Global Journal of Environmental Sciences. Vol. 4, No. 1, 2005: 5-10
ISSN 1596-6194.

ENHANCING CRUDE OIL DEGRADATION IN A SANDY SOIL: EFFECTS OF ADDITION OF POULTRY MANURE.

JUSTINA C. OKOLO

(Received 4 August 2004 ; Revision accepted 8 November 2004)

ABSTRACT

Natural restoration of crude oil polluted soils takes a long time, hence various soil treatments have been used to hasten the process. This study investigated the effects of the addition of poultry manure alone and in combination with surfactant (Goldcrew or Corexit) and/or alternate carbon substrate (glucose or starch) on crude oil degradation in a sandy soil. With poultry manure alone, optimal crude oil degradation was obtained at a concentration of 4.0% (w/w). At a poultry manure concentration of 1.0% (w/w), addition of either of the surfactants at 0.01% (v/w) did not significantly enhance crude oil degradation ($P>0.05$), while addition of either of the alternate carbon substrates at 0.5% (w/w) significantly increased the toluene extractable oil content ($P<0.05$). Enhanced degradation was observed with a combination of poultry manure (4.0%w/w) + Goldcrew (0.01%v/w) + glucose (0.5% w/w). Time course for crude oil degradation in samples which were given optimal treatments that contained poultry manure showed that after sixteen weeks incubation, the highest crude oil degradation of 7.92 ± 0.75% was observed from the sample treated with poultry manure alone at 4 % (w/w).

KEY WORDS: Poultry manure, Surfactants, Alternate carbon substrates, crude oil degradation.

INTRODUCTION

Pollution of soil by crude oil has deprived man of vast agricultural lands due to the adverse effects of crude oil on soil. Crude oil is rich in carbon but deficient in nitrogen and phosphorus, hence soil pollution by crude oil leads to increase in soil C: N and C: P ratios (Atlas, 1981). This affects the soil microbes that also depend on soil nutrients for their growth and proliferation. Different organic materials such as sewage sludge (Dibble and Bartha, 1979) and yeast extracts (Lehtomaki & Niemela, 1975) have been added to soils to serve as nutrient supplements providing nitrogen and phosphorus. Alternate carbon substrates have also been added to ensure adequate microbial number (Balba, 1993). Since crude oil sorbs to soil, it might not be bio-available for microbial utilisation, hence surfactants have also been added in some studies (Ellis *et al.*, 1990, Rithman and Johnson, 1989). The results of the effects of the addition of these soil treatments on the degradation of petroleum hydrocarbons have however been conflicting. Enhanced hydrocarbon degradations were reported upon addition of supplementary nutrients (Song *et al.*, 1990) and surfactants (Rithman and Johnson, 1989). The inhibition of mineralisation of aromatic compounds upon fertilizer addition (Morgan and Watkinson, 1990) and a significant increase in the apparent concentration of a polyaromatic hydrocarbon following surfactant addition (Litchfield *et al.*, 1992) have also been reported. This probably reflects the heterogeneity of soil and crude oil samples and indicates that there is no universal treatment regimen for the bioremediation of crude oil-polluted soils. There is therefore the need to optimize for soil treatments to be applied, to enhance the bioremediation of crude oil-polluted soils.

Poultry manure is rich in nitrogen, is easily available and has manure value in soil fertility (Amadi and UeBari, 1992). In this study, the impact of the addition of poultry manure alone and in combination with either surfactants and/or alternate carbon substrates on crude oil degradation was investigated.

MATERIALS AND METHODS

Collection of sample: Soil samples were collected randomly with a Dutch auger at a depth of 15cm from the agricultural farm of the Rivers State University of Science and technology, Port-Harcourt, Nigeria. Samples were homogonized, dried, sieved through a 2mm mesh and stored in polythene bags at room temperature (28±2°C) in the laboratory.

The crude oil which was used in contaminating the soil was a Nigerian Bonny medium blend obtained from Shell Petroleum Development Company (SPDC) Limited, Port-Harcourt, Nigeria.

Soil amendment materials included NPK (20:10:10) fertiliser obtained from National Fertilizer Company (NAFCON), Port Harcourt, Nigeria. Goldcrew and Corexit surfactants were obtained from SPDC, while poultry manure was obtained from a poultry farm in Port Harcourt. The poultry manure was air dried, crushed and stored in the laboratory at room temperature (28 ± 2°C) before use.

Soil characterization: The soil was characterized before contamination with the crude oil. Particle size determination was by the hydrometer method of Bouyoucos (1951) and pH was determined according to the modified method of McLean (1982). Total organic carbon was determined by the wet combustion method of Walkley and Black (1934) as modified by Nelson and Sommers (1982). Total nitrogen was determined by the semi-micro Kjeldhal method (Bremner and Mulvaney, 1982). Available phosphorous was determined by Brays No.1 method of Olsen and Sommers (1982). Exchangeable cations, calcium and magnesium were determined by EDTA complexiometric titrations (Heald, 1965) while sodium and potassium were determined by flame photometry. Ammonium-nitrogen was determined by the Nesslerizer method of Keeney and Nelson (1982) while nitrate-nitrogen was by the phenodisulphonic acid method (Bremner, 1965).

Soil microbial population was estimated by the ten-fold serial dilution method of Harrigan and McCance (1990). Population of total heterotrophic bacteria and fungi were estimated using nutrient agar (Oxoid) and potato dextrose agar respectively. Populations of petroleum hydrocarbon utilising bacteria and fungi were estimated using by the rapour phase transfer (Amanchukwu *et al.*, 1989) using the mineral salt medium of IPS (1987).

Contamination and amendment of samples: Twenty gram soil portions weighed into 100ml bottles were moistened to 60% of their field moisture capacity and left at room temperature (28 ± 2°C) in the laboratory for one week. Thereafter the samples were treated with 10% (v/w) crude oil and left at the same temperature for another two weeks. A basal dressing of NPK (20:10:10) fertilizer was applied at a concentration of 1250µg/g^{-1} soil. The effects of the various soil amendments were studied sequentially as follows:

i) Effects of soil treatments containing poultry manure at 1.0%(w/w)

ii) Effects of different concentrations of poultry

JUSTINA C. OKOLO, Department of Microbiology, Federal University of Technology, Owerri, Imo State, Nigeria.

Table 1: Soil properties before oil contamination and two weeks after contamination

Soil properties	Before oil contamination (Mean±SEM)	Two weeks after oil contamination.(Mean±SEM)
Chemical		
pH	7.10 ± 0.10[a]	5.10 ± 0.25[b]
Organic – C(%)	1.10 ± 0.01[b]	5.89 ± 0.02[a]
Total N(%)	0.08 ± 0.01[a]	0.10 ± 0.00[a]
C:N ratio	13.75	58.90
Nitrate –N(mg/l)	67.64 ± 0.02[a]	24.60 ± 0.01[b]
Ammonium –N(mg/l)	4.63 ± 0.00[b]	7.22 ± 0.00[a]
Available – P(mg/l)	51.94 ± 0.00[a]	42.81 ± 0.00[b]
Exchangeable bases		
Ca	1.42 ± 0.01[b]	3.62 ± 0.01[a]
Mg	0.13 ± 0.01[b]	0.50 ± 0.01[a]
Na	0.25 ± 0.01[b]	3.25 ± 0.01[a]
K	0.27 ± 0.01[b]	0.44 ± 0.01[a]
Microbiological		
Bacterial populations		
Total heterotrophs	0.65 ± 0.07[b]	3.90 ± 0.20[a]
Petroleum hydrocarbon	0.42 ± 0.05[b]	2.75 ± 0.02[a]
Fungal populations		
Total heterotrophs	0.32 ± 0.02[b]	1.85 ± 0.08[a]
Petroleum hydrocarbon	0.14 ± 0.00[b]	0.68 ± 0.10[a]

(a, b...) Within row, Mean ± SEM with different superscripts are significantly different at P<0.05

manure alone (0.5-4.0%w/w)

iii) Effects of different concentrations of poultry manure (0.5-4.0%w/w) supplemented with surfactant (Corexit at 0.001-1.00%v/w).

iv) Effects of different concentrations of poultry manure (0.5-4.0%w/w) supplemented with surfactant (Goldcrew at 0.001-1.00%v/w) and alternate carbon substrate (glucose at 0.05-2.0%w/w)

In each study, two control units of the soil were also set up. The contaminated control was treated with 10%(v/w) crude oil and NPK fertilizer while the uncontaminated control was treated with only NPK fertilizer. Both the amended soils and the controls were incubated at room temperature (28 ± 2°C) in the laboratory for four weeks. Thereafter the samples were air-dried, homogenized and oil content estimated. Changes in oil content in the treatments were calculated relative to the oil content in the contaminated control.

Time course for crude oil degradation in amended soils: The 60% moistened soil sample was left for two weeks and thereafter treated with 10%(v/w) crude oil and left at the same temperature for another four weeks. A basal dressing of NPK (20:10:10) fertilizer was applied at a concentration of 1250µg/g^{-1} soil. The samples were then variously treated with the soil amendments at the concentrations previously found to be optimal for crude oil degradation. Contaminated and uncontaminated soils were also set up and the samples incubated as previously described. Replicate samples were analysed at 0, 2, 6, 9, 12 and 16 weeks intervals and changes in oil content calculated relative to the oil content in the contaminated control.

Determination of oil content – Oil content was determined spectrophotometrically according to the toluene extraction method of Odu *et al.* (1989). This method provides an estimate of the available forms of total hydrocarbons in soils. One gram (1g) of the air-dried and homogenized soils was weighed into 50 ml conical flasks. Ten millilitres of toluene (solvent) was added into the flask to extract the oil in the soil. After shaking vigorously, the mixture was allowed to stand for 10 minutes after which it was filtered with Whatman No. 1 filter paper. The extracted oil was diluted appropriately with fresh toluene and the absorbance read at 420nm in Spectronic 21 spectrophotometer.

Determination of carbon dioxide evolution: Carbon dioxide production was determined and calculated according to the methods of Cornfield (1961) and Stotzky (1960). To absorb the carbon dioxide liberated during oil degradation, vials containing 10%(w/v) of barium peroxide in distilled water were placed inside 250 ml screw-capped bottles containing the soil treatments. The vials were withdrawn after four weeks and the amount of the carbon dioxide absorbed determined by titrating the barium carbonate formed with 1N HCl.

Statistical analysis: Each experiment was carried out in triplicates. Data collected were subjected to analysis of variance (ANOVA), and where differences existed, the Duncan's multiple range test (DMRT) was used to separate the means using the Statistical Analysis System (SAS, 1999). The relationships between the variables were established using the correlation analysis.

RESULTS AND DISCUSSION

Soil characterisation before oil application and two weeks after (Table 1) showed that the contamination led to reductions in pH, nitrate nitrogen and available phosphorus contents of the soil. It also led to an increase in the populations of total heterotrophic bacteria and fungi. This increased microbial population is however transient (Morgan

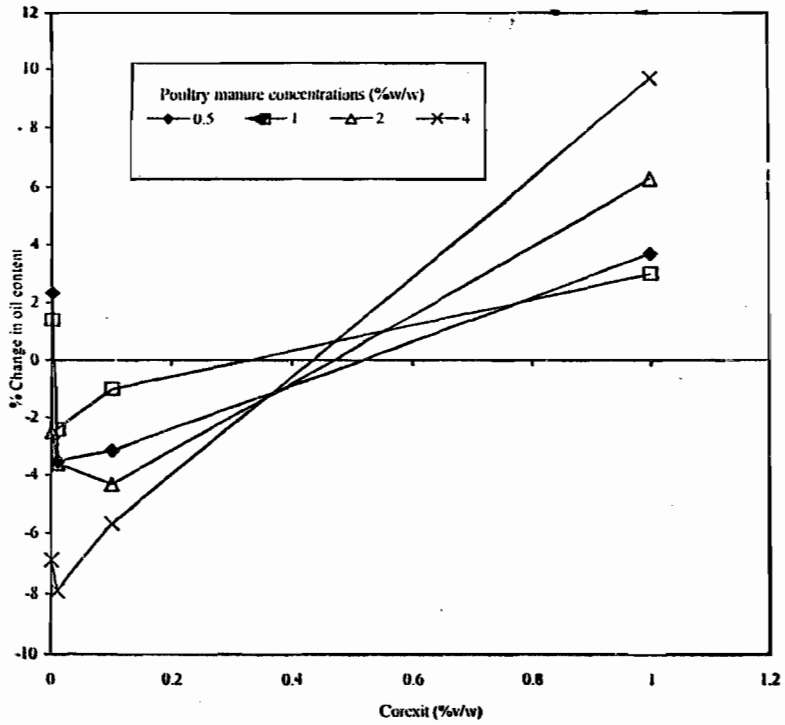

Fig. 1: Effects of different concentration of Corexit with the same concentration of poultry manure on crude oil degradation.

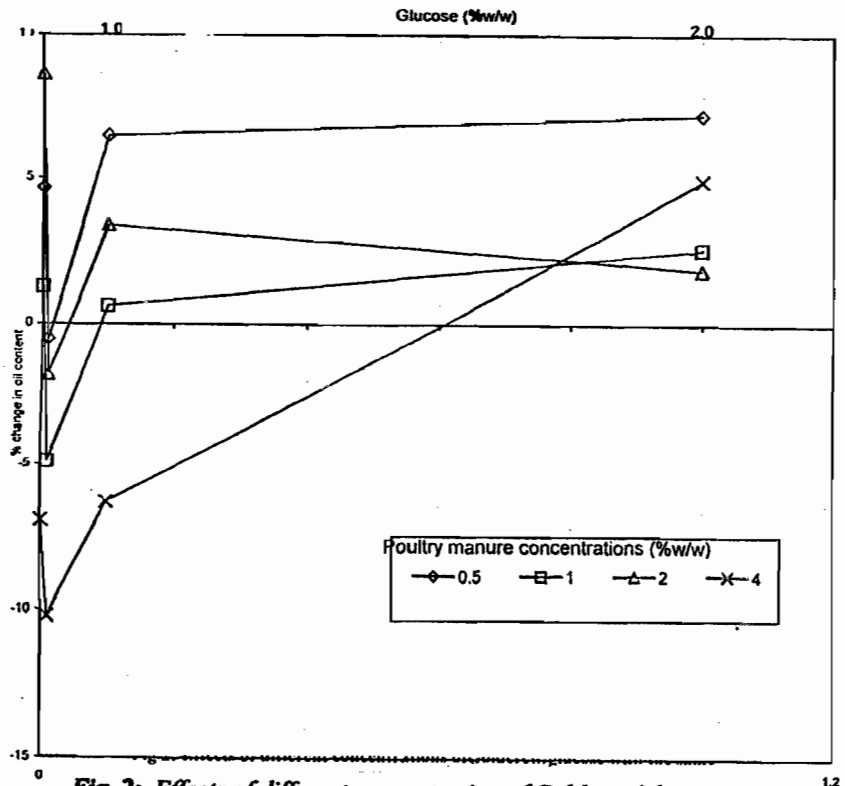

***Fig. 2:** Effects of different concentration of Goldcrew/glucose with the same concentration of poultry manure on crude oil degradation. goldcrew (% v/w)*

and Watkinson, 1989) since the microbes will utilize the already limited soil nutrients and reduce in number due to nutrient deficiency in the soil. The net effect of this, coupled with the chemical nature of crude oil is that microbial degradation of oil in un-amended soil is hampered.

The results of the effects of the addition of poultry manure alone as a nutrient supplement, and in combination with different surfactants and/or alternate carbon substrates to crude oil polluted soils are presented in Table 2. The highest crude oil degradation of 10.03 ± 0.33 % relative to the

Table 2: Effects of treatments containing poultry manure alone and with surfactants and/or alternate carbon substrates on crude oil degradation.

*Soil treatments	Oil contents (ppm) (Mean±SEM)	Change in oil contents (ppm) (Mean±SEM)	% Change in oil contents (Mean±SEM)	CO_2 production (mg/20g soil) (Mean±SEM)
Poultry manure	62,855.20 ± 0.00^{bc}	-1,491.36 ± 97.35^{c}	-2.32 ± 0.15^{c}	33.0 ± 1.0^{b}
Poultry manure + Goldcrew	62,465.81 ± 292.04^{b}	-1,880.75 ± 194.70^{cd}	-2.93 ± 0.30^{cd}	30.8 ± 1.0^{e}
Poultry manure + Corexit	62,222.44 ± 340.72^{b}	-2,124.12 ± 438.07^{cd}	-3.30 ± 0.68^{cd}	30.4 ± 0.5^{e}
Poultry manure + Glucose	67,575.32 ± 48.67^{a}	+3,228.77 ± 48.67^{e}	+5.02 ± 0.08^{e}	39.6 ± 1.0^{d}
Poultry manure + Starch	68,545.26 ± 535.41^{a}	+4,198.71 ± 632.76^{e}	+6.53 ± 0.99^{e}	57.2 ± 0.8^{a}
Poultry manure + Goldcrew + Glucose	57,888.06 ± 292.04^{d}	-6,458.50 ± 194.70^{a}	-10.03± 0.33^{a}	22.0 ± 0.0^{gh}
Poultry manure + Goldcrew + Starch	60,124.72 ± 243.37^{bc}	-4,221.84 ± 146.02^{b}	-6.56± 0.24^{b}	26.4 ± 1.0^{f}
Poultry manure + Corexit + Glucose	59,346.39 ± 486.74^{cd}	-5,000.17 ± 584.08^{b}	-7.77 ± 0.90^{b}	39.6 ± 1.0^{e}
Poultry manure + Corexit + Starch	60,806.16 ± 292.04^{c}	-3,540.40 ± 194.70^{bc}	-5.50 ± 0.31^{bc}	63.4 ± 1.4^{b}
Control	64,346.56 ± 36.79^{b}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	26.4 ± 0.4^{f}

(a, b...) Within column, Mean ± SEM with different superscripts are significantly different at P<0.05

*Soil treatments at concentrations added-Poultry manure: 1.0%w/w; Goldcrew and Corexit (Surfactants): 0.01%v/w; Starch and glucose (Alternate carbon substrates): 0.5%w/w

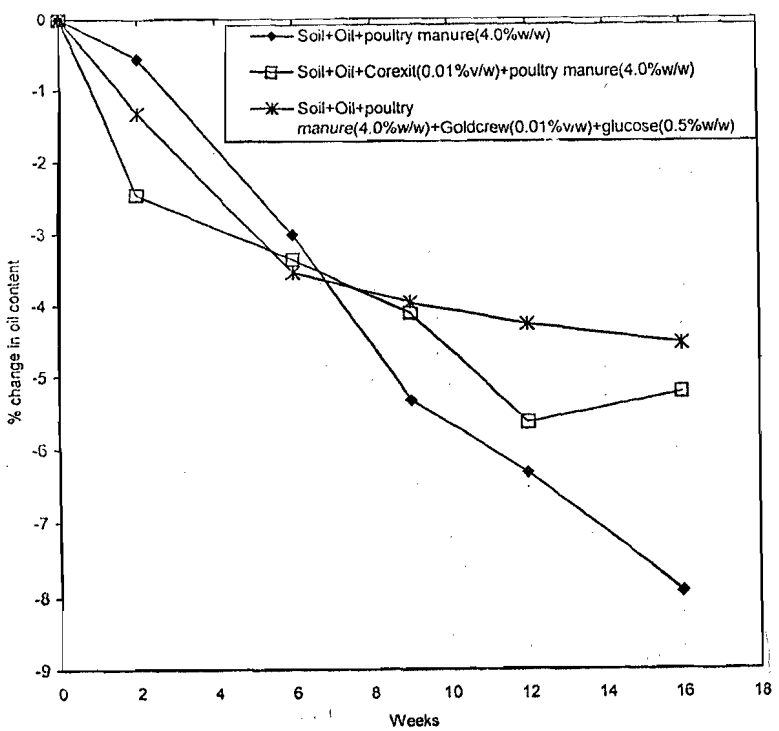

Fig. 3: Time course for crude oil degradation in a sandy soil given optimal treatments containing poultry manure

Table 3: Effects of different concentrations of poultry manure on crude oil degradation.

Poultry manure (%w/w)	Oil content (ppm) Mean ± SEM	Change in oil content (ppm) Mean ± SEM	% Change in oil content Mean ± SEM
0.5	57,760.04 ± 340.72^{ab}	-550.99 ± 146.02^{cd}	-0.95 ± 0.26^{cd}
1.0	57,209.05 ± 0.00^{ab}	-1,101.98 ± 194.70^{c}	-1.89 ± 0.33^{c}
2.0	56,545.15 ± 243.37^{b}	-1,765.88 ± 48.67^{b}	-3.03 ± 0.09^{b}
4.0	52,734.98 ± 194.69^{c}	-5,576.05 ± 0.	-9.56 ± 0.03^{a}
Control	58,311.03 ± 194.69^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}

(a, b...) Within column, Mean ± SEM with different superscripts are significantly different at P<0.05

contaminated control was obtained from sample treated with poultry manure + Goldcrew + glucose. The addition of either of the surfactants alone with poultry manure did not enhance crude oil degradation while the addition of either of the alternate carbon substrates alone with the poultry manure led to a significant increase in toluene extractable oil content (P>0.05) relative to the un-amended control. An increase in oil content in this study meant that greater crude oil degradation was observed in the control sample than in the treatment. This increase in oil content or reduced crude oil degradation following the addition of the alternate carbon substrate alone could be attributed to these substrates being more readily available for microbial growth and metabolism than the crude oil. Morgan and Watkinson (1990) had observed that in the presence of readily degradable substrates, microbes might ignore other available compounds and metabolise the substrates that need less energy to degrade. In contrast to these observations, Brown *et al.* (1986) reported enhanced microbial degradation of pentachlorophenol upon addition of cellobiose.

The significantly greater crude oil degradations observed upon addition of both surfactants and alternate carbon substrates than the addition of either implies that there could be an interaction between the effects of the two amendments on crude oil degradation. The surfactant increased the availability of the otherwise sorbed crude oil in the soil while the alternate carbon substrate provided the easily degradable carbon substrate that ensured the maintenance of a vibrant microbial population in the polluted soil. Knaebel *et al.* (1994) had observed that under realistic soil conditions, microbial mineralisation of organic chemicals in soil is partly controlled by interaction between the chemicals and the soil constituents. The amount of carbon dioxide production correlated positively with the residual oil content in the samples, however this is not significant (P<0.05).

Table 3 showed that the highest crude oil degradation of 9.56 ± 0.03% relative to the control was obtained from the sample treated with poultry manure at 4.0% (w/w). Apparently the supply of the limiting nutrients to the polluted soil increased with increasing concentration of poultry manure. Apart from the nutrient supply, as an organic waste, poultry manure could also have provided other non-specific ancillary compounds that would have encouraged the co-metabolic transformation of the crude oil.

The results of the effects of varying the concentrations of Corexit and poultry manure applied to crude oil polluted soil showed similar patterns of response to increasing concentrations of Corexit in all the concentrations of poultry manure added (Fig. 1). There was an initial reduction in oil content as the Corexit concentration increased until a peak was attained beyond which a further increase in Corexit concentration led to an increase in percentage change in oil content. The highest percentage reduction in oil content of 7.89 ± 0.89% was observed in the sample treated with Corexit + poultry manure concentration of 0.01% (v/w) + 4.0% (v/w). At the Corexit concentration of 1.0%(v/v), increases in oil contents were observed with all the concentrations of poultry manure added. A similar observation was also made even when glucose was added to the soil as an alternate carbon substrate (Fig. 2). It is possible that at the high surfactant concentration, microbial cells were lysed and the hydrocarbon contents of the cell wall (Trudgill, 1978) contributed to the toluene extractable hydrocarbons in the soil. The high surfactant concentration could also have reduced the microbial population in the treated soil relative to the population in the control, leading to more crude oil degradation being effected in the control. Litchfield *et al.* (1992) also reported a statistically significant increase in the apparent concentration of poly aromatic hydrocarbon in a creosote-contaminated site at high surfactant concentration.

The result of the time course for crude oil degradation in soils given different optimal treatments containing poultry manure (Fig.3), showed that the highest crude oil degradation of 7.92 ± 0.75 % relative to the contaminated control was obtained from sample treated with only poultry manure at 4.0%(w/w) after sixteen weeks incubation.

Crude oil degradation in the sandy soil studied was enhanced by the addition of poultry manure. The use of poultry manure provides a cheap and easily available source of soil amendment material for the bioremediation of crude oil polluted soil. Its effectiveness however depends on its application at the optimal concentration alone or in combination with both a suitable alternate carbon substrate and surfactant.

ACKNOWLEDGEMENT

The author thanks Professor C.T.I.Odu and Dr E.N.Amadi of the Rivers state University of Science and Technology, Nkpolu, Port Harcourt for useful suggestions.

REFERENCES

Amadi, A. and UeBari, Y., 1992. Use of poultry manure for amendment of oil polluted soils in relation to growth of maize (*Zea mays L*). Environment International. 18: 521-527

Amanchukwu, C.C., Obafemi, A. and Okpokwasili G.C., 1989. Hydrocarbon degradation and utilisation by a palmwine yeast isolate. FEMS Microbiology Letters 57: 51-54.

Atlas, R.M., 1981. Biodegradation of petroleum hydrocarbon: an Environmental perspective. Microbiol. Rev. *45*: 180-209.

Balba, T.M., 1993. Micro-organisms and detoxification of industrial wastes. In: Exploitation of Microorganisms. Jones D. G. (ed) Chapman and Hall, London, p. 371-410.

Bouyoucos, G., 1951. A recalibration of the hydrometer for making mechanical analysis of soil. *Agron. J. 43*: 434-438.

Bremner, J. M., 1965. Total Nitrogen. In : Methods in Soil Analysis: Chemical and Microbiological Properties Part II. C.A. Black (ed) American Society of Agronomy. p. 1149-1178.

Bremner, J.M. and Mulvany, C.S., 1982. Total nitrogen determination. In: Methods of Soil Analysis vol. 2, A.L. Page, R.H. Miller and D.R. Keeney (eds) Am. Soc. Agron. p.595.

Brown, E.J., Pignatello, J. Martinson, M and Crawford, R., 1986. Pentachlorophenol degradation. A pure bacterial culture and an epilithic microbial consortium. Appl. Environ. Microbiol. *52:* 92-97.

Cornfield, A.H., 1961. A simple technique for determining mineralisation of carbon during incubation of soil treated with organic material. Plant and Soil, 14: 90-93.

Dibble, J.T. and Bartha, R., 1979. Effect of environmental parameters on the Biodegradation of oil sludge. Appl. Environ. Microbiol. *37*: 729-739.

Ellis, B., Balba, M.T. and Theile, P., 1990. Bioremediation of oil-contaminated land. Environ. Technol. 11: 433-455..

Harrigan, W.F. and McCance, M.C., 1990. Laboratory Methods in Food and Dairy Microbiology. Academic Press, London.

Heald, W.R., 1965. Determination of calcium and magnesium. In: Methods of Soil Analysis: Chemical and Microbiological properties. Part 2 C.A. Black (ed) Am. Soc. Agron. p. 999-1010.

IPS, 1987. Technical Report On Environmental Impact Assessment Of The Shell Petroleum Dev. Company Industrial Area Standby Central Power Plant. Institute Of Pollution Studies, Rivers State University of Sc. and Tech. Port Harcourt, p 237.

Keeney, D.R. and Nelson, D.W., 1982. Determination of mineral (inorganic) nitrogen. In: Methods of Soil Analysis. Vol 2. A.L Page, R.H. Miller and D.R.Keeney, (eds). Am. Sco. Agron. Madison, p. 643.

Knaebel, D.B., Federle, T.W., McAvoy,D.C. and Vestal, J.R., 1994. Effects of mineral and organic soil constituents on microbial mineralisation of organic compounds in natural soil. Appl. Environ. Microbiol. 60: 4500-4508.

Lehtomaki, M. and Niemela, S., 1975. Improving microbial degradation of oil in soil. *Ambio. 4*: 126-129.

Litchfield, C.D., Herson, D.S. Baker, K.H. Krishnamoorthy, R. and Zeli, W., 1992. Biotreatability study and field pilot design for a wood treatment site. IGT symposium on Gas, Oil and Environmetnal Biotechnology. Sept. 21-23 1992. Oklahoma, US.

McLean, E.O., 1982. Soil pH and lime requirement. In: Methods of Soil Analysis. Part 2. Chemical and Microbiological properties, 2nd ed. Am. Soc. Agron. Inc., Madison, Wisconsin.

Morgan, P. and Watkinson, R.J., 1989. Hydrocarbon degradation in soils and methods for soil biotreatment. CRC Critical Reviews in Biotechnology. 8: 305-328.

Morgan, P. and Watkinson, R.J., 1990. Assessment of the potential for *in situ* biotreatment of hydrocarbon contaminated soils. Water Sci. Technol. *22*: 63-68.

Nelson, D.W. and Sommers, L.E., 1982. Determination of organic carbon. In: Method of Soil Analysis vol 2. A. L. Page, R.H. Miller and D.R. Keeney (eds) *Am. Soc. Agron.*Madison, p. 539.

Odu, C.T.I. , Nwoboshi, L.C., Fagado, S.O. and Awani, P. E., 1989. Post-impact study of SPDC'S Nun River 8" delivery line oil spillage. Final report.SPDC, Nig. p95.

Olsen, S.R. and Sommers, L.E., 1982. Determination of available-phosphorus. In :Methods of Soil Analysis vol.2 A. L. Page, R.H. Miller and D.R. Keeney (eds) *Am. Soc. Agron.*Madison, p. 403.

Rithman, B.T. and Johnson, N.M., 1989. Rapid biological clean-up of soils contaminated with lubricating oil. Water Sci. Technol. 21: 209-219.

SAS, 1999. Statistical Analysis System User's guide: Statistics. SAS Institute Inc. Cary, N.C., U.S.A.

Stotzky, A., 1960. A simple method for determination of respiratory quotient of soil as a result of storage. Plant and Soil. *16*: 1-18.

Song, H.; Wang, X. and Bartha, R., 1990., Bioremediation potential of terrestrial fuel spills. Appl. Environ. Microbiol. *56*: 652-656.

Trudgill, P.W., 1978. Microbial degradation of alicyclic hydrocarbons. In: Developments in Biodegradation of Hydrocarbons 1 Watkinson, R.J. (ed). Applied Science Publishers Ltd, London, p. 47-84.

Walkey, A. and Black, A.I., 1934. An examination of the Degtjareff methods for determining soil organic matter and proposed modification of the chromic acid titration method. *Soil Sci. 37:* 29-38.