1. Enhanced microbial activities from laboratory and growth chamber studies. *Environ. Eng. Sci***.** 2, 157-168.

Igwo-Ezikpe, M. N., Gbenle, O. G., Ilori, M. O., Okpuzor, J. and Osuntoki, A. A. (2009) Evaluation of *Alcaligenes faecalis* degradation of chrysene and diesel oil with concomitant production of biosurfactant. *Res. J. Environ. Toxicol*. 3(4), 159-169.

Jawetz, E., Melnick, J. L., Adelberg, E. A., Brooks, G. F., Butel, S. J. and Ornston, L. N. (1991) Pseudomonads, Acinetobacter and uncommon gram-negative. In: Jwaetz E., Adelberg E. A., Brooks G.F., Butel S.J. and Ornston L.N. (Eds). *Review of Medical Microbiology*, 19 th edn. Appleton and Langue Publishing Prentice Hall, Los Altos, California, USA., pp: 224-229.

Le Dreau, Y., Jacquot, F., Doumenq, P., Guiliano, M., Bertrand, J. C. and Mille, G. (1997) Hydrocarbon balance of a site which had been highly and chronically contaminated by petroleum waste of a refinery from 1956-1992. *Mar. Pollut. Bull*. 34, 456-468.

Mandri, T. and Lin, L. (2007) Isolation and characterization of engine oil degrading indigenous microorganisms in Kwazulu-Natal, South Africa. *Afr. J. Biotechnol.* 6(1), 023-027.

Mbah, C. N., Nwite, J. N. and Nweke, I. A. (2009) Ameriolation of spent oil contaminated ultisol with organic wastes and its effect on soil properties and maize (*Zea mays L*) yield. *World. J. Agric. Sci***.** 5(2), 163-168.

Nwachukwu, S. C. U. (2000a) Modeling the recovery rate of a tropical soil ecosystem polluted with crude petroleum using *Pseudomonas* spp. as the test organism. *Int. J. Environ. Educ. Inf*. 19(1), 55-62.

Nwachukwu, S. C. U. (2000b) Enhanced rehabilitation of tropical aquatic environments polluted with crude petroleum using *Candida utilis. J. Environ. Biol*. 21(3), 241-250.

Nwachukwu, S. C. U. (2000c) The use of land for field evaluations of the impact of crude oil on the biotic and abiotic factors in developing bioremediation strategies for agricultural land upon pollution with crude petroleum or petroleum products. *J. Environ. Biol.* 21(4), 359-366.

Nwachukwu, S. C. U., James, P. and Gurney, T. R. (1999) Training of *Pseudomonas aeruginosa* as a polishing agent for cleaning environments polluted with hydrocarbons by step-up complex hydrocarbon utilization technique. *Int. J. Environ. Edu. Inf*. 18(1), 53-66.

Nwachukwu, S. C. U. (1998) Kinetics of biodegradation of crude petroleum pollutant in tropical Makaraba Creeks Water. *Proc. Nigerian Soc. Microbiol*. 1(1), 17-30.

Nwachukwu, S. C. U. and Ugoji, E. O. (1995) Impacts of crude petroleum spills on microbial communities of tropical soils. *Int. J. Ecol. Environ. Sci*. 21, 169-176.

Nwoko, C. O., Okeke, P. N., Agwu, O. O. and Apkan, I. E. (2007) Performance of *Phaseolus vulgaris* L. in a soil contaminated with spent-engine oil. *Afr. J. Biotechnol*. 6(16), 1922 – 1925.

Odjegba, V. J. and Sadiq, A. O. (2002) Effects of spent engine oil on the growth parameters, chlorophyll and protein levels of *Amaranthus hybridus* L. *The Environmentalist*. 22, 23-28.

Prescott, L. M., Harley, J. P. and Klein, D. A. (2005) Order *Pseudomonadales*. In: Microbiology, 6 th edn. McGraw Hill. Pp: 489 – 490.

Raven, R., Berg, L. R. and Johnson, G. B. (1993) Environment. Saunders College Publishing, Philadelphia. USA, pp.569.

Raymond, R. L., Audson, J. O. and Jamison, V. W. (1976) Oil degradation in soil. *Appl. Environ. Microbiol*. 31, 522-535.

Smith, G. (1969) Classification and identification of fungi In: Introduction to industrial mycology. 6 th edn. Edward Arnold, London, 1**,** 56-144.

Vazqeuz-Duhalt, R. (1989) Environmental impact of used motor oil. *Sci. Total Environ*. 79(1), 1-23.

Walker, M. V. and Crawford, R. L. (1997) Overview: Biotransformation and biodegradation. In: Hurst C. J., Kundsen G. R., Mcinerney M. J., Stetzenbach L. D., Walter M. V. (eds). Manual of Environmental Microbiology. American Society for Microbiology Press, Washington, D.C., pp: 707-708.

Wang, J., Jia, C. R., Wong, C. K. and Wong, P. K. (2000) Characterization of polycyclic aromatic hydrocarbon created in lubricating oils. *Water Air Soil Pollut*. 120(3-4), 381-396.

Wang, Z., Fingas, M. and Sergy, G. (1994) Study of 22 year-old arrow oil samples using biomarker compounds by GC/MS. *Environ. Sci. Technol*. 28, 1733-1746.