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Assessment of Cow Dung on Fungal Growth Isolated from Spent Engine Oil Contaminated Soil Obtained from Mechanic Workshop in Minna, Nigeria

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Abstract

Samples of the spent engine oil contaminated soils were collected from Shanchaga, Maikunkele, Shiroro, Tunga and Bosso mechanic workshops located in Minna Local Government Area. Four samples of 0.5kg each per location were collected making a total of 2kg of soil sample per site and 10kg from the five sites. Non-oil contaminated soil samples were collected from Biological Garden of the Department of Plant Biology, Federal University of Technology, Minna, Nigeria. The fungi were isolated from the mechanic workshop soils using dilution plate method in mineral salt medium. Five treatments (25g/kg, 50g/kg, 75g/kg, 100g/kg and 125g/kg) of different weights of steam sterilized cow dung were applied to the soil in the pots measuring 15cm × 35cm filled with one kilogram (1kg) of steam sterilized soil. Standard suspension (10.02×10^4cfu/ml) of each of the three pure fungal isolates (R. stolonifer, A. flavus and T. harzianum) obtained among the fifteen fungi (Aspergillus niger, Rhizopus stolonifer, Fusarium oxysporium, Aspergillus flavus, Penicillium notatum, Aspergillus fumigatus, Trichoderma harzianum, Penicillium griseofulvum, Rhodotorula sp, Cunninghamella echinulata, Trichoderma viride, Penicillium chrysogenum, Mucor hiemalis, Mucor racemosus and Mucor plumbeus) screened for enhancement were added to the pots and thoroughly mixed with each of the 5ml of spent engine oil contaminated soil. The contaminated soils were allowed to stand for one week before the application of the different treatments. All treatments were replicated three times and the contents of each pot were watered and tilled twice a week for aeration. All the fungi were identified based on macroscopic and microscopic features of the fruiting

bodies, spores and hyphal mass. The spent oil utilising fungal population counts (SOUFPC) were determined with the test fungi. The studies revealed that the population of the three hydrocarbon degrading T. harzianum, R. Stolonifer, and A. flavus counts in all the soils amended with the various weights of cow dung were higher compared to that of un-amended control pots. These increases in all the amended pots were higher in CD75 counts.

Key words: *Contamination, cow dung, hydrocarbon, enhancement, remediation*

Introduction

In Nigeria, as in many other oil producing countries, petroleum hydrocarbon contamination is widespread. Pollution arising from the disposal of spent engine oil (S.E.O) is one of the environmental problems in Nigeria and is more widespread than crude oil pollution (Odjegba and Sadiq, 2002). The prevalent mode of indiscriminate disposal of these spent engine oils in the environment calls for urgent attention. Though, studies have been carried out on auto-repair workshop sites in the area (Ipeaiyeda and Dawodu, 2008), most of them focused on the implications of heavy metal contamination in soils caused by spent engine oil without recourse to the effects of other petroleum hydrocarbon pollutants such as the polycyclic aromatic hydrocarbons (PAH) present in spent engine oils (Christopher, 2008). These spent engine oils form part of the most hazardous wastes commonly generated in auto-repair shops around cities in Nigeria (Ipeaiyeda and Dawodu, 2008).

Since the late 1980s, after the chemical and mechanical treatments of lands and water bodies and thermal treatment (incineration) of hazardous wastes proved economically and environmentally unsustainable, focus shifted towards the biological methods which are cost-effective as well as environmentally sustainable and also socially acceptable. One of such biological methods is Bioremediation. Bioremediation is a soft bioengineering technique to clean up contaminated lands/sites using microbes (bacteria or fungi), plants (terrestrial and aquatic) and earthworms (Bijofp, 2003). Bioremediation works carried out by the microorganisms are called 'micro-remediation' while those performed by plants are called Phytoremediation (Perfumo *et al.*, 2007).

This spent engine oil constitutes a potential threat to humans, animals, and vegetation (Adelowo *et al.*, 2006). Spent engine oils are made up of hydrocarbons which modify the physical and chemical properties of soil and its structure (Chi Yuan and Krishnamurthy, 2000). The nutrient deficiencies which arise due to petroleum hydrocarbon contamination of soil may however be offset by addition of

cow dung to the soil (Osazee and Adebola, 2016). In this study, the aim is to assess the influenced of cow dung on fungi growth isolated from mechanic workshop soils contaminated with spent engine oil.

MATERIALS AND METHODS

Study Site

The study was carried out in Minna, Nigeria. Improper disposal of spent engine oil is the major source of oil pollution in this locality. Five mechanic workshops (about 5 km apart) contaminated with spent engine oil were randomly selected for this study and sites were selected from the following areas in Minna; Chanchaga, Tunga, Bosso, Maikunkele and Shiroro.

Sample Collection

Spent engine oil contaminated soil samples was randomly collected from each of the selected site using a pre-cleaned hand auger at a depth of 0 –15cm. Four replications of 0.5kg each per location making a total of 2kg of soil sample per site and 10kg from the five sites. The samples from each point per site was pooled together, homogenised, air dried, sieved through a 2-mm mesh screen and stored in a polythene bag at room temperature in the laboratory for further studies (Goddey and Dami, 2013).

Isolation of Fungi from Soil Obtained from Mechanic Workshops

Media used for isolation of fungi

Bushnell Hass agar medium was prepared according to the mineral salts medium (MSM) composition of Mohsenzadeh *et al*. (2009).The composition of the medium was NaCl, 10.0g; $MgSO_4.7H_2O$, 0.42g; KCl, 0.29g; KH_2PO_4, 0.83g; Na_2HPO_4, 1.25g; $NaNO_3$, 0.42g; agar, 20g; distilled water, 1 litre; spent engine oil, 2ml and pH of 7.2. The medium was used for isolation of the fungi (oil-degraders).

Isolation and identification of fungal isolates

One millilitre of the aliquot of serially diluted soil sample obtained from mechanic workshops was added onto Bushnell Hass agar containing 2ml of spent engine oil by spread plate method. The plates were incubated at 28 ± 2^0C and those isolates that showed good growth on oil agar medium were used for the determination of Spent Oil Utilising Fungi (SOUF).

The fungal isolates were identified based on the colony morphology, nature of hyphae, nature of conidia and shape. A portion of the mycelial mat of the fungi was picked with sterile needle and placed on a clean slide containing a drop of lactophenol cotton blue stain. The mycelial growth was teased gently to allow it mix with the stain, covered with cover slip and observed under a low to high power objectives (x40 and

×100) of the light microscope. The fungal isolates were identified by comparing their characteristics with those of known taxa using the schemes of Kiiyukia (2003).

Determination of population of hydrocarbon utilising fungi

The medium used to determine the fungal population was prepared by the addition of one (1mL) of spent engine oil sterilized with 0.22µm pore size millipore filter paper to mineral salt medium, which has been cooled to 45^0C under aseptic condition. Chloramphenicol (250 mg/L) was added to prevent bacterial growth. The mineral salt medium and spent engine oil were then mixed thoroughly and dispensed into sterile Petri dishes (Obire *et al.*, 2008). The oil agar plates were inoculated with 1ml aliquots of 10^{-4} dilutions of the soil samples incubated at $28 \pm 2\ ^0$C for 6 days. Colonies which developed and showed growth and zones of clearance of oil on the oil-agar plates were counted as spent oil utilizing fungi. The colonies counted were computed and expressed as colony forming units (cfu) per gram of soil (Akoachere *et al.*, 2008).

RESULTS

Isolation of fungi from the various mechanic workshop soils

The different types of fungal isolates obtained from the mechanic workshops used for this study were *Rhizopus stolonifer, Trichoderma harzianum* and *Aspergillus flavus*

Enhancement of *Rhizopus stolonifer* population growth in soil contaminated with spent engine oil amended with cow dung

Table 1 shows the counts of *Rhizopus stolonifer* in uncontaminated (negative control), contaminated (positive control) and amended soils. There were significant enhancement ($P<0.05$) of *R. stolonifer* obtained in CD75, CD100 and CD125 relative to the controls. At month 0, the highest level of enhancement of *R. stolonifer* was obtained in CD125 (59.33±3.28) while the least enhancement was observed in CD25 with a count of 17.67±2.03. At month 2, there were significant enhancement in *R. stolonifer* obtained in CD125 (48.33±2.19) and the least enhancement was obtained in CD25 (16.67±1.76). At month 4, the highest level of significant in enhancement with *R. stolonifer* was obtained in CD125 (67.00±1.73) while the least enhancement was obtained in CD25 with a count of 26.67±1.76. At month 6, the highest enhancement of *R. stolonifer* was obtained in CD125 (81.67±4.41) while the least was obtained in CD25 with population count of 37.67±5.04. At month 8, there were significant enhancements of *R. stolonifer* population growth obtained in CD100 (77.00±5.86) and CD125 (85.33±4.41) relative to the controls. At month 10, the enhancement in population growth of *R. stolonifer* ranged from 16.00±2.65 to 62.67±3.71. The highest level of enhancement of *R. stolonifer* was obtained in CD125

(62.67±3.71) followed by CD100 (43.67±8.76) while the least enhancement was obtained in CD50 (27.33±3.71).

Table 1: Effect of cow dung supplements on population growth of *Rhizopus stolonifer* (10^{-4}cfu/g)

Treatment (g/kg)	Time (Month)					
	0	2	4	6	8	10
CD25	17.67±2.03^{b}	16.67±1.76^{ab}	26.67±1.76^{ab}	37.67±5.04^{b}	41.67±5.89^{b}	27.67±3.76^{b}
CD50	20.00±0.58^{c}	21.00±3.61^{c}	32.00±5.29^{c}	40.33±4.84^{c}	43.67±6.89^{c}	27.33±3.71^{b}
CD75	44.33±4.70^{d}	41.33±4.81^{d}	49.33±4.67^{d}	56.67±3.33^{d}	64.00±1.15^{d}	35.33±2.91^{c}
CD100	57.00±1.73^{e}	48.00±2.08^{e}	57.33±4.06^{e}	64.67±2.60^{e}	77.00±5.86^{e}	43.67±8.76^{d}
CD125	59.33±3.28^{f}	48.33±2.19^{e}	67.00±1.73^{f}	81.67±4.41^{f}	85.33±5.81^{f}	62.67±3.71^{e}
NC (0)	13.00±4.36^{a}	15.33±3.71^{a}	27.33±4.33^{b}	31.67±6.94^{ab}	24.33±6.49^{a}	16.00±2.65^{a}
PC (0)	13.67±4.49^{a}	17.33±3.71^{b}	25.33±0.88^{a}	30.67±4.37^{a}	30.67±3.53^{ab}	18.67±2.40^{ab}

Values are Mean ± Standard Error of mean. Values with the same superscript(s) along the same column are not
significantly different ($p > 0.05$) tested by DMRT

Enhancement of *Trichoderma harzianum* population growth in spent engine oil contaminated soil amended with cow dung

The populations which were represented in colony forming units per gram (cfu/g) of soil ranged from 17.00±3.22 to 64.00±3.22 in month 0. The highest enhancement in *T. harzianum* population was observed in CD75 with mean counts of 64.00±3.22. In month 2, the enhancement in mean population count of *T. harzianum* ranged from 20.33±3.71 to 53.00±2.31. There were significant enhancement ($P<0.05$) in the mean count of *T. harzianum* at CD75 when compared to other treatment options. At month 4, CD75 also proved to be the best enhancer when compared to other treatment options like CD100 (49.00±8.08) and CD125 (48.67±3.76). There were significant enhancement ($P<0.05$) in the growth of *T. harzianum* at CD75 when compared to other treatment options. At month 6, the enhancement in population growth of *T. harzianum* followed the same trend with CD75 having the highest enhancement in population count with 94.33±9.33. There were no significant differences ($P>0.05$) in the

enhancement of *T. harzianum* among the treatment options except in CD75 (94.33±9.33). At month 8, the enhancement in population mean count of *T. harzianum* ranged from 27.00±4.16 to 86.00±2.65. The highest enhancement in mean count of *T. harzianum* was observed in CD75 (86.00±2.65) followed by CD125 (58.67±4.10) while the least was observed in CD25 with 27.00±4.10. There were significant differences ($P<0.05$) in the enhancement of *T. harzianum* in CD75 when compared to other experimental treatments applied. The enhancement in population at month 10 ranged from 19.00±2.16 to 50.33 ±7.42. The highest enhancement in population count of *T. harzianum* was observed in CD75 (50.33±7.42) while the least enhancement was recorded in control 1 (19.00±2.16). There was significant difference ($P<0.05$) in the enhancement of *T. harzianum* population count at CD75 when compared to other treatment options like CD25, CD50 and the controls.

Table 2: Effect of cow dung supplements on population growth of *Trichoderma harzianum* (10^{-4}cfu/g)

Treatment (g/kg)	Time (Month)					
	0	2	4	6	8	10
CD25	19.33±2.03^{b}	26.00±4.73^{b}	36.67±3.48^{b}	42.67±5.04^{b}	27.00±4.16^{a}	24.33±4.37^{b}
CD50	25.00±0.58^{bc}	27.33±4.63^{bc}	39.67±4.06^{bc}	45.33±7.31^{c}	28.00±4.51^{ab}	25.67±6.36^{bc}
CD75	64.00±3.22^{e}	53.00±2.31^{e}	75.67±1.76^{e}	94.33±9.33^{e}	86.00±2.65^{e}	50.33±7.42^{e}
CD100	45.33±7.13^{d}	33.00±4.58^{c}	49.00±8.08^{d}	43.00±8.51^{bc}	52.00±6.66^{c}	42.00±3.00^{d}
CD125	42.33±6.17^{c}	36.67±5.61^{d}	48.67±3.76^{c}	51.00±4.93^{d}	58.67±4.10^{d}	40.00±2.89^{c}
NC (0)	18.00±4.36^{ab}	20.33±3.71^{a}	32.33±4.33^{a}	36.67±6.94^{a}	29.33±6.49^{b}	21.00±2.65^{ab}
PC (0)	17.00±3.22^{a}	22.33±3.28^{ab}	34.33±2.73^{ab}	38.33±6.69^{ab}	32.33±7.17^{bc}	19.00±2.65^{a}

Values are Mean ± Standard Error of mean. Values with the same superscript(s) along the same column are not
significantly different ($p > 0.05$) tested by DMRT

Enhancement of *Aspergillus flavus* population growth in soil contaminated with spent engine oil amended with cow dung

Table 3 shows counts of *Aspergillus flavus* obtained from the soil sample analysed. At month 0, the average population growth in enhancement of *A. flavus* ranged from 31.33±4.67 to 47.33±7.45. The highest enhancement in population growth of *A. flavus* was obtained in CD125 (47.33±7.45) followed by CD75 with enhancement of

43.33±3.84 and the least was obtained in CD25 with a population growth of 31.33±4.67. There was significant difference (P<0.05) in the enhancement of *Flavus* growth obtained from CD125 and CD50. On month 2, the enhancement in population growth of *A. flavus* ranged from 28.67±3.71 to 40.00±4.73. The highest enhancement of *A. flavus* was obtained in CD75 (40.00±4.73) followed by CD125 with enhancement in population growth of 38.67±4.67 and the least was obtained in positive control with population growth of 28.00±3.06. There were no significant differences (P>0.05) in the enhancement of *A. flavus* obtained from all the treatments. On month 4, the enhancement in population growth of *A. flavus* ranged from 34.33±2.91 to 70.67±9.21. The highest enhancement of *A. flavus* was obtained in CD75 (70.67±9.21) followed by CD100 (54.33±3.71) and the least was obtained in positive control (34.33±2.91). There was significant difference (P< 0.05) in the enhancement of *A. flavus* count obtained from CD75, CD100 and positive control. At month 6, the average enhancement of *A. flavus* ranged from 39.67±2.03 to 85.33±5.04. The highest enhancement in population growth was obtained from CD75 (85.33±5.04) followed by CD50 with count of 64.67±2.19 while the least was recorded in positive control, having 39.67±2.02. There was significant difference (P<0.05) in the enhancement of *A. flavus* count obtained from CD75 and CD50. At month 8, the enhancement in population growth of *A. flavus* ranged from 44.00±4.04 to 100.67±1.76. The highest enhancement in population growth was obtained in CD75 with population growth of 100.67±1.76 followed by CD100 (62.00±4.73) and least enhancement was obtained in positive control (44.00±4.04). There were significant differences (P<0.05) in the enhancement of *A. flavus* population growth obtained from positive control, CD100 and CD75. At month 10, the enhancement in population growth of *A. flavus* ranged from 35.33±2.40 to 66.33±6.69. The highest enhancement in population growth was obtained in CD75 (66.33±6.69) followed by CD50 (44.67±5.18) while the least enhancement was obtained in positive control (35.33±2.40). There were no significant differences (P>0.05) in the enhancement of *A. flavus* obtained from the experimental values (treatments) and the control values.

Table 3: Effect of cow dung supplements on population growth of *Aspergillus flavus* (10^{-4}cfu/g)

Treatment (g/kg)	Time (Month)					
	0	2	4	6	8	10
CD25	31.33±4.67^{a}	30.67±3.53^{ab}	35.33±4.67^{ab}	49.00±4.58^{b}	44.67±2.96^{b}	38.00±5.57^{b}
CD50	32.67±4.49^{ab}	28.67±3.71^{a}	53.00±0.58^{c}	64.67±2.19^{d}	53.33±5.18^{b}	44.67±5.18^{b}
CD75	43.33±3.84^{c}	40.00±4.73^{b}	70.67±9.21^{d}	85.33±5.04^{e}	100.67±1.76^{d}	66.33±6.69^{d}
CD100	36.67±4.06^{b}	33.67±5.49^{bc}	54.33±3.71^{cd}	56.33±3.28^{cd}	62.00±4.73^{c}	43.67±3.18^{b}
CD125	47.33±7.45^{d}	38.67±4.67^{b}	51.00±4.04^{bc}	55.33±5.70^{c}	56.00±3.46^{bc}	39.00±4.73^{b}

NC (0)	31.33±2.40[a]	28.00±3.06[a]	34.33±2.91[a]	39.67±2.03[a]	44.00±4.04[a]	35.33±2.40[ab]
PC (0)	32.33±2.03[ab]	31.67±3.84[b]	38.00±4.36[b]	42.67±2.03[ab]	44.67±3.28[a]	34.33±3.53[a]

Values are Mean ± Standard Error of mean. Values with the same superscript(s) along the same column are not significantly different ($p > 0.05$) tested by DMRT

Discussion

The studies revealed that the population of the five viable hydrocarbon degrading *T. harzianum, A. niger, R. Stolonifer, F. oxysporium* and *A. flavus* counts in all the soils amended with the various weights of cow dung were higher compared to that of un-amended control pots. The fungi population in amended pots did not follow the pattern observed in the population of non-amended contaminated pots. These increases in all the amended pots were higher in CD75 counts. This may be due to the time it used to produce extracellular enzymes to degrade the oil as well as the cow dung. This result is in agreement with earlier work by Stephen *et al.* (2013). They observed higher fungi counts in amended soil compared to oil polluted soil and oil free soil. These counts are comparable to those of Ijah and Antai (2003b), who observed counts of hydrocarbon degraders in oil polluted soil to be $x10^6$ cfu/g. The low counts observed in all the un-amended control pots may be due to differences in fungal characteristics and soil ecology of the unamended pots. The reason for the higher counts of fungi in amended soil could be as a result of the presence of appreciable quantities of nitrogen and phosphorus in the cow dung, especially high nitrogen content in CD75, which is a necessary nutrient for fungi biodegradative activities (Adesodun and Mbagwu, 2008). Uncontaminated pots (NC) showed significantly lower fungal growth relative to the contaminated pots (PC). This indicates that the presence of spent oil either attracted hydrocarbon degrading organisms or served as substrate for the multiplication of the exogenous hydrocarbon degrading fungi. Jelena *et al.* (2009) observed similar findings and reported that the presence of gasoline (a hydrocarbon) in the soil resulted in significant increase in microbial population and metabolic activities. Avidano *et al.* (2005) further reported that the number of hydrocarbon-utilizing organisms were higher in oil polluted sites than in the unpolluted sites. It was also observed that viable count of spent oil degrading fungi in amended pots yielded significantly ($P < 0.05$) higher populations than in un-amended pots

Conclusion

The results of this study showed that fifteen fungi belonging to eight genera were associated with spent engine oil contaminated soils in Minna. Thus, the isolated fungi as mentioned above could be used as potential fungi to degrade petroleum

hydrocarbons, especially those spill out as a result of anthropogenic activities. The results also showed that among the different weights of treatment applied, the poultry amended CD75 had significant enhancement on *A. flavus*, *F. harzianum* and *R. Stolonifer* populations.

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