ISOLATION OF INDIGENOUS MICROBES FROM DIESEL CONTAMINATED SOIL IN OLUKU, BENIN CITY, EDO STATE.

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ABSTRACT

The microbial characterization of diesel oil contaminated soil in Oluku, Edo State was investigated. The microbial growth for bacteria and fungi were 5.2x 10^6 cfu/g and 3.8x10^6 cfu/g respectively for the contaminated and 2.2 x10^6 Cfu/g and 2.4 x10^6 Cfu/g for the control soil samples respectively. The most frequently encountered bacterial were *Pseudomonas aeruginosa* 27(31.4%), others in frequency of occurrence include *Bacillus subtilis* 21(24.4%), *Micrococcus* spp. and *Corynabacterium* spp. with 17(19.8%) and *Proteus* spp. 4(4.6%) while the most frequently isolated fungal is *Fusarium* spp. 22(32.9%). Other fungi isolate encountered includes *Aspergillus* spp. 15(22.4%), *Penicillium* 14(20.9%), *Rhizopus* 10(14.9%) and *Mucor* 6(8.9%). These changes could affect the geological cycle of nutrient transformation and uptake by microbes and inevitably man, it's environment and health. The identification and use of indigenous microbes remains the most effective method of clean up of hydrocarbon spills.

Keywords: *Pseudomonas*, Diesel contaminated soil, microbial growth, Oluku, Indigenous Microbes.

INTRODUCTION

Diesel, a fractional distillation product of crude oil, like all other fossil fuels consist of a complex mixture of molecules called hydrocarbons. In large concentrations, they are toxic to the many organisms including humans. The states in Nigeria in which crude oil has been found and consistently drilled include Abia, Akwa Ibom, Anambra, Bayelsa, Cross River, Delta, Edo, Imo and Rivers (Ujowundu *et al*., 2011).

Diesel, like all other petroleum products can be introduced into the environment through seepage into the soil and contaminates ground water. Run off from uncontaminated sites can carry petroleum contaminants off site into nearby water ways (Malik and Ahmed, 2012). It could also be due to oil spills, leaking unplugged wells, disposal ponds of waste, pipeline ruptures, discharge during transport in tanks and ship failures. Petroleum production, drilling operations and improperly sealed and abandoned wells and tanks, contamination of surface, ground waters and soils (USEPA, 2008; Kharaka and Hanor, 2003). With the continuous usage of diesel in generators and engines of lorries, a lot of quantities have been transported, hence the contamination of the environment. Crude oil is destructive to soil not only because of the contact toxicity but also due to the fact that the hydrocarbons reduce oxygen tension and increases anaerobiosis which is harmful to plant roots (Ekpo and Udofia, 2008). As with the development of petroleum industry, the area of oil polluted soils is enlarged continuously.

Thus, it would come to a burning question to treat the oil polluted soils to recovery level (Turgay *et al*., 2010; Zhang *et al*., 2012). Large scale contamination of both terrestrial and aquatic environments, consequent on the activities of oil exploration and refining industries have been documented (Aluyi *et al*., 2006; Chikere *et al*., 2009; Okpokwasili, 2006).

Microbial communities exposed to hydrocarbons become adapted, exhibiting selective enrichment and genetic changes resulting in increased proportions of hydrocarbon degrading bacteria and bacterial plasmids encoding hydrocarbon catabolic genes (Okerentugba and Ezeronye, 2003).

However, various microorganisms are able to use some crude oil fractions as sole source and convert these components to non- toxic materials such as Carbon (iv) oxide and water. The oil consuming microorganisms are widely distributed in soils which play important roles (Zhang *et al*., 2012).The genera *Pseudomonas*, *Arthrobacter*, *Alcaligens*, *Flavobacterium*, *Micrococcus* and *Mycobacterium* appear to be the most consistent (Jain *et al*., 2005; Chikere *et al*., 2009; Ma'an *et al*., 2011; Sebiomo *et al*., 2011; Malik and Ahmed 2012), while *Aspergillus* and *Penicillium* are known to be the most efficient mobilisers of hydrocarbon (Obire and putheti, 2008).

The biodegradation capabilities of bacteria have been recognized but that of fungi have been a subject of intense research due to their ability to synthesize relatively unspecific enzymes involve in cellulose, lignin, high molecular weight and more recalcitrant compounds including the aromatics (Sebiomo *et al*., 2011).

This study is aimed at identifying and characterizing the microbial isolates present at the chronic diesel contaminated soils in Oluku.

MATERIALS AND METHODS

The Study Area: located along Benin Lagos expressway. It is one of the communities of Ovia North East Local Government Area of Edo State. It's a popular one because Trailers and tankers drivers have been using that spot as their park for years. It is a nodal town which has attracted a lot of human and economic activities. The area has been chronically subjected to diesel contamination for over ten years.

Sample collection: The Soil samples which have been contaminated with diesel for over ten years were used for this study. They were obtained from mechanic workshops in Oluku, Edo State at a depth of between 15-20cm. Non-contaminated soil samples collected were from a distance of not less 1km from the contaminated sites. All soil samples were collected in triplicates with sterile black cellophane bag and transported to the laboratory where analysis began within 24 hours of collection.

Isolation and characterization of hydrocarbon utilizing bacteria and fungi

The method of Sebiomo *et al*., 2011 was used. The bacterial species indigenous to the diesel contaminated and uncontaminated soils were isolated by pour plate technique. A 10ml fold was prepared and serially diluted and 0.1ml aliquot of appropriately 10^{-3} and 10^{-5}ml dilutions were inoculated into nutrient agar plates. Individual cultures were identified through cultural, morphological and biochemical techniques using the taxonomic scheme of the Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994).

The fungi species indigenous to the diesel contaminated site were isolated using Potato Dextrose Agar (PDA) into which streptomycin (50mg/ml) was added to suppress the growth of bacterial species. Fungal isolates were identified and characterized as described by Barnett and Hunter (1998).

Determination of soil pH: The method of Ujowundu *et al*., 2011 was used. The pH of the soil samples were measured using a digital precision model S-25C pH meter with 1: 1 of water to soil ratio.

Statistical analysis: This was carried out using the method of Ogbeibu (2005).

RESULTS

The diesel contaminated soil had bacteria and fungi counts of 5.2 x 10^6 cfu/g and 3.8 x 10^6 cfu/g respectively, while the uncontaminated (control) had bacteria and fungi counts of 2.2 x10^6 cfu/g and 2.4 x10^6 cfu/g respectively. Five bacterial genera capable of utilizing diesel oil as a carbon source were isolated from the contaminated soils. Two isolates, *Pseudomonas aeruginosa* and *Bacillus subtilis* were the most frequently encountered with a percentage prevalence of 31.4% and 24.4% respectively, while *Proteus* spp. had the least frequency of occurrence with 4.6%. *Fusarium* had the highest count of 32.9% while *Mucor* had the least of 8.9% among the fungal genera. The pH for the contaminated and control soils were 6.7 and 6.9 respectively while the temperature for the contaminated and control are 27°c and 29 °c respectively. There was no significant

difference the between the contaminated and control values of pH and temperature at 95% probability level.

Table 1: Mean total viable count of Microorganisms isolated from Contaminated and control soil (Cfu/g)

Sample	Bacteria	Fungi
Contaminated soil	5.2×10^6	3.8×10^6
Control soil	2.2×10^6	2.4×10^6

Table 2: Frequency of occurrence of Microbes isolated from soil contaminated with diesel.

Bacteria isolates	Frequency of occurrence (%)
Pseudomonas aeruginosa	27 (31.4)
Bacillus subtilis	21 (24.4)
Micrococcus spp.	17 (19.8)
Corynebacterium spp.	17 (19.8)
Proteus spp.	4 (4.6)
Fungal isolates	
Fusarium spp.	22 (32.9)
Aspergillus spp.	15 (22.4)
Penicillium spp.	14 (20.9)
Rhizopus spp.	10 (14.9))
Mucor spp.	6 (8.9)

Table 3: pH and Temperature (°c) of contaminated soil and control

Source	pH	Temperature (°c)
Contaminated	6.7	27
Control	6.9	29

DISCUSSION

The microbial diversity of diesel oil contaminated soil from Oluku community was investigated. The microorganisms capable of utilizing oil and oil products as a sole source of carbon and energy practically occurs everywhere in air, water and soil, a term referred to as ubiquitous by Oliver and Magot (2005); Malik and Ahmed (2012). This study showed bacteria and fungi counts of 5.2×10^6 cfu/g and 3.8×10^6 cfu/g respectively.

This report corroborates with report of Rosenberg and Ran (1996) where they estimated that in 1g of unpolluted soil, there are $1x10^2$ to $1x10^3$ cells of hydrocarbon degrading microorganisms whereas in 1g of oil polluted soil, their numbers increases to between $1x10^6$ to $5x10^7$. This study revealed that the diesel oil contaminated sites played host to a lot of microorganisms more than the control. This observation is related to findings made by Ujowundu *et al.,* 2011 who revealed that oil contaminated soils are enriched with more nutrients especially carbon which is the building block of life. Hence, more microorganisms. The utilization of the diesel oil as sole source of carbon and energy by these microorganisms resulted in their greater population.

Although, some studies have shown that hydrocarbon (diesel) contaminated sites habour mainly gram negative bacteria (Macnaughton *et al*., 1999; Kaplan and Kitts, 2004). The dominant hydrocarbon utilizing bacteria isolated from this study were mainly gram positive belonging to the genera *Bacillus subtilis*, *Corynabacterium* spp. and *Micrococcus* spp. with the exception of *Pseudomonas aeruginosa*. These are similar to the findings from other studies. Ujowundu *et al*. (2011) on diesel petroleum contaminated soil in Southeastern Nigeria reported the presence of *Bacillus* spp., *Micrococcus* spp. and *Corynebacterium* spp. Also, Chikere *et al*. (2009) isolated the presence of similar organisms including *Norcadia* spp., *Rhodococcus* spp., *Arthrobacter* spp. and *Mycobacterium* spp.

The variations in the isolates of this study and those of previous findings could be attributable to various factors like media, method of culturing, time and method of sampling, period of transportation and storage (Akortha *et al*., 2011).

Pseudomonas aeruginosa (31.4%) and *Bacillus subtilis* (24.4%) being the most frequently encountered bacteria in this study agrees with the findings from other studies. Sebiomo *et al*. (2011) reported *Pseudomonas* and *Bacillus* spp. as the most prevalent in their studies carried out in Ijebu –Ode.

However, in constrast to this study, Ujowundu *et al*. (2011) isolated *Staphylococcus* from their study. Sebiomo *et al*. (2011) and Ujowundu *et al*. (2011) reported *Aspergillus* as the most predominant genera of fungi in their work in addition to other fungi genera. Ijah and Antai (2003) reported *Bacillus* spp. as being the predominant isolate of all the crude oil utilizing bacteria characterized from highly polluted soil samples. *Bacillus* spp. are more tolerant to high levels of hydrocarbon in soil due to their resistant endospores. There is known evidence that isolates belonging to the *Bacillus* spp. could be effective in cleaning oil spills (Ghazi *et al*., 2004; Singh and Lin, 2008).

The pH and temperature of the soil was 6.7 and 27°c respectively. This invariably favours microbial growth. This corresponds with the report of Ma'an *et al*. 2011 where they revealed a pH of 6.9 and temperature of 28°c on an isolated bacterial consortium for crude oil biodegradation and also Chikere *at al*., 2009, where they reported a pH of 6.5 and temperature of 26°c in their studies on bacterial diversity in tropical crude oil polluted soil undergoing bioremediation.

The pH reported in this study is near neutrality which inevitably favours the growth and proliferation of microorganisms. Their presence could be attributable to the ubiquitous nature of microorganisms and the fact that the contaminated sites contains high organic content thereby providing excellent nutritional source for the propagation of microorganisms (Singh and Lin, 2008).

Conclusively, this study revealed that diesel oil contaminated sites habour more microorganisms because it contains more nutrients particularly carbon which support microbial growth.

REFERENCES

1. Akortha, E.E., Aluyi, H.S.A. and Enerijiofi, K.E. (2011). Transfer of amoxicillin
2. resistance gene among bacterial isolates from sputum of pneumonia patients attending the University of Benin Teaching Hospital, Benin City, Nigeria. *Journal of Medicine and Medical Sciences*.2 (7): 1003-1009.
3. Aluyi, H.S.A., Ekhaise, F.O. and Adelusi, D.M. (2006). Effect of human activities and oil
4. pollution on the Microbiological and Physiological Quality of Udu River, Warri, Nigeria. *Journal of Applied Sciences* 6 (5): 1214 1219.
5. Barnett, H.L. and Hunter, B.B. (1998). Illustrated genera of imperfect fungi. 4th APS Press, St. Paul, Minnesota. 218pp.
6. Chikere,C.B., Okpokwasili, G.S.C. and Chikere, B.O.(2009). Bacterial diversity in a
7. tropical crude oil-polluted soil undergoing bioremediation. *African Journal of Biotechnology*. 8 (11): 2535-2540.
8. Ekpo, M.A. and Udofia,U.S. (2008). Rate of biodegradation of crude oil by
9. microorganisms isolated from oil sludge environment. *African Journal of Biotechnology*. 7(24): 4495-4499.
10. Ghazali, F.M. Zaliha, Adbul Rahman, R.N., Salleh, A.B., Basri, M. (2004).
11. Biodegradadtion of hydrocarbons in soil by microbial consortium. *International. Biodeterioration and Biodegradation*.54: 61- 67.
12. Hamamura, N., Olsun, S.H., Ward, D.M. and Inskeep, W.P. (2006). Microbial population
13. dynanics associated with crude oil biodegradation in diverse soils. *Applied Environmental Microbiology*. 72: 6316-6324.
14. Holt, G.T., Krieg, R.N., Sneath, P.H.A., Staley, T.J. and Williams, T.S. (1994). Manual of
15. Determinative Bacteriology. 9th edition. Williams and Wilkins Baltimore Md, USA. pp176-211.
16. Ijah, U.J.J. and Antai, S.P. (2003). Removal of Nigerian light crude oil in soil over a 12 month period. *International. Biodeterioration and Biodegradation*. 51: 93-99.
17. Jain, R.K., Kapur, M., Labana, S., Lal, B., Sarma, P., Mhattacharya ,D. and Thakur, I.S.
18. (2005). Microbial diversity: Application of microorganisms for the biodegradation of xenobiotics. *Curricular Science.* 89: 101-112.
19. Kaplan, C.N. and Kitts, C.L. (2004). Bacterial succession in a petroleum land treatment unit. *Applied Environmental Microbiology*. 70: 1777- 1786.
20. Kharaka, Y.K. and Hanor, J.S. (2003). Deep fluids in the continental: In sedimentary basins, in Drever, J.I., ed. Treatise on Geochem. 5: 499-540.
21. Ma'an, F.A., Alam, M.Z., Suleyman, A. M. and Iman, A. F. (2011). An isolated bacterial consortium for crude oil Biodegradation. *African Journal of Biotechnology*. 10(81): 18763-18767.
22. Macnaughton, S.J., Stephen, J.R., Venosa, A.O., Davis, G.A., Chang, Y.J. and White,
23. D.C. (1999). Microbial population changes during bioremediation of an experimental oil spill. *Applied Environmental Microbiology*. 65: 3516-3574.
24. Malik, Z .A. and Ahmed, S. (2012). Degradation of petroleum hydrocarbons by oil field isolated bacteria**l**

consortium. *African Journal of Biotechnology*. 11(3): 650-658.

25. Obire, O. and Putheti, D.R. (2008). Fungi in Bioremediation of oil polluted environments.
26. Ogbeibu, A.E. (2005). Biostatistics: A practical Approach to Research and Data Handling. Mindex Publishing Company Limited. 264pp.
27. Okerentugba, P.O and Ezeronye, O.U. (2003). Petroleum degrading potentials of single
28. and mixed microbial cultures isolated from rivers and refinery effluent in Nigeria. *African Journal of Biotechnology*. 2 (9): 288-292.
29. Okpokwasili, G.S.C. (2006). Microbes and the Environmental Challenge. Inaugural lecture series No. 53. University of Port Harcourt Press.Port Harcourt. 56pp.
30. Oliver, B. and Magot, M. (2005). Indigenous microbial communities in oil fields. In

a. petroleum microbiology, Washington, D.C. ASM press. pp 21-34.

31. Quatrini, P., Scaglione, G., DePasquale, C., Reila, S., and Puglia, A.M. (2008). Isolation of gram positive n-alkane degraders from a hydrocarbon contaminated mediterrarean shoreline. *Journal of Applied Microbiology*. 104: 251-259.
32. Rosenberg, E. and Ron, E.Z. (1996). Bioremediation of petroleum contamination. In:
33. Crawford, R.L. and Crawford, D.L. (eds). Bioremediation: principles and Application. Cambridge University Press. pp 100- 124.
34. Sebiomo, A., Awosanya, A.O and Awofodu, A.D. (2011). Utilisation of crude oil and gasoline by ten bacterial and five fungal isolates. *Journal of Microbiology and Antimicrobials*. 3(3): 55-63.
35. Shoal, S.H. and Srivastava, A.K. (1994). Environment and Biotechnology. Role of
36. biotechnology in pollution control. Ashish Publishing House. New Delhi. Pp 163-170.
37. Singh, C. and Lin, J. (2008).Isolation and characterization of diesel oil degrading
38. indigenous microrganisms in Kwazulu-Natal, South Africa. *African Journal of Biotechnology*. 7 (12): 1927-1932.
39. Turgay, O.C., Erdogan, E.E. and Karaca, A. (2010). Effect of humic deposit (leonardite)
40. on degradation of semi-volatile and heavy hydrocarbons and soil quality in crude-oil-contaminated soil. *Environmental Monitoring Assessment*. 170: 45-58.
41. Ujowundu,C.O., Kalu, F.N., Nwaoguikpe,R.N., Kalu, O.I., Ihejirika, C.E., Nwosunjoku,
42. E.C. and Okechukwu, R.I. (2011). Biochemical and physical characterization of Diesel Petroleum Contaminated Soil in Southeastern Nigeria. *Research Journal of Chemical Sciences*. 1(18): 57-62.
43. USEPA Emergency Response Programme. (2008). Physical properties of petroleum based oil. www.epa.gov.
44. Watanabe, K. (2002). Linking genetics, physiology and ecology: an interdisciplinary
45. approach for advancing bioremediation. *Journal of Bioscience and Bioengineering*. 94 (6): 557-562.

Zhang, L., Huo, M. and Zhu, S. (2012). Enzymatic remediation of the polluted crude oil by *Rhodococcus* African *Journal of Microbiology Research.* 6(7):1540-1547.