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Bioremediation of Soil Contaminated with Crude Oil using different Weights of Poultry Manure.

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Abstract

The exploration of petroleum has led to a huge influx of carbon and decrease in nitrogen and phosphor contents into the impacted environment. Utilization of this carbon will require external supplies nitrogen and phosphorus for the oil-consuming microorganisms. Hence, this research investigated t effect of different weights of cow dung on the microbiological properties of crude oil contaminated soil The crude oil research used was Bonny light crude oil obtained from Kaduna Refining and Petrochemic Company (KRPC). The poultry manure was collected from a poultry farm situated at Kafin Hausa Loc Government Area, Jigawa State. The soil samples were collected from the main campus of Sule Lamid University, Kafin Hausa. Two hundred milliliters (200ml) of crude oil with initial weight of 25g/kg w added to sterile bag containing 1Kg of soil and thoroughly mixed. The experiments were allowed to sta in screen house for two weeks with adequate watering at 2days interval before application of poult manure at various weights of 50g/kg, 100g/kg and 150g/kg of soils. The mean total aerobic bacte present in the soil samples at the beginning of the experiment (week zero) and subsequently at 1-wee intervals for each of the treatment. Associated bacteria were Isolated, identified and estimated usin spread plate method on Nutrient Agar. The extent of crude oil biodegradation by the isolates w determined using the gravimetric analysis. From the results, nine bacterial species from 5 Genera we isolated and characterized from amended and control soil used in this research. Genus from *Bacillus* h the highest representation of five species. The results showed that there was a steady increased bacterial population in all the amended soil and control from week0 to week 3 and decreased from week to week 6. However, the bacterial population from soil treated with manure at 150g/Kg was significantl higher($P<0.05$) while that of control was low. The soil amended with poultry manure at 150g/kg gave th highest reduction (77.6%) of crude oil. This was followed by soil amended with 100g/kg and 50g/k poultry manure with percentage reduction of 59.84% and 42.00% respectively. Also, 35.80% reductio was observed from the control experiment (non-amended sample). From the study, it can be conclude that crude oil contaminated soil may result in reduced microbial population in soil but this can b remedied by the addition of organic nutrient supplements especially using poultry manure at 150g/kg a biostimulating agent.

Key words: Crude oil, Poultry Manure, Amendment, Bioremediation, Biostimulation

Introduction

Oil spillage is one of the greatest environmental problems Nigeria is currently battling with especially in the Niger Delta zone. Oil communities have been at the receiving end of this environmental problem. The problem has generated a lot of concer within the three tiers of government especiall in oil producing states (Onuoha *et al.*, 2003) The main sources of oil spill in Niger Delt are: vandalisation of the oil pipelines by th local inhabitants; ageing of the pipelines; oil

blow outs from the flow stations; cleaning of oil tankers on the high sea and disposal of used oil into the drains by the road side mechanics (Stephen *et al.*, 2013). More so, the exploration of petroleum has led to the pollution of land and water ways. The agricultural lands have become less productive (Dabbs, 1996) and the creeks and the fishing waters have become more or less dead (Odokuma and Ibo, 2002). Several civil unrests due to oil exploration have also been witnessed in the Niger Delta region of Nigeria (Inoni *et al.*, 2006).

The adverse effects of crude oil on soil cannot be overemphasized. Upon decreasing the nitrogen and phosphorus contents, crude oil makes available excessive hydrocarbon to the soil which affects soil enzymatic activities due to the inability of soil microbes to degrade this excess hydrocarbons (Ijah *et al.*, 2008). Also, Anene and Chika (2006) reported that the rate and efficiency of biodegradation depend on the occurrence of adequately numerous and active microflora in the contaminated or polluted environment. To improve crude oil polluted soils for enhanced and sustainable ecosystems, several efforts which include physicochemical and biological methods have been employed in the remediation of the polluted soils (Erdogan and Karaca, 2011).

Biostimulation involves the addition of rate-limiting nutrients to accelerate degradation by indigenous microbes. This assumes that every organism needed to accomplish the desired treatment-results is present (Akanni and Ojeniyi, 2008). Though it is not certain that those organisms present are the most suitable to degrade the pollutant. When an oil spill occurs, it results in a huge influx of carbon into the impacted environment. Supplying enough nitrogenous nutrients to the cultures to enable their proliferation present serious problems, since sea, river, or lake water and soil have little nitrogen and phosphorus, rapid oil utilization will require external supplies of these elements for the oil-consuming microorganisms. Carbon is the basic structural component of living matter, and in order for the indigenous microorganisms to be able to convert this carbon into more biomass, they need significantly more nitrogen and phosphorous than is normally present in the soil (Okolo *et al.*, 2005). Both of these essential elements are ingredients of protein and nucleic acids of living organisms, and therefore should be adequately provided. Hence, this research became necessary in order to study the effect of different weights of Cow dung on the microbiological properties of crude oil contaminated soils.

MATERIALS AND METHODS

Sample Collection

The crude oil used was Bonny light crude oil. It was obtained from Kaduna Refining and Petrochemical Company (KRPC), Kaduna State, in sterile sample bottles and transported to the laboratory for the study. The poultry manure was collected from a poultry farm situated at Kafin Hausa Local Government Area, Jigawa State. The Poultry manure was air dried and crushed before use.

The soil samples were collected from the main campus of Sule Lamido University, Kafin Hausa. The topsoil (0-15cm) with no previous history of crude oil contamination was collected in polythene bags from four different locations. From each location, 5.0kg of the soil was collected, bulked together and homogenized. Four sterilized bags were perforated and filled with 1.0kg of the soil from each of the location making a total of 16 bags from all the locations.

Description and Treatment of Samples

Methods of Osazee *et al.* (2014) was adopted with little modifications. Two hundred milliliters (200ml) of crude oil with initial weight of 25g/kg was added to each bag containing 1Kg of soil from each of the different locations and thoroughly mixed. Four replicates were made from each of the location while no crude oil was added to the remaining 4 bags which served as control. The experiments were allowed to stand in screen house for two weeks with adequate watering at 2days interval before application

of poultry manure at various weights of 50g/kg, 100g/kg and 150g/kg of soils.

Determination of Hydrocarbon Utilizing Bacteria

The mean total aerobic bacteria present in the soil samples at the beginning of the experiment (week zero) and subsequently at 1-week intervals for each of the treatment options were isolated, identified and estimated using spread plate method on Nutrient Agar (Anon, 2010). Gram staining reaction, Indole test, Gluconate oxidation test, Urease test, Citrate utilization test, Methyl red test, Motility test and Voges-Proskauer test were all carried out using the methods as described by Oyeleke and Manga (2008).

Determination of Extent of Crude Oil Degradation by the Isolates

The extent of crude oil biodegradation by the isolates was determined using the gravimetric analysis method of Odu (1972).

The ability of microbial isolates to degrade crude oil was demonstrated in terms of reduction in the quantity of crude oil introduced to pollute the soil samples. Carbon tetrachloride was employed as the extractant. The quantity of residual crude oil extracted from the soil samples was carried out as described by Udeme and Antai (1988).

After 6 weeks, three samples per single treatment were analysed for the quantity of residual crude oil. Each of the 1.0kg soil treatment samples was mixed with 300ml of carbon tetrachloride, placed in a separating flask, shaken vigorously for 3 minutes and allowed to settle for 5 minutes. The liquid phase separated by allowing the mousses (crude oil-carbon tetrachloride) to pass gradually through a funnel fitted with filter paper (Whatman No 1). Anhydrous sodium sulphate spread on the filter paper was employed to remove any moisture in the mixture. The liquid phase was collected in a pre-weighed Pyrex beaker. The beaker containing the extract was placed in an oven and the extractant allowed evaporating at 50°C. The beaker with the residual crude oil was allowed to cool to room temperature and weighed to determine the quantity of residual crude oil by difference. The percentage of crude oil degraded after six weeks was determined from the equation:

$$\% \text{ crude oil degraded} = \frac{\text{weight of crude oil degraded}}{\text{Original weight of crude oil}} \times 100$$

weight of crude oil degrad
= original weight of crude oil - weight of residual crude oil

Statistical Analysis

Analysis of variance (ANOVA) was used to test whether the different levels of nutrient amendments given to the crude oil contaminated soil will be statistically significant and means were separated using Duncan Multiple Range Test (DMRT).

Results and Discussion

From the results, a total of 9 species from 5 Genera of bacteria were isolated and characterized from amended and control soil used in this research (Table 1). Genus from *Bacillus* has the highest representation of five species. Some of these bacterial species has earlier been reported by Ijah *et al.* (2008); Stephen *et al.* (2013) and Osazee *et al.* (2015).

The mean counts of heterotrophic bacterial populations (HBPs) are presented in Table 2. The results showed that there was a steady increased in HBPs in all the amended soil and control from week 0 to week 3 and decreased from week 4 to week 6. However the bacterial population from soil treated with manure at 150g/Kg was significantly higher ($P<0.05$) while that of control was reversed But the difference in HBPs between the bag amended with 50g and 100g was no significant ($P>0.05$). This agreed with the position of Njoku *et al.* (2009) that nutrien deficiencies which arise due to petroleum hydrocarbon contamination of soil may however be offset by addition of cow dung to the soil. This low population observed at the weeks 0 and 1 might be attributed probably to initial inhibition of water and nutrient uptake due to the hydrophobic character of crude oil.

Table 1: Biochemical Characterizations of Bacteria Isolated from Crude Oil Contaminated Soil

IC	Morphology	GR	Cit	MR	VP	Mot	UT	Indole	Suc	Lac	Glu	H_2S	GP	ST	Org
K	Rod	+	+	+	-	+	+	-	+	-	+	+	-	+	*Bacillus sp*
L	Rod	-	-	+	+	+	+	-	+	-	+	+	+	-	*Proteus mirabilis*
P	Rod	+	-	-	+	+	-	-	+	-	+	-	+	-	*Bacillus laterosporus*
Q	Rod	-	+	-	+	+	-	-	+	+	+	-	-	-	*Enterobacter sakazakii*
R	Rod	+	+	+	-	+	+	-	+	-	+	+	-	+	*Bacillus megaterium*
S	Rod	+	-	-	+	+	+	+	+	-	+	+	-	+	*Bacillus alvei*
T	Rod	-	-	+	-	+	-	-	-	-	+	-	-	-	*Morganella sp*
U	Rod	-	-	-	+	+	-	-	-	-	+	-	-	-	*Serratia sp*
V	Rod	-	-	-	+	+	-	-	+	-	+	-	-	-	*Bacillus laterosporus*

Key: IC : Isolates code; GR : Gram reaction; Cit : Citrate; MR : Methyl red; VP : Voges proskauer; Mot : Motility; UT : Urease test; Suc : Sucrase Lac: Lactose; Glu: Glucose; GP: Gas production; ST: Starch test; Org: Organism +: Positive; -: Negative

Table 2: Bacterial Populations (10^{-5}cfu/g) in Amended and Non-amended Crude Oil Contaminated Soil

Poultry manure (g/kg)	0	1	2	3	4	5	6
50	52.00±2.22^{b}	50.00±2.08^{b}	59.00±3.51^{b}	66.67±4.63^{b}	61.00±8.19^{b}	42.00±6.08^{b}	36.00±1.16^{b}
100	53.00±3.04^{b}	51.00±3.51^{bc}	61.00±8.19^{b}	68.00±2.08^{b}	63.00±6.43^{bc}	54.00±5.69^{b}	39.00±2.00^{bc}
150	55.00±2.22^{c}	53.00±3.61^{c}	65.00±4.16^{c}	76.00±5.51^{c}	64.00±2.52^{c}	56.00±6.66^{c}	40.00±6.56^{c}
Control	40.00±5.56^{a}	37.00±1.16^{a}	46.00±8.89^{a}	55.00±5.03^{a}	47.00±2.65^{a}	35.00±2.65^{a}	29.00±1.16^{a}

Means in the same column followed by similar alphabets are significantly the same (P≥0.05), DMRT (1951). Values are means of four replicates

Table 3: The populations of Bacteria (10^{-5}cfu/g) in each of the treatment and control

	WEEKS						
Poultry manure (g/kg)	0	1	2	3	4	5	6
50	7.00±1.16^{a}	11.00±2.52^{a}	18.00±2.08^{a}	19.00±5.13^{b}	27.00±2.52^{b}	34.00±3.22^{b}	47.00±5.2^{b}
100	8.00±0.58^{a}	13.00±2.65^{a}	17.00±2.08^{a}	18.00±1.16^{b}	29.00±5.13^{b}	35.00±3.61^{b}	53.00±4.58^{c}
150	9.00±1.00^{a}	25.00±1,53^{b}	33.00±1.53^{b}	34.00±0.58^{c}	48.00±5.51^{c}	54.00±6.66^{c}	58.00±2.31^{d}
Control	7.00±0.58^{a}	9.00±0.58^{a}	15.00±0.58^{a}	14.00±0.58^{a}	24.00±1.16^{a}	30.00±1.16^{a}	32.00±1.16^{a}

Means in the same column followed by similar alphabets are significantly the same (P≥0.05), DMRT (1951). Values are means of four replicates

The results in Table 3 showed a progressive rise in Hydrocarbon Utilizing Bacterial Populations (HUBPs) was observed over the course of the experiment in all the contaminated soils. There was an enhanced growth in HUBPs due to the presence of poultry manure amendment in all the amended bags when compared to the control. The difference in the increased in HUBPs between the bags amended with 50g, 100g and the control was not significant ($p>0.05$). But soil amended with150g/Kg manure was significantly higher ($p<0.05$). This finding was in consistency with the report of Thieman and Palladino (2009) that addition of nutrients into the soil, leads increase in number of microorganism, enhanced their growth and increased the rate of biodegradation. It was also observed that the population increased with increase in weight of poultry manure. The increase in effects of manure on population growth of soil microorganisms according to Brandli *et al.* (2008) may not be unconnected with the composition of the poultry manure.

Table 4: Percentage loss of Crude Oil in each of the Treatment after Six Weeks of Amendment

Poultry manure (g/kg)	Weight of Residual Crude Oil (g/kg)	Percentage Loss in Crude Oil
50	14.50	42.00
100	10.04	59.84
150	5.60	77.60
Control	16.05	35.80

Original weight of crude oil = 25.0g/kg

Table 4 shows the crude oil (g/kg) content remaining in the soil and the percentage of crude oil removed from the soil per treatment. The soil amended with poultry manure at 150g/kg gave the highest reduction (77.6%) of crude oil.. This was followed by soil amended with 100g/kg and 50g/kg poultry manure with percentage reduction of 59.84% and 42.00% respectively. Also, 35.80% reduction was observed from the control experiment (non-amended sample). This agreed with Frederic *et al.* (2005) who reported77 – 95% loss of total alkanes and 80% of PAHs from hydrocarbons contaminated soil amended with nutrients within the period of 180days. The reduction was observed to increase with increased weight of Poultry manure as earlier reported by Osazee *et al.* (2015) that 90g of cow dung fertilizer proved the best treatment option with the removal of 52.59% of crude oil from amended soil sampled. These observations might be connected with the composition and quantity of the physicochemical properties of the Poultry manure.

Conclusion

From the study, it can be concluded that crude oil contaminated soil may result in reduced microbial population in soil but this can be remedied by the addition of poultry manure supplements especially using poultry manure at 150g/kg as biostimulating agent.

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