Remediation Capacities of Selected Fibrous Waste on Crude Oil Contaminated Soil

I.H Ifijen
Rubber Research Institute of Nigeria, P.M.B. 1049, Benin-City, Nigeria

Abstract
The use of groundnut and melon seed husk in the remediation of crude oil contaminated soil was examined. The remediation was carried out at different concentrations (0.654mg/kg, 0.706mg/kg, 1.581mg/kg & 3.460mg/kg) of poly aromatic hydrocarbon (PAHs) in the soil sample C ($C_{10,}$ C_{15}, C_{20} & C_{25}), which represents the control. The levels of PAHs in the soil samples before and after one month of amendments application were determined. The PAH levels of the soil that was amended with melon seed husk for A_{10ml}, A_{15ml}, A_{20ml} and A_{25ml} were reduced by 81%, 53%, 61% and 86% respectively, while that amended with groundnut seed husk containing variable volumes of crude oil (sample $B_{10ml,}$ B_{15ml}, B_{20ml} and B_{25ml}) were reduced by 74%, 76%, 69% and 91% respectively.
Keywords: remediation; contaminated soil; polycyclic aromatic hydrocarbon; melon seed husk; groundnut seed husk.

1.0 INTRODUCTION

Pollution of the soil through anthropogenic activities been associated with increasing amounts of petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAH's), halogenated hydrocarbons, pesticides, solvents of different chemical nature, metal complexes and salts in the environment. The adverse effects of these chemicals on soil flora, fauna, and human health is well documented[1, 2].Hence, various methods of remediating crude oil contaminated soils have been designed including bio-augmentation, bio-stimulation, natural attenuation, phytoremediation, and rhizoremediation[3-7]. Remediation of crude oil contaminated soils usually involves the degradation of these contaminants with the aid of bacteria or their phytoextraction/degradation by plants[1, 8,9]. In Phytoremediation, volatile contaminants can also be removed from the soil via evapotranspiration in a process known as phytovolatilization[1].The contaminant degradation pathways of microbes typically involves the secretion of contaminant degrading enzymes such as dioxygenases, phosphatases, dehalogenases, P450 monooxygenases, laccases, nitrilases, peroxidases, and nitroreductases[1, 8, 10-12]. Though certain microbial strains can successfully degrade particular compounds alone (e.g mineralization of pentachlorophenol by *Sphingobiumchlorophenolicum* strain ATCC39723), most other microbes work in synergy to degrade contaminants[13, 14]. Several microbes capable of degrading contaminants in crude oil polluted soils have been identified[1, 15-17].In fact, it is a general postulate that bacteria capable of degrading contaminants can be found in virtually all soil samples[1, 18]. Some examples of microbes previously identified for their potentials in treatment of PAH contaminated soils include bacteria of the genera*Acinetobacter, Achromobacter, Bacillus, Actinomyces, Exiguobacterium, Burkholderia, Klebsiella, Nocardia, Microbacterium, Spirillum, Pseudomonas, Vibrio and Streptomyces, and fungi, Aspergillus, Allescheria, Debayomyces, Candida, Penicillium, Mucor, Saccharomyces and Trichoderma[19]*. Previous research efforts at bio-remediation of contaminant polluted soils have focused on the characterization of available microbes in a soil sample and stimulation of their degradation pathways via inoculation with effective contaminant degrading bacteria[1, 3].However, some attempts at maximizingthe use of contaminant degrading bacteria viainoculation of soil samples (bio-augmentation), have been relatively unsuccessful[1, 15,20-22]. Reasons for the lack of success in these attempts include the inability of the introduced inoculant to compete with the already existing microflora/fauna in the soils, the inability of the inoculant to grow sufficiently to reach sub-surface contaminants, inadequate nutrients in the soil to support microbial growth, preferential metabolism of other carbon based compounds in the soil instead of the contaminant of interest, and presence of toxic chemicals in the soil that could impede microbial growth[1, 15, 23]. One successful mechanism of bypassing some of these challenges is analogue enrichment which involvesthe introduction of metabolites that are usually formed during the degradation process, so as to stimulate the degradation pathway[1, 24,25]. The introduction of nitrates, phosphates, and aeration of samples have also been identified as means of improving the effectiveness of contaminant degrading bacteria[1, 3]. Other factors that affect the performance of contaminant degrading bacteria/plants include residual oil concentration, population density of the microbes, environmental conditions and chemical composition of the hydrocarbons present[1, 3]. In the case of phytoremediation, the concentration of contaminants could affect the effectiveness of the bioremediation process through the introduction of oxidative stress[1]. This coupled with the fact that contaminant rich soils are usually poor in nutrient levels and the diversity of the microbial consortium, result in poor plant biomass accumulation, and remediation rates in such samples[1].

In this work, natural attenuation and bio-stimulation of crude oil contaminated soil using groundnut seed husk and melon seed husk (which are typically agricultural waste) for degradation of PAHs was investigated and statistical analysis of the obtained result was done.

2.0 Materials and Methods

2.1 Sample Collection

The crude oil (unrefined forcados blend) was obtained from the forcados terminal of shell company. The study was carried out in biological garden in Earth Quest laboratory in Warri, Delta State.

The soil used in this study was required to be free of PAHs contamination. It was collected from a virgin site at University of Benin. The soil was sieved with 2mm mesh before use and was analyzed for any PAHs content and physiochemical properties.

Melon shell and groundnut husk were collected from an open market in Auchi, Edo State. Both agricultural materials were grounded to powdered form and then sieved to a size of 0.84mm.

2.2 Bioremediation Experiments

One kilograms of soil sample was measured into 26 plastic buckets (PB) labeled A and D in replicate. Samples A to C was contaminated with variable volumes of crude oil each (A_{10ml}, A_{15ml}, A_{20ml}, A_{25ml}, B_{10ml}, B_{15ml}, B_{20ml}, B_{25ml} & C_{10ml}, C_{15ml}, C_{20ml}, C_{25ml}) in duplicate, sample C being the control while D had no crude oil. The samples were allowed to stand for 30days with daily watering and mixing for proper acclimatization of the microorganism. The samples were analyzed for PAH contents and physicochemical properties. Samples A and B were treated with 1.0g of groundnut seed husk and melon seed husk respectively. The samples were stirred and left for another 30days with daily watering.

2.3 Physico-chemical properties of the soil

The physical-chemical properties soil (The pH, organic carbon, conductivity, total nitrogen content, phosphorous content) were determined using standard methods.

2.4 PAHs Determination

The amount and type of PAHs content present in each soil sample was determined using a gas chromatography

3.0 Result and Discussions

Table 1: Physico-chemical properties of the melon/groundnut seed husk and the uncontaminated soil sample

Component	Melon seed husk	Groundnut seed husk	Uncontaminated Soil Sample
PH	7.800	8.200	5.08
Total organic carbon (%)	18.530	19.210	0.59
Phosphorous (%)	0.021	0.031	0.019
Nitrogen (%)	0.090	0.160	0.032

The agricultural amendments with melon and groundnut seed husks were observed to posses appreciable quantities of organic carbon of about 18.53% & 19.21% respectively. This is a major nutrient requirement by bacteria in the degradation of organic substances in the environment [3].

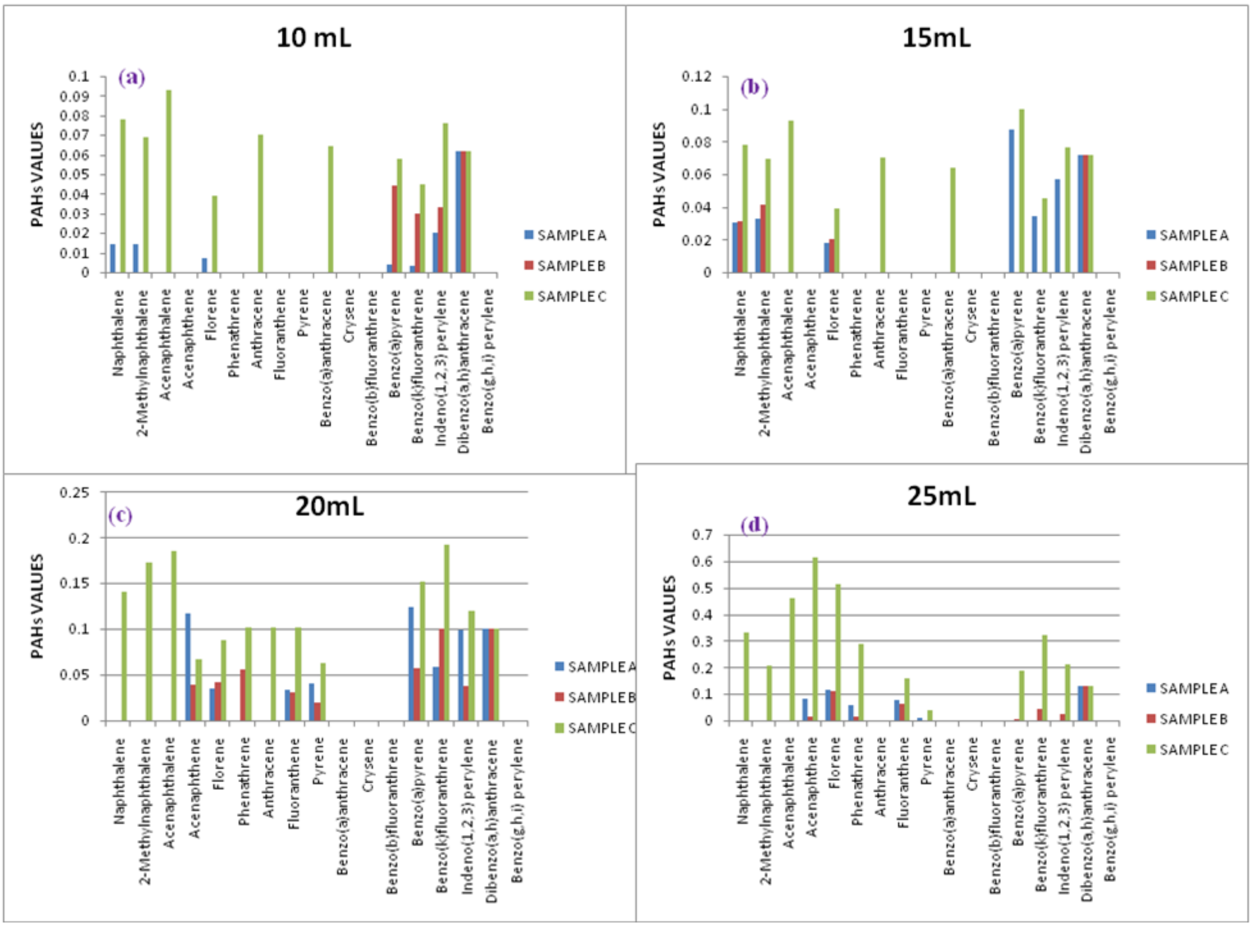

Figure 1**:** A bar chart showing the comparison of the PAHs fractions (10ml, 15ml, 20ml and 25ml of sample A, B & C)

A represents the contaminated soil with variable volume of crude oil amended with melon seed husk.
B represents the contaminated soil with variable volume of crude oil amended with groundnut seed husk.
C represents the contaminated soil sample with variable volume of crude oil without any agricultural amendment.
D represents the uncontaminated soil.

Analysis of results from the remediation of the selected PAH's in the contaminated soils according to the bar chat displayed in Figure 1, showed that melon and groundnut seed husk are good stimulants for promoting the natural attenuation of contaminants in soils. The effectiveness of the seed husks in treatment of the contaminated soils may be attributed to the amount of organic carbon, nitrogen and phosphorus present in them (Table 1). These will naturally serve as sources of nutrients aiding the metabolism of the microbes present in the soil samples [4]. In the bar charts it was observed that of all the PAHs monitored at the 10ml and 15 ml crude oil amount, acenaphthene, phenanthrene, flouranthene, pyrene, chrysene, benzo(b)flouranthene, benzo(g,h,i)perylene were degraded via natural attenuation without the use of bio-stimulants (melon seed husk or groundnut seed husk), however as the amount of crude oil was increased to 20ml and 25ml contamination level, only chrysene, benzo(b)flouranthene and benzo(g,h,i)perylene were degraded by natural attenuation. The effect of the bio-stimulants on the ease of degradation was noticed at all contamination levels. With melon seed husk, in addition to the PAHs which were degraded by natural attenuation, Napthalene, 2-methyl nathalene, Acephthene, florene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(k)fluoranthrene, indeno(1,2,3) perylene, acenapthene, phenathene and pyrene were also degraded, likewise with groundnut seed husk, Napthalene, 2-methyl nathalene, Acephthene, florene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(k)fluoranthrene, indeno(1,2,3) perylene, acenapthene, phenathene and pyrene were also degraded in addition to the PAH's degraded by natural attenuation, however, the extent to which they were degraded was different. At the 10ml, 15ml, 20ml amd 25ml contamination level naphthalene, 2-methylnaphthalene, Acephthalene, florene and anthracene were the most degraded. Generally, groundnut seed husk proved to be better bio-stimulants according to the bar chat shown in Figure 2. This may be attributed to the higher amounts of total organic carbon (%), phosphorous (%), nitrogen (%) in the groundnut seed husk (Figure 1). This trend agrees with previous reports by Bento *et al*. [3] who also noticed an improvement in bioremediation capacities as a result of the addition of nutrient sources (bio-stimulants) for soil microbes.

Table 2: Total PAHs content of samples at different levels of contamination

	10mL	15 mL	20 mL	25 mL
SAMPLE A	**0.124**	**0.331**	**0.611**	**0.468**
SAMPLE B	**0.169**	**0.164**	**0.483**	**0.299**
SAMPLE C	**0.654**	**0.706**	**1.581**	**3.460**

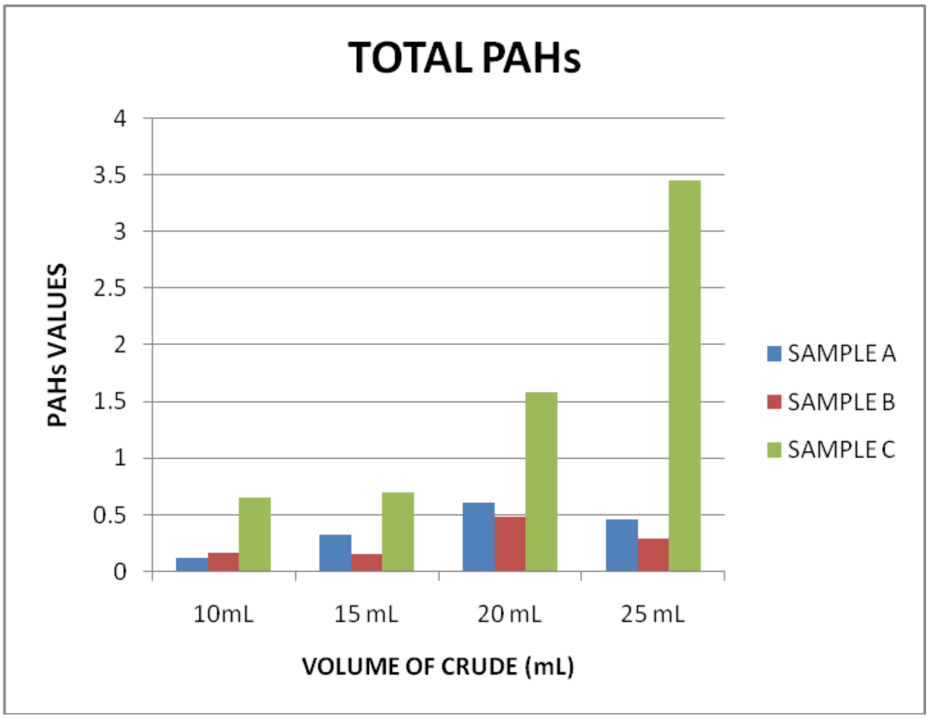

Figure 2**:** A bar chart showing the comparison of the total PAHs fractions (sample A, B and sample C)

Figure 2 shows the total PAHs that were degraded after 30days of remediation of 10ml, 15ml, 20ml and 25ml crude oil contaminated soil. The sample that was contaminated with 10ml of crude oil (C_{10ml}) contained total PAHs of 0.654mg/kg, after 30days of remediation, the PAHs level was noticed to have decreased to 0.124mg/kg and 0.169mg/kg for soil amended with melon seed husk (A_{10mL}) and groundnut seed husk (B_{10mL}) respectively. Similar trends were observed for sample $C_{15ml,}$ $C_{20ml,}$ C_{25ml} as their total PAHs levels decreased from 0.706mg/kg, 1.581mg/kg and 3.46mg/kg to 0.164mg, 0.483mg/kg and 0.299mg/kg after 30days of remediation for soil amended with melon seed husk and groundnut seed husk respectively. This is probably due to the continual ventilation and bio-stimulant addition (melon and groundnut seed husk) which had considerable effect on the growth of hydrocarbon degrading bacteria in soil [19].

In summary, the PAHs content of the soil samples (A & B) contaminated with 10ml of crude oil were respectively degraded by 81% and 74%, 15ml of crude oil contamination were degraded by 53% and 76% , 20mL crude oil contamination were degraded by 61% and 69%. Lastly, 25mL of crude oil contamination were degraded by 84% and 91%.

These result showed that, melon and groundnut seed husk amendments increased the microbial activity in the soil for degradation of organic material.

4.0 CONCLUSION

The results of this study show that melon and groundnut seed husks possess the ability to enhance the remediation of crude oil in contaminated soil. This has shown that agricultural wastes of no economic value which caused environmental menace can have industrial potential to become a source of income to rural dwellers. Their use in reclaiming oil polluted soil should be encouraged in the Nigerian environment.

Reference

1. K.E. Gerhardt, X.-D.H., B.R. Glick, B.M. Greenberg, *Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges.* Plant science, 2009. **176**: p. 20-30.
2. R.B. Meagher, *Phytoremediation of toxic elemental and organic pollutants.* Curr. Opin. Plant Biol., 2000. **3**: p. 153-162.
3. F.M. Bento, F.A.O.C., B.C. Okeke, W.T. Frankenberger, *Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation.* Biores Technol,

2005. **96**: p. 1049–1055.
4. Skipper, H.D., *Bioremediation of contaminated soils.* Principles and Applications of Soil Microbiology., ed. D.M.E. Sylvia. 1999, Prentice Hall, Upper Saddle River, NJ.
5. E. Seklemova, A.P., K. Kovacheva *Biostimulation based bioremediation of diesel fuel: field demonstration.* . Biodegradation, 2001. **12**: p. 311–316.
6. J.Y. Richard, T.M.V., *Characterization of a soil bacterial consortium capable of degrading diesel fuel.*
. Int. Biodet. Biod., 1999. **44**: p. 93–100.
7. S. Barathi, N.V., *Utilization of petroleum hydrocarbons by Pseudomonas fluorescence isolated from a petroleum contaminated soil.* Environ Int., 2001. **26**: p. 413-416.
8. S. Susarla, V.F.M., S.C. McCutcheon, *Phytoremediation: an ecological solution to organic chemical contamination.* Ecol. Eng., 2002. **18**: p. 647–658.
9. T. Macek, M.M., J. Kas, *Exploitation of plants for the removal of organics in environmental remediation.* Biotechnol. Adv., 2000. **18**: p. 23–34.
10. Q. Chaudhry, M.B.-Z., S. Gupta, E.J. Joner, *Utilizing the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment.* Environ. Sci. Pollut. Res., 2005. **12**: p. 34-48.
11. A.C. Singer, I.P.T., M.J. Bailey, *The tritrophic trinity: a source of pollutant-degradaing enzymes and its implications for phytoremediation.* Curr. Opin. Microbiol., 2004. **7**: p. 239–244.
12. S. Cherian, M.M.O., *Transgenic plants in phytoremediation: recent advances and new possibilities.* Environ. Sci. Technol., 2005. **39**: p. 9377–9390.
13. M. Cai, L.X., *Organization and regulation of pentachlorophenol-degrading genes in Sphingobium chlorophenolicum ATCC 39723.* J. Bacteriol., 2002. **184**: p. 4672–4680.
14. M.-H. Dai, J.B.R., J.R. Warner, S.D. Copley, *A previously unrecognized step in pentacholorophenol degradation in Sphingobium chlorophenolicum is catalyzed by tetrachlorobenzoquinone reductase (PcpD).* J. Bacteriol., 2003. **185**: p. 302–310.
15. I. Kuiper, E.L.L., G.V. Bloemberg, B.J.J. Lugtenberg, *Rhizoremediation: a beneficial plant–microbe interaction* Mol. Plant Microbe Interact., 2004. **17**: p. 6–15.
16. D.L. Johnson, K.L.M., D.R. Anderson, S.P. McGrath, *Enhanced dissipation of chrysene in planted soil: the impact of a rhizobial inoculum.* Soil Biol. Biochem., 2004. **36**: p. 33–38.
17. Lovley, D.R., *Cleaning up with genomics: applying molecular biology to bioremediation.* Nat. Rev., 2003. **1**: p. 35–44.
18. A.C. Singer, I.P.T., M.J. Bailey, *The tritrophic trinity: a source of pollutant-degradaing enzymes and its implications for phytoremediation.* Curr. Opin.Microbiol., 2004. **7**: p. 239–244.
19. H-S. Joo, P.M.N., M.Shoda, C-G. Phae, *Bioremediation of oil-contaminated soil using Candida catenulata and food waste.* Environmental Pollution, 2008. **156**: p. 891-896.
20. Pilon-Smits, E., *Phytoremediation.* Annu. Rev. Plant Biol, 2005. **56**: p. 15-39.
21. M. Dua, A.S., N. Sethunathan, A. Johri, *Biotechnology and bioremediation: successes and limitations.* Appl. Microbiol. Biotechnol., 2002. **59**: p. 143-152.
22. H. Harms, T.N.P.B., *Mass transfer limitation of microbial growth and pollutant degradation.* J. Ind. Microbiol. Biotechnol., 1997. **18**: p. 97-105.
23. P. Van Dillewijn, A.C., J.A. Paz, M.M. Gonza´ lez-Pe´ rez, J.M. Oliva, J.L. Ramos,, *Bioremediation of 2,4,6-trinitrotoluene under field conditions.* Environ. Sci. Technol., 2007. **41**: p. 1378-1383.
24. W. Brunner, F.H.S., D.D. Focht, *Enhanced biodegradation of polychlorinated biphenyls in soil by analog enrichment and bacterial inoculation.* J. Environ. Qual., 1985. **14**: p. 324-328.
25. P.A. Holden, M.K.F., *Soil microorganisms in soil cleanup: how can we improve our understanding?* . J. Environ. Qual., 1997. **26**: p. 32-40.