EFFECTS OF SOIL TREATMENTS CONTAINING POULTRY MANURE ON CRUDE OIL DEGRADATION IN A SANDY LOAM SOIL

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Abstract. The impacts of crude oil pollution on a sandy loam soil and the influence of incorporation of poultry manure alone and in combination with alternate carbon substrates (glucose or starch) and surfactants (Goldcrew or Corexit) were investigated. Oil pollution increased soil organic carbon and reduced soil nitrates and phosphorus, thus imposing a condition that impaired oil degradation in the soil. Treatment of the soil with poultry manure alone, enhanced oil degradation but the extent of this was influenced by the incorporation of alternate carbon substrates or surfactants. Addition of glucose or Corexit encouraged crude oil degradation, while addition of starch or Goldcrew reduced the extent of degradation obtained. The soil amendments interacted in affecting crude oil degradation. This was optimal with a combination of poultry manure (2.0%w/w) + glucose (2.0%w/w) which yielded a crude oil degradation of 7.42±1.02% after sixteen weeks incubation.
Keywords. *alternate carbon substrates, crude oil degradation, poultry manure, surfactants*

Introduction

Crude oil pollution adversely affects the soil ecosystem through adsorption to soil particles, provision of an excess carbon that might be unavailable for microbial use and induction of a limitation in soil nitrogen and phosphorus [3, 4]. These cause a delay in the natural rehabilitation of crude oil polluted soils and various soil treatments have been used in bioremediation strategies to hasten the process. These include surfactants, alternate carbon substrates and organic and inorganic nitrogen and phosphorus. The effectiveness of these treatments has however been conflicting [8, 10, 15]. This might be related to the heterogeneity of soils and crude oil samples as well as possible interactions between the soil amendments and the natural soil constituents [14]. The effectiveness of each treatment in any soil therefore needs to be evaluated on a case specific basis.

Poultry manure has over time been used to improve soil fertility [23]. Its efficacy in promoting plant growth in crude oil polluted Nigerian soils has also been reported [1, 21]. This study investigates the impact of addition of poultry manure alone and in combination with surfactants or alternate carbon substrates to enhance crude oil degradation in a sandy loam soil.

Materials and methods

Samples and sample collection

Soil sample was collected randomly with a Dutch auger at a depth of 15 cm from an agricultural farm in Port-Harcourt, Nigeria. Samples were homogenized, dried, sieved

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through a 2 mm mesh and stored in polythene bags at room temperature (28±2 °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 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. The poultry manure was obtained from a poultry farm in Port Harcourt. It was air dried, crushed and stored in the laboratory at room temperature (28±2°C) before use.

Soil characterization

The soil was characterized before pollution and two weeks after pollution with crude oil. Particle size determination was done by the hydrometer method [5]; pH was determined according to the modified method of McLean [16]; total organic carbon was determined by the modified [19] wet combustion method [26] and total nitrogen was determined by the semi-micro Kjeldhal method [7]. Available phosphorous was determined by Brays No.1 method [22]. The exchangeable cations, sodium and potassium were determined by flame photometry. Ammonium-nitrogen was determined by the nesslerisation method [13] while nitrate-nitrogen was by the phenoldisulphonic acid method [6].

Soil microbial population was estimated by the ten-fold serial dilution method [11]. 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 the vapour phase transfer method [2] a mineral salt medium [12].

Pollution and amendment of samples

Twenty gram soil portions weighed into 100 ml 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 1250 μg/g soil. The effects of the various soil amendments were studied as in *Tables 2* and *3*, and *Fig. 1*.

In each study, two control units of the soil were also set up. The polluted control was treated with 10%v/w crude oil and NPK fertilizer while the unpolluted 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 soils were air-dried, homogenized and oil content estimated. Changes in oil content in the treatments were calculated relative to the oil content in the polluted and un-amended control.

Crude oil degradation and carbon dioxide production in amended soils

This study investigates the extent of crude oil degradation and carbon dioxide production obtained with time using the soil treatment that gave optimal crude oil degradations in the previous studies (poultry manure + glucose at 2.0%w/w + 2.0%w/w. Twenty gram soil portions weighed into 100 ml bottles were moistened to 60% of their field moisture capacity and left at room temperature (28±2 °C) in the laboratory for two weeks. Thereafter the samples were 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 1250 μg/g soil. The samples were then variously treated

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

chemical	soil properties	
	before oil pollution (mean±SEM)	**two weeks after oil pollution (mean±SEM)**
pH	6.10±0.10[a]	4.90±0.01[b]
organic C(%)	2.14±0.02[b]	7.06±0.06[a]
total N(%)	0.15±0.00[a]	0.18±0.01[a]
C : N ratio	14.27	39.22
nitrate N (ppm)	55.35±0.35[a]	12.30±0.05[b]
ammonium N (ppm)	5.93±0.01[b]	7.22±0.01[a]
available P (ppm)	20.00±0.50[a]	10.88±0.01[b]
Exchangeable cations (meq/100g)		
Na	0.17±0.01[b]	6.08±0.01[a]
K	0.78±0.01[a]	1.28±0.01[a]
Microbiological		
Bacterial populations ($\times 10^8$ $cfug^{-1}$soil)		
total heterotrophs	1.88±0.14[b]	4.00±0.30[a]
petroleum hydrocarbon utilisers	0.76±0.13[a]	0.85±0.05[a]
Fungal populations ($\times 10^5$ $cfug^{-1}$soil)		
total heterotrophs	0.72±0.08[b]	1.72±0.08[a]
petroleum hydrocarbon utilisers	0.41±0.06[b]	1.63±0.19[a]

Within row, mean±SEM with different superscripts are significantly different at P<0.05

with the soil amendments at the concentrations previously found optimal for crude oil degradation (*Fig. 2*). Polluted and unpolluted controls 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 polluted and un-amended control. Samples for carbon dioxide production were similarly treated and set up in 250 ml screw-capped bottles.

Determination of oil content

Oil content was determined spectrophotometrically according to the toluene extraction method [20]. One gram (1 g) of air-dried and homogenized soil was weighed into 50 ml conical flasks 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 and then it was filtered through Whatman No. 1 filter paper. The extracted oil was diluted appropriately with fresh toluene and the absorbance read at 420 nm in Spectronic 21 spectrophotometer.

Determination of carbon dioxide evolution

Carbon dioxide production was determined and calculated according to the methods of Cornfield [9] and Stotzky [25]. 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 soil treatments. The vials were withdrawn after four weeks during the pollution and amendment studies (section 2.3) and 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 1 N hydrochloric acid.

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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) [24]. The relationships between the variables were established using the correlation analysis.

Results and discussion

Pollution of the sandy loam soil by crude oil led to an increase in soil organic carbon from 2.74±0.02% to 7.06±0.06% as well as in the populations of total heterotrophic microorganisms (*Table 1*). There was however a reduction 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 pollution therefore adversely affected the soil properties. The increased microbial population – despite this – represented an immediate

Table 2: *Effects of soil treatments containing poultry manure on crude oil degradation.*

soil treatment*	oil content (ppm) (mean±SEM)	change in oil content (ppm) (mean±SEM)	% change in oil content (mean±SEM)	CO_2 production (mg / 20 g soil) (mean±SEM)
poultry manure	57014.36±389.39^{c}	-2659.53±94.69^{b}	-4.46±0.34^{b}	28.6±1.0^{c}
poultry manure +Goldcrew	58863.96±194.69^{bc}	-809.93±0.00^{d}	-1.36±0.01^{d}	33.0±1.0^{b}
poultry manure +Corexit	55649.63±292.04^{d}	-4024.26±486.74^{ab}	-6.74±0.78^{ab}	24.2±0.2^{d}
poultry manure +glucose	55064.98±194.69^{d}	-4608.91±0.00^{a}	-7.72±0.05^{a}	24.2±0.2^{d}
poultry manure +starch	61876.98±11362.86^{a}	+2203.09 ±1168.17^{f}	+3.69±1.95^{f}	8.8±1.0^{e}
poultry manure +Goldcrew +glucose	58181.31±194.69^{bc}	-1492.58±0.00^{c}	-2.50±0.01^{c}	52.8±1.0^{a}
poultry manure +Goldcrew +starch	57009.26±48.67^{c}	-2664.63±146.02^{b}	-4.47±0.22^{b}	26.4±1.0^{cd}
poultry manure + Corexit + glucose	57889.27±584.08^{bc}	-1784.62±389.39^{c}	-2.99±0.67^{c}	30.8±1.0^{b}
poultry manure + Corexit + starch	55938.79±778.78^{d}	-3735.10±584.39^{ab}	-6.23±1.01^{ab}	55.0±0.5^{a}
control (Un-amended)	59673.89±194.69^{b}	0.00±0.00^{e}	0.00±0.00^{e}	22.0±0.5^{de}

Within column, mean±SEM with different superscripts are significantly different at P<0.05.
**Concentrations of soil treatments: Poultry manure: 1.0%w/w; Goldcrew and Corexit (surfactants): 0.01% v/w; glucose and starch (alternate carbon substrates): 0.5%w/w*

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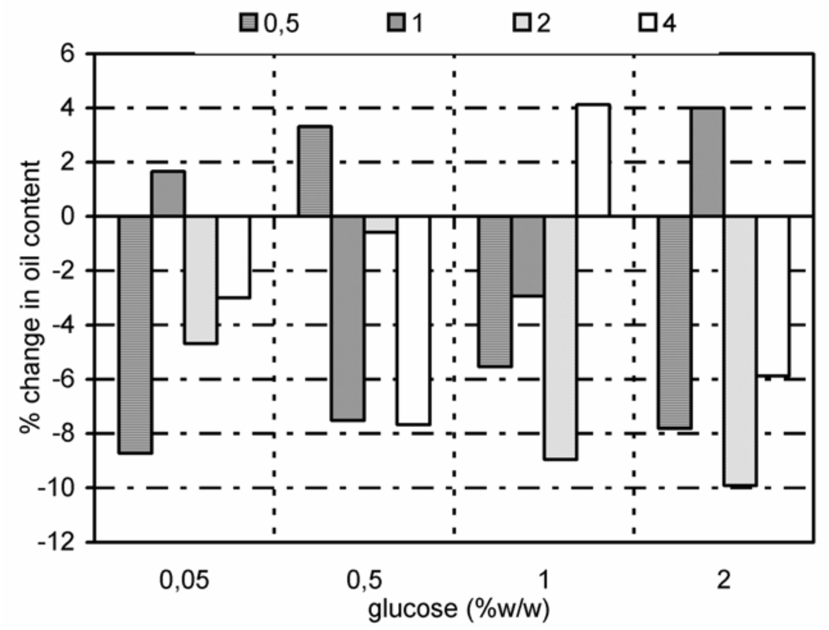

Figure 1. *Effects of different concentrations of glucose and poultry manure on crude oil degradation.*

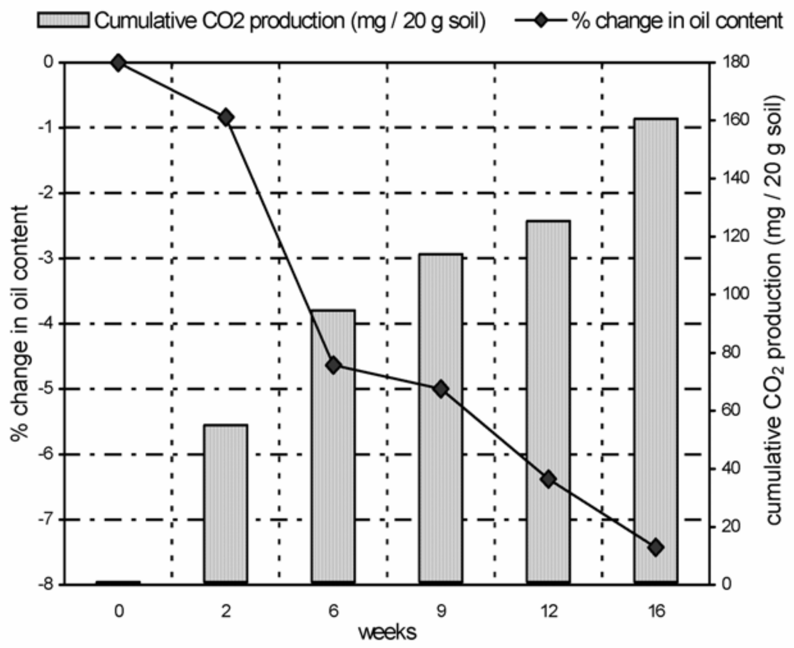

Figure 2. *Crude oil degradation and cumulative carbon dioxide production in soil treated with poultry manure (2%w/w) + glucose (2%w/w)*

response to the added organic carbon, which provided an additional carbon substrate for microbial growth and multiplication. This increase is however transient since these microbes will utilize the already depleted soil nitrogen and phosphorus which eventually will become limiting and cause a reduction in microbial population with time [17].

The results of the effects of the addition of poultry manure alone and in combination with surfactants and/or alternate carbon substrates are presented in *Table 2*. Apart from the sample treated with poultry manure + starch in which a reduction in crude oil.

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degradation relative to the un-amended control was observed, enhanced crude oil degradations were obtained from all the other treatments. The enhanced oil degradations were obtained from all the other treatments. The enhanced oil degradation observed with the addition of either of the surfactants to poultry manure + starch mixture indicate that the soil amendments interacted in enhancing crude oil degradation. Maximum oil reduction of 7.72±0.05% relative to the un-amended control was observed from sample treated with poultry manure + glucose. A significant (P<0.05) positive correlation (r = 0.59) existed between carbon dioxide production and extent of crude oil degradation. Glucose must have provided a more bio-available alternate carbon substrate that ensured a vibrant microbial population that led to the increased crude oil degradation. This is contrary to the observation that in the presence of a more readily degradable carbon substrate, microbes will prefer the carbon source that needs less energy to degrade [18]. It has been observed that microbial mineralisation of organic matter added to soil depends on the interaction between the chemicals in the soil [14]. In this case, it is therefore possible that an interaction between poultry manure, glucose and the soil constituents provided a soil condition most suitable for crude oil degradation in the sandy loam soil.

Results presented in *Fig. 1* showed that this interaction was optimal at a poultry manure + glucose concentration of 2.0%w/w + 2.0%w/w where the greatest crude oil degradation of 9.91±1.17% was obtained. The result of the extent of crude oil degradation observed with time in the sandy loam soil treated with the optimal concentration of poultry manure + glucose is presented in *Fig. 2*. There was a consistent reduction in oil content with time. After sixteen weeks incubation, under the prevailing natural environmental condition, a crude oil degradation of 7.42±1.02% relative to the un-amended control was obtained while the cumulative amount of carbon dioxide produced was 160.0±1.0mg / 20g soil.

Conclusion

Poultry manure enhanced crude oil degradation in the sandy loam soil. The extent of this depended on the presence of other soil amendments. Maximum degradation was achieved with the addition of a mixture of poultry manure and glucose at optimal levels.

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