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RESEARCH ARTICLE

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Bioremediation of crude oil contaminated soils using cow dung as bioenhancement agent

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ABSTRACT

Studies were conducted in the Department of Biological Sciences, Sule Lamido University, Kafin Hausa, Nigeria, to determine the effects of Cow dung on the microbiological properties of crude oil contaminated soil for six weeks. Cow dung treatments applied were Control, 30g/kg, 60g/kg, 90g/kg and the soils were amended after two weeks of crude oil contamination. Soil samples were collected from the plastic bowls for microbiological analyses. The isolates were cultured to test their ability to grow on crude oil. The Fungal Growth as monitored by the measurement of total fungal populations, hydrocarbon utilizing fungal populations, and the quantitative hydrocarbon losses were also determined at different weights of Cow dung. Results indicated that the remediation effect was nutrient dependent. The following fungal groups of crude oil utilizers were isolated, namely, as Trichoderma harzanium, Aspergillus flavus, Rhizopus sp, Penicillium sp, Aspergillus niger, Rhizopus sp and Trichothercium roseum.

Key words: Crude oil, Cow dung, Amendment, Bioremediation, Biostimulation

INTRODUCTION

Nigeria is one of the major producers of crude oil in the world and pollution of the environment due to oil spillage has steadily increased. Contamination of the environment is frequently associated with hydrocarbon pollution because of the increasing global demand for petroleum hydrocarbons and its products. In Nigeria, this growing demand for petroleum hydrocarbon as the major energy source of domestic cooking and lighting has evidently led to disturbing cases of crude oil spillages.In the Niger Delta Area alone, there have been over 550 reported cases of crude oil spillage since 1976, releasing about 2.8 million barrels of crude oil into the environment [1,2]. The presence of heavy metals in some environments has, therefore, been attributed to petroleum prospecting and mining as well as oil spills [3]. It is estimated that more than four thousand incidents of crude oil spills have occurred in the Niger Delta region of Nigeria since 1960, releasing several million barrels of crude oil (some containing heavy metals) into the surrounding areas [4]. These metals however, can inhibit various cellular processes and their effects are often concentration dependent and also vary in their individual toxicity [5].

The adverse effects of crude oil on soil cannot be overemphasized. Upon decreasing the nitrogen and phosphorus contents, crude oil provides to the soil excessive hydrocarbon which affects soil enzymatic activities due to the

inability of soil microbes to degrade the excess hydrocarbons [6, 7, 8]. 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 [9, 10]. Several reports have shown that bioremediation method, among other treatment options is the most cost effective and environmentally friendly way of restoring contaminated soils [11, 12, 13, 14].

Consequently, biostimulation of indigenous microorganisms through nutrient supplementation has gained wide acceptance in bioremediation works. Ijah and Okang [15] reported that the growth and proliferation of oil utilizing microorganisms in polluted soil is greatly influenced by the availability of nutrients. This has led to bioremediation of petroleum – hydrocarbon contaminated soils becoming an attractive method of treatment due to its advantage, which include environment friendly nature of the process. 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, Nigeria. The Cow dung manure was collected from a ranch situated at Toyawa village along the Sule Lamido University Road, Jigawa State.

The soil samples were collected from the main campus of Sule Lamido University, Kafin Hausa, Jigawa State. The top soil (0-15cm) with no previous history of crude oil contamination was collected from three different locations. The soil samples were bulk together, homogenized and 1.0kg weighed into perforated labelled plastic bowls. This perforation allows for proper drainage (i.e. avoid water logging) and better aeration of the experimental soil. A total of 48 bowls filled with experimental soil were used for the experiment.

Description and treatment of samples

Crude oil was added to the soil in the bowls and thoroughly mixed with the soil. The contaminated and uncontaminated soils were allowed to stand under natural environment for two weeks before application of different levels of Cow dung. During this period, the soil samples were watered at interval of two days. A total of 36 bowls with soil were contaminated with crude oil and 12 bowls without crude oil contamination. After two weeks of contamination, Cow dung was carefully weighed into the bowls containing the crude oil at various weights (control, 30g/kg, 60g/kg and 90g/kg of soils). The Cow dung was crushed before use. Soil samples were replicated 3 times, and arranged in Completely Randomised Design.

Microbiological analysis

The enumeration and identification of total aerobic fungal populations, total hydrocarbon utilizing fungal populations as well as the extent of crude oil utilization by fungal isolates were determined.

Determination of Fungal Populations

The mean total aerobic fungi present in the samples at the beginning of the experiment and subsequently at 1-week intervals for each of the treatment options were estimated using spread plate method with Potato Dextrose Agar (PDA) as medium.

The Potato Dextrose Agar plates were prepared according to manufacturer's specifications. About 1g of each of the soil samples was serially diluted up to ten-fold dilution. An aliquot of 0.1ml (at 10^{-4} dilution) of the ten-fold dilution of the contaminated crude oil soil suspension was seeded onto PDA plates each for the determination of viable fungal cell counts. These plates were incubated at room temperature (31 ± 3^0C) for 5 days. The counts obtained were multiplied by the dilution factor to obtain the fungal cell counts per gram of soil.

Determination of Hydrocarbon Utilizing Fungal Populations

An aliquot of 0.1ml (at 10^{-4} dilution) of the crude oil soil suspension was seeded onto modified mineral salts medium of Mills *et al.* [16]. The vapour phase transfer technique of Okpokwasili [17] was adopted, which employs the use of sterile filter paper soaked in crude oil, which served as the carbon and energy sources. The soaked sterile filter papers were then aseptically placed onto covers of the inoculated inverted plates and incubated for 6 days at

37^0C. Average mean counts of colonies from triplicates were recorded and used for the calculation of colony forming unit multiplied by the dilution factor for hydrocarbon utilizers within the fungal population.

The isolated colonies of the mineral salts medium were further purified by subculturing onto Potato Dextrose Agar (PDA) medium to obtain a pure culture. These were examined both macroscopically and microscopically for the identification of the fungi.

Determination of Extent of Crude Oil Biodegradation by the Isolates

The extent of crude oil bioaccumulation by the isolates was determined using the gravimetric analysis method of Odu [18].

The ability of microbial isolates to degrade or accumulate 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 below.

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 50^0C. 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 by noting its absorbance reading at 520nm. The percentage of crude oil degraded after six weeks was determined using Udeme and Antai [19] equation:

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

Weight of crude oil degraded = Original weight of crude oil – weight of residual crude oil

RESULTS

The mean counts of heterotrophic fungal populations are presented in Table 1. At week 0, the mean counts of fungal populations decreased in all the treatment options from week 0 to week 1. The mean counts of fungal populations increased again from week 2 to week 4 before reducing in weeks 5 and week 6. The increment observed in mean counts of fungal populations were significant ($p<0.05$) in most cases.

Table 1: Fungal Populations (10^{-4}cfu/g) in Amended and Non-amended (Control) Crude Oil Contaminated Soil

	Weeks						
Different weights of cow dung (g/kg)	**0**	**1**	**2**	**3**	**4**	**5**	**6**
C.D30	42.00 ± 2.89^{ab}	27.00 ± 1.53^{a}	48.00 ± 1.16^{a}	47.00 ± 2.65^{ab}	49.00 ± 2.08^{a}	35.00 ± 2.08^{a}	30.00 ± 2.52^{a}
C.D60	42.00 ± 4.49^{ab}	28.00 ± 3.06^{a}	52.00 ± 3.47^{ab}	50.00 ± 2.65^{ab}	53.00 ± 2.65^{ab}	37.00 ± 2.65^{a}	32.00 ± 2.31^{ab}
C.D90	43.00 ± 1.53^{ab}	29.00 ± 3.22^{a}	66.00 ± 3.06^{c}	65.00 ± 2.65^{c}	62.00 ± 6.43^{b}	54.00 ± 3.22^{c}	42.00 ± 4.04^{c}
Control	34.00 ± 2.65^{a}	32.33 ± 5.61^{a}	46.00 ± 2.08^{a}	45.00 ± 2.31^{a}	40.00 ± 1.53^{a}	39.00 ± 1.53^{a}	24.00 ± 2.52^{a}

a b c mean in a column with different superscripts are significantly different ($p<0.05$), values are means of three replicates ± standard error.

Table 2: Hydrocarbon Utilizing Fungal Populations (10^{-4}cfu/g) in Amended and Non-amended (Control) Crude Oil Contaminated Soil

	Weeks						
Different weights of Cow dung (g/kg)	**0**	**1**	**2**	**3**	**4**	**5**	**6**
C.D30	4.00 ± 0.58^{a}	14.00 ± 3.06^{a}	18.00 ± 2.31^{a}	24.00 ± 2.52^{ab}	29.00 ± 1.73^{ab}	33.00 ± 2.08^{ab}	36.00 ± 1.16^{a}
C.D60	4.33 ± 0.88^{a}	15.00 ± 1.16^{a}	19.00 ± 2.08^{a}	26.00 ± 3.61^{ab}	31.00 ± 3.61^{ab}	37.00 ± 4.73^{b}	48.00 ± 5.29^{b}
C.D90	4.67 ± 1.20^{a}	23.00 ± 4.73^{b}	31.00 ± 3.22^{b}	37.00 ± 2.89^{c}	48.00 ± 5.29^{c}	54.00 ± 4.58^{c}	68.00 ± 1.16^{c}
Control	4.00 ± 2.65^{a}	10.00 ± 1.16^{a}	14.00 ± 1.16^{a}	18.00 ± 3.06^{a}	21.00 ± 1.53^{a}	23.00 ± 4.58^{a}	30.00 ± 0.58^{a}

a b c mean in a column with different superscripts are significantly different ($p < 0.05$), values are means of three replicates $\pm$ standard error.

Table 2 showed the results of the Hydrocarbon Utilizing Fungal Populations (HUFPs) in amended and non-amended crude oil contaminated soil. The HUFP increased over the period of the study. The results indicated that at week 0, there was no significant increase ($p>0.05$) in HUFP in amended and non-amended crude oil contaminated soil. More so, the results showed that the HUFP increased correspondingly from week 1 up to week 6 as the treatment weights increases. These increments differ significantly ($p<0.05$) in all the cases.

The results of the weight losses of crude oil due to microbial attack are presented in Table 3. The weight loss of crude oil in the different treatment options was showed by the percentage reduction of crude oil in the amended and non-amended samples. The application of 90g of Cow dung proved to be the best treatment option with the removal of 52.59% of crude oil from the samples, followed by 60g of Cow dung (44.35%). There was also 37.06% removal of crude oil in bowls treated with 30g of Cow dung, and 29.41% of crude oil removal was also observed in the control experiment (non-amended).

Table 3: Loss of Crude Oil from the Crude Oil Contaminated Soil Samples for each Treatment after Six Weeks of Treatment

Different weights of Cow dung (g/kg)	Weight of Residual Crude Oil (g/kg)	Percentage Loss in Crude Oil
C.D30	10.70	37.06
C.D60	9.46	44.35
C.D90	8.06	52.59
Control	12.00	29.41

Original weight of crude oil = 17.0g/kg

DISCUSSION

There was a decreased in the mean counts of fungal populations in the crude oil contaminated soil in week 0 to week 1. The mean counts for fungal populations increased again from week 2 up to week 4 before reducing again in week 5 up to week 6. This observation agrees with that of Atlas [20] who reported that the drop in the total heterotrophic counts in the contaminated soil in the first week can be attributed to selective inhibition of members of the microbial community as a result of the toxic components of petroleum and also as a result of reduced aeration and upset of carbon/inorganic nutrient balance for the indigenous population caused by the presence of petroleum.

The mean counts of the HUFPs increased over the period of the study. This shows that the soil amended with 90g of Cow dung gave the highest mean count of hydrocarbon utilizing fungal population (68.00 ± 1.16). The increase in HUFP in response to the increasing addition of organic nutrients has been reported by Henry *et al*. [21] and Abu and Ogiji [22]. This finding is consistent with the work done by William *et al*. [23] who used poultry litter to enhance the degradation of petroleum hydrocarbons in the soil. The results showed that a significant first order rate of total petroleum hydrocarbon biodegradation was measured for all the treatment units containing the poultry litter.

The bioremediation potential of the treatment options was showed by the percentage reduction of crude oil in the samples. The application of 90g of Cow dung fertilizer proved the best treatment option with the removal of 52.59% of crude oil from the sample, followed by 60g of Cow dung and 30g of Cow dung with percentage reduction of 44.35% and 37.06% respectively. There was also 29.41% removal of crude oil in the control experiment (non-

amended sample). This also agreed with the work done by Osazee *et al*. [24] who reported that the quantity of Cow dung added to crude oil contaminated soil has a significant effect on the remediation process.

CONCLUSION

The results indicated that Cow dung at the different weights tested was effective in biostimulation of fungal species in crude oil contaminated soil leading to corresponding increased in microbial population. Therefore, attention should be given to the utilization of optimum application levels as the results of this study indicated that biodegradation respond to differences in treatment application for soil quality similar to the one used for this study.

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