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# EFFECTS OF COW DUNG ON CRUDE OIL DEGRADATION IN A SANDY LOAM SOIL

***Okolo[1], Justina C. and Emmanuel N. Amadi[2]**

[1]*Department of Microbiology, Federal University of Technology, P. M. B. 1526 Owerri. Imo State.*

[2]*Department of Biological Sciences, Rivers State University of Science and Technology, Port Harcourt. Rivers State.* **Corresponding author" email:chiokolo@yahoo.com*

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## Abstract

*The effects of addition of cow dung alone and in combination with surfactant and/or alternate carbon substrate on crude oil degradation in a sandy loam soil were investigated. Cow dung added alone at a concentration of 1.0% (w/w) to the oil-contaminated soil, reduced the extent of crude oil degradation obtained relative to the degradation in contaminated soil not containing cow dung. Enhanced crude oil degradations were obtained in samples containing cow dung at 1.0% (w/w) with either or both of surfactants (Goldcrew or Corexit) at 0.01% (v/w) and alternate carbon substrates (Glucose or Starch) at 0.5% (w/w). Optimal crude oil degradation was obtained in contaminated soil treated with a combination of cow dung at 0.5% (w/w) and Corexit at 0.01% (v/w). This combination effected a crude oil degradation of 9.41± 2.69% relative to the contaminated soil not containing cow dung, after sixteen weeks incubation.*

**Key words:** Alternate carbon substrates, Cow dung, Crude oil degradation, Surfactants.

## Introduction

The adverse effects of crude oil on the ecology and aesthetic appeal of contaminated beaches, creeks, waters and soils of the Niger delta region of Nigeria have deprived man of vast agricultural and recreational areas. It has been reported that natural rehabilitation of contaminated lands is often prolonged due to the alteration of soil properties by the crude oil. Notable among these is its effect on the diversity and abundance of microorganisms, the primary agents for its degradation (Atlas, 1981; Okolo *et al*., 2004). Numerous reports which appear to be conflicting abound on the dynamics of crude oil degradation in soil and the effects of management practices employed to enhance the process. Such practices include the addition of nutrients, surfactants and alternate carbon substrates (Li *et al.*, 2000, Ellis *et al.*, 1990, Brown *et al*., 1986). The variations in the effects of the soil additives reflect the diversity and complexity of crude oil and soil from different geographical and climatic regions (Bossert and Bartha, 1984).

Manure application is an age-long practice that enhances soil fertility. It alters the availability of soil nutrients by stimulating biological activities and mineralisation (Sikora and Adler, 2003). The effectiveness of poultry manure at improving the fertility of crude oil-polluted soils has been reported (Ogboghodo *et al*., 2004). Cow dung contains nitrogen and phosphorus and has been found useful in enhancing soil fertility (Kuepper, 2003).

This study investigates the effects of cow dung as a soil additive for enhanced crude oil degradation in a sandy loam soil. The crude oil biodegradative potential of the hydrocarbon degrading bacterial population isolated from this soil has been previously reported (Okolo *et al*., 2004).

## Materials and methods

*Samples and sample collection* **-** Soil samples were collected randomly with a Dutch auger (10 cm diameter) at a depth of 15 cm from a periodically ponded agricultural farm in Port-Harcourt, Nigeria. The samples were homogenized, dried, sieved through a 2mm mesh and stored in polythene bags at room temperature (28 ± 2$^{o}$C) in the laboratory. The crude oil was a Nigerian Bonny medium blend obtained from Shell Petroleum Development Company (SPDC) Limited, Port-Harcourt, Nigeria.

Soil treatment materials included NPK (20:10:10) fertiliser obtained from National Fertilizer Company of Nigeria (NAFCON), Port Harcourt, Nigeria. Goldcrew and Corexit surfactants were obtained from SPDC. Cow dung was obtained from an abattoir in Port Harcourt. It was air dried, crushed and stored in the laboratory at room temperature (28 ± 2$^{0}$C) before use.

*Soil characterization*

The soil was characterized before contamination and at two weeks after contamination with crude oil (10%v/w). Particle size was determined by the hydrometer method (Bouyoucos 1951) while pH was determined according to the modified method of McLean (1982). Total organic carbon was determined by the wet combustion method (Walkey and Black, 1934) as modified by Nelson and Sommers (1982), and total nitrogen was determined by the semi-micro Kjeldhal method (Bremner and Mulvaney, 1982). The available phosphorous was determined by Brays No.1 method (Olsen and Sommers, 1982) and the exchangeable cations, sodium and potassium were determined by flame photometry. The ammonium-nitrogen was determined by the nesslerisation method (Keeney and Nelson, 1982) while nitrate-nitrogen was by the phenoldisulphonic acid method (Bremner, 1965).

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

*Contamination and treatment of samples*

Twenty gram soil portions were weighed into 100ml bottles, moistened to 60% of their field moisture capacity and left at room temperature (28 ± $2^0$C) in the laboratory for one week. Thereafter, the samples were contaminated 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 to all the samples. The effects of the various soil treatments containing cow dung were studied sequentially as follows:

a) Effects of soil treatments containing cow dung alone (1.0% w/w); cow dung (1.0% w/w) + surfactant (Goldcrew or Corexit at 0.01% v/w); cow dung (1.0% w/w) + alternate carbon substrate (glucose or starch at 0.5% w/w); cow dung (1.0% w/w) + surfactant (Goldcrew or Corexit at 0.01% v/w) + alternate carbon substrate (glucose or starch at 0.5% w/w)

b) Effects of soil treatments containing different concentrations of cow dung (0.5-4.0%w/w) + Corexit (0.001-1.00% v/w).

For each study, a control sample contaminated with 10% (v/w) crude oil and treated with only NPK fertilizer was also set up. Both the soils treated with cow dung with or without surfactants and/or alternate carbon substrates, and the controls not treated with cow dung were incubated at room temperature (28 ± $2^0$C) in the laboratory for four weeks. Thereafter, the soils were air-dried, homogenized and oil contents estimated.

*Crude oil degradation and carbon dioxide production with time in oil-contaminated soil treated with cow dung (0.5% w/w) and Corexit (0.01% v/w).*

This study investigated the extent of crude oil degradation obtained over a sixteen week incubation period in a sample treated with cow dung (0.5% w/w) + Corexit (0.01% v/w). This treatment gave optimal crude oil degradation in the previous study (section 2.3b).

Twenty gram soil portions were weighed into 100ml bottles, moistened, contaminated and treated with NPK fertilizer as previously described (section 2.3). Each sample was then treated with cow dung (0.5% w/w) + Corexit (0.01% v/w). Control samples contaminated with 10% (v/w) crude oil and treated with only NPK fertilizer were also set up. Both the contaminated soils treated with cow dung (0.5% w/w) + Corexit (0.01% v/w), and the contaminated controls not treated with cow dung were incubated at room temperature (28 ± $2^0$C) in the laboratory. Replicate samples were analyzed at 0, 2, 6, 9, 12 and 16 weeks intervals and changes in oil content in the samples containing cow dung (0.5% w/w) + Corexit (0.01% v/w) were calculated relative to the oil content in the controls. The samples for carbon dioxide production were similarly treated and set up in 250ml screw-capped bottles.

*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 the 250 ml screw-capped bottles containing the treated soils. The vials were withdrawn for titration after four weeks during the contamination and treatment study (section 2.3a), and were withdrawn and replaced with fresh ones at 0, 2, 6, 9, 12 and 16 weeks intervals during the degradation studies (section 2.4). The amount of the carbon dioxide absorbed was determined by titrating the barium carbonate formed with 1N hydrochloric acid.

*Determination of oil content*

Oil content was determined spectrophotometrically according to the toluene extraction method of Odu *et al.* (1989). One gram (1g) of air-dried and homogenized soil was weighed into 50 ml conical flask and ten millilitres of toluene (solvent) added to extract the oil in the soil. After shaking vigorously, the mixture was allowed to stand for 10 minutes after which it was filtered through Whatman No. 1 filter paper. The extracted oil was diluted appropriately with fresh toluene and the absorbance read at 420nm in Spectronic 21D (The Bausch and Lomb) spectrophotometer using a standard curve of Bonny medium crude oil as the reference.

The extents of crude oil degradation in the treatments containing cow dung were expressed as percentage changes in oil content, calculated relative to the oil content in the contaminated sample not treated with cow dung (control).

*Analysis of findings*

Each experiment was carried out in triplicates. Data collected were subjected to analysis of variance (ANOVA) and the Duncan's Multiple Range Tests (DMRT) (SAS, 1999).

## Results and discussion

Contamination of the sandy loam soil by crude oil increased the soil organic carbon content from 2.74 ± 0.02% to 7.06 ± 0.06%. It also increased the populations of total heterotrophic microorganisms (Table 1). There were, however, reductions in nitrate-nitrogen and available phosphorus from 55.35 ± 0.35 ppm to 12.30 ± 0.05 ppm and 20.00± 0.50 ppm to 10.88± 0.01 ppm respectively. Crude oil contamination therefore adversely affected the soil properties. Bachoon *et al.* (2001) had previously reported increases in total microbial abundance in soil in response to petroleum hydrocarbon contamination. Our present findings therefore support this earlier report. The increased microbial population observed may have represented an immediate response to the added organic carbon, which provided an additional carbon substrate for microbial growth and multiplication. This increase is however usually transient because as reported by Morgan and Watkinson (1989), the increased population will utilize the already depleted soil nitrogen and phosphorus which would eventually become limiting and cause a reduction in microbial population.

The results of the effects of different soil treatments containing cow dung on crude oil degradation show that maximum oil degradation (8.10 ± 1.02%) was obtained in oil-contaminated sample treated with cow dung + Corexit (Table 2). The highest amount of carbon dioxide (61.6 ± 1.0 mg/20g soil) was produced in contaminated soil treated with cow dung + Corexit + Starch. Table 2 also shows that crude oil degradation was significantly ($P<0.05$) lower in contaminated soil treated with cow dung alone than in the contaminated and untreated control, hence, the increase in percentage change in oil content (5.44 ± 0.44). This observation is in agreement with that of Bachoon *et al.* (2001). Although cow dung may have provided some of the limiting nitrogen and phosphorus needed for microbial crude oil degradation, it is rich in lignocellulose. This lignocellulose may have provided additional carbon source that worsened the already increased soil C:N ratio resulting from crude oil contamination, thereby limiting biodegradation. Bachoon *et al.* (2001) observed that oiled sediments treated with plant detritus contained more quantities of all aliphatic and most aromatic hydrocarbons than untreated oiled sediments.

Enhanced crude oil degradation was obtained in contaminated samples treated with a combination of cow dung and either or both of surfactant and alternate carbon substrate (Table 2). Brown *et al.* (1986), also observed enhanced degradation of pentachlorophenol upon addition of cellobiose as an alternate carbon substrate. Enhancement by the alternate carbon substrates may be attributed to the ready availability of the carbon in the substrates (glucose and starch), which in consequence ensured dynamic microbial activities that improved oil degradation in the oil-contaminated soil.

In the case of surfactants, Rithman and Johnson (1980), in line with the observations of the present study, reported increased biodegradation of lubricating oil following addition of surfactant. On the contrary, Litchfield *et al.* (1992) observed that the addition of surfactants to a creosote contaminated site did not significantly increase the biodegradation of polyaromatic hydrocarbons. The effects of surfactants on hydrocarbon biodegradation may therefore depend on the nature of the hydrocarbon substrate. The enhancement observed in the present study may have resulted from the surfactant-induced increased bioavailability and degradability of crude oil (Alexander, 1994), and the release of other nonspecific ancillary carbon compounds from both the crude oil and the cow dung. These compounds would then have served as co-metabolic substrates for enhanced crude oil degradation (Baker, 1994).

The application of cow dung with either of the surfactants or the alternate carbon substrates to crude oil contaminated soils led to significantly ($P<0.05$) greater oil degradation being obtained in samples treated with cow dung + Corexit than with cow dung + Goldcrew, and with cow dung + glucose than with cow dung + starch (Table 2). However, when cow dung was applied with both of surfactant and alternate carbon substrate, significantly ($P<0.05$) greater degradation was obtained in the sample treated with cow dung + Corexit + starch than with cow dung + Corexit + glucose. This might imply some form of synergy among the treatments in affecting crude oil degradation. This is in agreement with the observations of Knaebel *et al.* (1994), that possible interactions between soil additives and natural soil constituents affect biodegradation of hydrocarbons.

Studies of crude oil degradation using different concentrations of cow dung and Corexit showed that optimal degradation (8.45 ± 0.24%) was obtained with cow dung at 0.5% (w/w) + Corexit at 0.01% (v/w) (Fig. 1). In all the samples treated with Corexit at 1.0 %

(v/w), significantly ($P<0.05$) lower crude oil degradations were obtained than in the contaminated and untreated control. This is in line with the observations of Litchfield *et al*. (1992) in which an increase in the apparent concentration of benzo-a-pyrene was reported at high surfactant concentrations. The greater oil degradation in the control may be attributed to the high microbial population in the control which led to more crude oil degradation. Conversely, the samples treated with Corexit at 1.0 % (v/w) may have experienced microbial cell lysis, leading to reduced microbial activities and lower crude oil degradation.

The extent of crude oil degradation in the contaminated soil treated with a combination of cow dung (0.5%w/w) + Corexit (0.01%w/w) showed consistent crude oil degradations and a steady increase in cumulative carbon dioxide production with time. After sixteen weeks incubation, a crude oil degradation of 9.41± 2.69% relative to the untreated sample was obtained and the amount of carbon dioxide produced was 193.6 ± 0.01mg/20g soil (Fig.2).

## Conclusion

Since the application of some additives might decrease the extent of oil degradation achieved relative to an untreated soil, optimization studies are necessary before the application of soil additives to enhance crude oil degradation. The optimal use of cow dung as a soil additive for the purpose of achieving enhanced crude oil degradation in the sandy loam soil studied relies on the incorporation of a surfactant (Corexit) at the right concentration. The implications of the present findings are enormous and of direct relevance to environmental management regimens as improper applications of additives may worsen the condition of already contaminated soils.

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

| Soil properties | Before oil contamination (Mean±SEM) | Two weeks after oil contamination .(Mean±SEM) |
|---|---|---|
| Chemical | | |
| pH | $6.10 \pm 0.10^a$ | $4.90 \pm 0.01^b$ |
| Organic – C(%) | $2.14 \pm 0.02^b$ | $7.06 \pm 0.06^a$ |
| Total N(%) | $0.15 \pm 0.00^a$ | $0.18 \pm 0.01^a$ |
| C:N ratio | 14.27 | 39.22 |
| Nitrate –N(ppm) | $55.35 \pm 0.35^a$ | $12.30 \pm 0.05^b$ |
| Ammonium –N(ppm) | $5.93 \pm 0.01^b$ | $7.22 \pm 0.01^a$ |
| Available – P(ppm) | $20.00 \pm 0.50^a$ | $10.88 \pm 0.01^b$ |
| Exchangeable cations (Meq/100g) | | |
| $Na^+$ | $0.17 \pm 0.01^b$ | $6.08 \pm 0.01^a$ |
| $K^+$ | $0.78 \pm 0.01^a$ | $1.28 \pm 0.01^a$ |
| Microbiological | | |
| Bacterial populations ($x10^8 cfug^{-1}$soil) | | |
| Total heterotrophs | $1.88 \pm 0.14^b$ | $4.00 \pm 0.30^a$ |
| Petroleum hydrocarbon utilisers | $0.76 \pm 0.13^a$ | $0.85 \pm 0.05^a$ |
| Fungal populations ($x10^5 cfug^{-1}$soil) | | |
| Total heterotrophs | $0.72 \pm 0.08^b$ | $1.72 \pm 0.08^a$ |
| Petroleum hydrocarbon utilisers | $0.41 \pm 0.06^b$ | $1.63 \pm 0.19^a$ |

*(a, b, …) Within row, mean ± SEM with different superscripts are significantly different at $P<0.05$*

**Table 2: Effects of soil treatments containing cow dung 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/20gsoil) (Mean±SEM) |
|---|---|---|---|---|
| Soil + oil +Cow dung | $62{,}921.87 \pm 486.73^{a}$ | $+3{,}247.98 \pm 92.04^{e}$ | $+5.44 \pm 0.44^{e}$ | $24.2 \pm 1.0^{d}$ |
| Soil + oil +Cow dung +Goldcrew | $56{,}496.95 \pm 194.70^{c}$ | $-3{,}176.94 \pm 0.00^{b}$ | $-5.32 \pm 0.02^{b}$ | $41.8 \pm 1.0^{b}$ |
| Soil + oil +Cow dung + Corexit | $54{,}840.17 \pm 778.78^{e}$ | $-4{,}833..72 \pm 584.08^{a}$ | $-8.10 \pm 1.02^{a}$ | $35.2 \pm 0.2^{c}$ |
| Soil + oil +Cow dung + Glucose | $56{,}203.69 \pm 0.00^{c}$ | $-3{,}470.20 \pm 94.70^{b}$ | $-5.82 \pm 0.30^{b}$ | $26.4 \pm 0.4^{d}$ |
| Soil + oil +Cow dung + Starch | $58{,}843.54 \pm 179.70^{b}$ | $-830.35 \pm 15.00^{c}$ | $-1.39 \pm 0.02^{c}$ | $37.4 \pm 1.0^{bc}$ |
| Soil+ oil +Cow dung + Goldcrew + Glucose | $56{,}106.34 \pm 486.73^{c}$ | $-3{,}567.55 \pm 292.04^{b}$ | $-5.98 \pm 0.52^{b}$ | $44.0 \pm 0.5^{b}$ |
| Soil + oil +Cow dung + Goldcrew + Starch | $58{,}663.84 \pm 48.67^{b}$ | $-1{,}040.05 \pm 146.02^{c}$ | $-1.74 \pm 0.24^{c}$ | $22.0 \pm 0.0^{d}$ |
| Soil + oil +Cow dung + Corexit + Glucose | $57{,}177.16 \pm 194.69^{bc}$ | $-2{,}496.73 \pm 0.00^{bc}$ | $-4.18 \pm 0.02^{bc}$ | $17.6 \pm 1.0^{e}$ |
| Soil + oil +Cow dung + Corexit + Starch | $55{,}614.20 \pm 584.08^{d}$ | $-4{,}059.69 \pm 389.39^{ab}$ | $-6.80 \pm 0.69^{ab}$ | $61.6 \pm 1\ 0^{a}$ |
| Soil + oil (Control ) | $59{,}673.89 \pm 194.69^{ab}$ | $0.00 \pm 0.00^{d}$ | $0.00 \pm 0.00^{d}$ | $22.0 \pm 0.5^{d}$ |

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