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INFLUENCED OF ORGANIC WASTE ON PHYSICOCHEMICAL PROPERTIES OF SPENT ENGINE OIL CONTAMINATED SOIL SUPPLEMENTED WITH FUNGI ISOLATED FROM MECHANIC WORKSHOPS 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, Nigeria. 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. The influenced of *spent engine oil on soil properties supplemented with fungi was investigated. Five treatments (25g/kg, 50g/kg, 75g/kg, 100g/kg and 125g/kg) of different weights of 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 pure fungal isolates obtained from the mechanic workshop soils were added to the pots and thoroughly mixed with each of the 5ml of spent engine oil contaminated soil. The*

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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. The results of the analysis of the soil supplemented with Rhizopus stolonifer revealed that spent engine oil had no effect on the silt, sand and clay properties in all the soils. The increased in concentration of the organic carbon as a results of spent engine oil contamination was significantly decreased in the treated pots when compared to control 2. Significant increased was observed in moisture content, pH, nitrogen, potassium, and phosphorus in control 2 when compared to CD75. Soils supplemented with Aspergillus flavus showed that spent engine oil had no effect on the silt, sand, clay, moisture and nitrogen content while significant increased were observed in pH, potassium and phosphorus contents of the soil due to spent engine oil contamination. Significant decreased was observed in carbon content of the soil.

INTRODUCTION

Naturally, soil is the richest reservoir of microorganisms and a key component of ecosystems because environmental sustainability depends largely on a sustainable soil ecosystem. Whenever soil is polluted, the ecosystem is altered and agricultural activities are affected (Adedokun and Ataga, 2006; Igwo-Ezikpe *et al.*, 2009). Contaminated lands generally result from past and present industrial activities with little or no awareness of the health and environmental consequences of their production processes and waste disposal methods. The problem is worldwide, but more severe in the developing countries such as Nigeria where there are no effective regulatory policies on the environment, thus encouraging unwholesome industrial practises (Adelowo *et al.*, 2006). This directly affects the rate at which spent engine oil enters and pollutes the environment as disposal of the spent engine oil into gutters, water drains and vacant plots is a common practice among automobile mechanics that change oil from motor vehicles and power generating machines. The indiscriminate disposal of this waste oil increases pollution incidents in the environment (Odjegba and Atebe, 2007). 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 organic waste on SEO contaminated soils supplemented with fungi isolated from mechanic workshop soils in Minna, Nigeria.

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 5km 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 Oil Degrading Fungi

Oil degrading fungi were isolated from the soil samples by the enrichment culture technique using sterile motor oil as carbon and energy source (Amund *et al.*, 1994).To do this, 1.0g of the soil sample was poured into a test tube containing 10ml of distilled water. One (1) millilitre of the suspension was pipette into a test tube containing 9ml of distilled water. The sample was serially diluted to 10^{-5} dilution. One (1) millilitre aliquots of the second-fold dilution 10^{-2} (cfu) was added to the mineral salts medium containing 10% V/V sterile motor oil as the sole carbon and energy source (enriched culture medium) and incubated at (28±2°C) for 5 days. Fungal isolates obtained from the enrichment culture were sub-cultured onto potato dextrose agar incorporated with tetracycline. Colonies were further sub-cultured severally on the basis of their colonial characteristics to obtain pure culture isolates (Nwachukwu and Akpata, 2003).

Identification of associated fungi

The isolated fungi were identified based on the isolates colonial characteristics on culture plate and microscopic features such as nature of mycelium, types of fruiting bodies and the spore structure. The isolates were identified according to Kora *et al*, (2005)

RESULTS

Isolation of fungi from the various mechanic workshops

There were fifteen fungal species isolated belonging to eight genera and identified as *Rhizopus stolonifer, Aspergillus niger, A. fumigatus, Trichoderma harzianum, Aspergillus flavus, Penicillium notatum, Trichoderma viride, Penicillium chrysogenum, Rhodotorula rubra, Cunninghamella echinulata, Fusarium oxysporium, Mucor hiemalis, Penicillium griseofulvum, Mucor plumbeus and Mucor racemosus*. Among all the fifteen fungal isolates that were screened, three fungi (*Rhizopus stolonifer*, *Trichoderma harzianum* and *Aspergillus flavus*) were selected among the fungi that showed high mycelia growth in medium containing spent engine oil and were used for this study.

Influence of cow dung on the physicochemical properties of spent engine oil contaminated soil supplemented with *Rhizopus stolonifer*

The physicochemical properties of spent engine oil contaminated soil are presented in Table 4.5. In moisture content, CD75 displayed the highest moisture content among the treatments with a value of 8.97±1.07%. The least value of moisture content was recorded for control 1 (NC) with 4.53±1.44%. There were no significant differences (P>0.05) among the treatments and the control values. In pH, CD75 had the highest value of 8.17±0.71 followed by CD125 with 7.07±1.18. However, the pH of the sample tends towards alkaline with the least value of 4.57±0.58 being obtained in control 1 (NC).There were no significant differences ($P>0.05$) among the treatments except in CD75 when compared with the controls. The organic carbon content analyses varied from 1.73±0.64 in CD75 to 6.80±0.90 in control 2 (PC). The organic carbon content in control 2 was significantly higher ($p <0.05$) than those recorded for all the treatments. The nitrogen content similarly, varied from 2.52±0.30 in control 2 to 5.33±0.86 in control 1. The highest (5.47±0.79) nitrogen content was recorded for CD75. The nitrogen content of CD75 (5.47±0.79) was significantly higher ($p <0.05$) than that of CD25 (3.23±0.34). The potassium content ranged from 25.73±3.54 in control 2 to 50.67±5.81 in CD75. Meanwhile, high potassium contents were recorded for other treatments. There were no significant differences ($P>0.05$) among the treatments. The range value of phosphorus recorded among the treatments was 1.47±0.83 to 4.00±1.50. The highest phosphorus content was recorded in CD75 (4.73±1.42) followed by CD125 (4.00±1.50) and the least value was observed in control 2. The soil particle size analyses of the sand, silt and clay showed that there were no significant differences ($P>0.05$) in the values obtained.

Table 3.1: Physicochemical properties of soil samples as influenced by *Rhizopus stolonifer*

Parameter	Control(NC)	Control (PC)	CD25 (g/kg)	CD50 (g/kg)	CD75 (g/kg)	CD100 (g/kg)	CD125 (g/kg)
Moisture (%)	4.53 ± 1.44^{a}	5.27 ± 1.71^{ab}	5.33 ± 1.80^{ab}	6.10 ± 2.05^{b}	8.97 ± 1.07^{c}	6.60 ± 1.86^{b}	6.40 ± 1.56^{b}
pH	4.57 ± 0.58^{a}	5.00 ± 0.83^{ab}	5.20 ± 0.80^{ab}	5.83 ± 0.95^{ab}	8.17 ± 0.71^{d}	6.47 ± 1.08^{b}	7.07 ± 1.18^{c}
C (%)	6.80 ± 0.90^{c}	4.10 ± 1.11^{bc}	4.20 ± 0.84^{bc}	2.13 ± 0.75^{ab}	1.73 ± 0.64^{a}	3.70 ± 0.95^{b}	2.90 ± 0.67^{ab}
N (%)	2.52 ± 0.30^{a}	3.23 ± 0.34^{ab}	3.93 ± 0.38^{ab}	4.43 ± 0.34^{b}	5.47 ± 0.79^{c}	4.10 ± 0.29^{b}	5.33 ± 0.86^{c}
K(mg/kg)	38.67 ± 6.36^{b}	25.73 ± 3.54^{a}	37.00 ± 7.18^{ab}	38.17 ± 6.98^{b}	50.67 ± 5.81^{d}	48.33 ± 7.27^{c}	38.80 ± 5.92^{b}
P (mg/kg)	2.83 ± 1.21^{ab}	1.47 ± 0.83^{a}	2.47 ± 1.29^{ab}	3.33 ± 1.38^{b}	4.73 ± 1.42^{c}	3.97 ± 1.57^{b}	4.00 ± 1.50^{bc}
Sand (%)	774.67 ± 33.65^{a}	774.33 ± 32.92^{a}	774.00 ± 34.31^{a}	773.67 ± 33.74^{a}	774.33 ± 33.53^{a}	773.67 ± 34.11^{a}	774.00 ± 33.71^{a}
Silt (%)	68.67 ± 6.72^{a}	67.73 ± 6.74^{a}	67.83 ± 6.34^{a}	68.10 ± 6.41^{a}	68.23 ± 6.39^{a}	68.23 ± 6.29^{a}	67.83 ± 6.34^{a}
Clay (%)	93.37 ± 4.44^{a}	93.03 ± 3.48^{a}	93.10 ± 3.77^{a}	93.47 ± 3.85^{a}	93.00 ± 3.38^{a}	93.17 ± 3.70^{a}	93.60 ± 3.72^{a}

Values are Mean ± Standard Error of mean. Values with the same superscript(s) along the same row are not significantly different ($p> 0.05$) tested by DMRT. **PC:** Positive control (2); **NC:** Negative control (1)

The moisture content was highest in CD75 with the value of 8.20±1.10% (Table 4.7). This is significantly higher ($p<0.05$) than the moisture content recorded for control 1. The pH content ranged from 3.50±0.67 in control 1 to 7.67±1.51 in CD125. The pH content in CD125 was not significantly higher ($P>0.05$) than those recorded for other treatments. The carbon content varied from 1.73±0.64 in CD75 to 5.80±0.64 in control 2. The highest (5.80±0.64) carbon content was recorded for control 2 (PC). There were no significant differences ($P>0.05$) among the various treatment options. The nitrogen content ranged from 2.13±0.24 in control 2 to 7.00±0.81 in CD75. Meanwhile, the high nitrogen content recorded for CD75 was statistically significant ($P<0.05$) when compared to other treatments. The range value of potassium recorded among the treatments was 31.33±1.45 to 57.00±5.51. The highest potassium content was recorded in CD75 followed by control 1, and the least value in control 2. There were no significant differences ($P>0.05$) among the treatments except in CD75 which recorded a significant increased ($P<0.05$). The highest phosphorus content was recorded for CD75 with 4.93±0.48 while the lowest was recorded for control 2 with 1.07±0.56. Similarly, There were no significant differences ($P>0.05$) among the treatments except in CD75 which recorded a significant increase. The soil particle size analyses of the sand, silt and clay showed that there were no significant differences ($P>0.05$) in values obtained.

Parameter	Control1(NC)	Control 2(PC)	CD25(g/kg)	CD50(g/kg)	CD75(g/kg)	CD100(g/kg)	CD
Moisture (%)	3.50±1.26^{a}	4.03±1.30^{ab}	4.10±1.25^{ab}	4.70±1.49^{ab}	8.20±1.10^{c}	6.50±0.61^{b}	7.2
pH	3.50±0.67^{a}	4.70±1.01^{ab}	5.77±0.82^{b}	5.30±0.46^{b}	7.57±0.38^{c}	5.60±0.32^{b}	7.6
Carbon (%)	5.80±0.64^{c}	4.17±1.01^{b}	4.10±1.01^{b}	1.82±0.35^{a}	1.73±0.64^{a}	2.63±0.44^{ab}	2.9
Nitrogen (%)	2.67±0.18^{a}	2.13±0.24^{a}	3.70±0.36^{ab}	3.27±0.37^{ab}	7.00±0.81^{c}	5.53±0.79^{b}	3.2
Potassium (mg/kg)	40.40±5.55^{bc}	29.53±3.07^{a}	31.37±3.56^{ab}	31.33±1.45^{a}	57.00±5.51^{c}	36.33±2.33^{b}	36.
Phosphorus (mg/kg)	2.47±0.94^{a}	1.07±0.56^{a}	1.50±0.64^{a}	1.70±0.69^{a}	4.93±0.48^{b}	1.73±0.66^{a}	3.1
Sand (%)	775.00±35.47^{a}	773.67±34.04^{a}	774.33±33.84^{a}	773.67±33.98^{a}	774.33±33.84^{a}	774.00±34.24^{a}	77 71
Silt (%)	65.67±4.18^{a}	65.67±3.92^{a}	65.83±3.35^{a}	65.50±3.55^{a}	64.67±2.73^{a}	64.33±3.38^{a}	65
Clay (%)	91.33±2.91^{a}	91.37±2.70^{a}	91.43±2.62^{a}	91.66±2.40^{a}	90.67±3.18^{a}	91.00±2.08^{a}	90

Table 3.2: Physicochemical properties of soil samples with different weights of treatment supplemented with *Trichoderma harzianum*.

Values are Mean ± Standard Error of mean. Values with the same superscript(s) along the same row are not significantly different ($p > 0.05$) tested by DMRT. **PC:** Positive control; **NC:** Negative control

Influence of cow dung on the physicochemical properties of spent engine oil contaminated soil supplemented with *Aspergillus flavus*

The analysis from Table 4.10 indicated the presence of moisture, pH, carbon, nitrogen, potassium, phosphorus, sand, silt, and clay as influenced by *A. flavus*. The moisture content ranged from 5.00±1.03 to 7.73±1.54. The moisture content was highest in CD125 with the value of 7.73±1.54. This increase in moisture content in CD125 was not significantly enhanced ($p>0.05$) when compared with the moisture content recorded for other treatments. The pH varied from 3.30±0.32 in control 1 to 7.17±0.60 in CD125. The pH content in CD125 was significantly enhanced ($P<0.05$) than those recorded for other treatments. The carbon content similarly, ranged from 2.17±0.34 in control 1 to 7.67±1.20 in control 2. The highest (7.67±1.20) carbon content was recorded for control 2. Most of the carbon contents in all the treatment pots were significantly enhanced ($P<0.05$). The nitrogen content ranged from 2.37±0.88 in CD25 to5.67±1.45 in CD75.The nitrogen content was not significantly enhanced ($P>0.05$) in most of the treatment options except in CD75 when compared with CD25.The range value of potassium recorded among the treatments was 21.00±3.79 to 48.00±9.82. The highest enhancement of potassium content was recorded in CD75 followed by CD50, and the least value in control 2. There was significant enhancement ($P<0.05$) between CD75 and CD100. The highest phosphorus content was recorded in CD75 (5.30±0.61), followed by CD125 with 4.03±1.13 while the lowest enhancement of phosphorus content was recorded in control 2 (0.93±0.41). The phosphorus content were not significantly enhanced ($P>0.05$) in most of the treatments except in CD75 and CD25. The soil particle size analyses of the sand, silt and clay showed that there were no significant differences (P>0.05) in the values obtained.

Table 3.3: Physicochemical properties of soil samples with different weights of treatment as influenced by *Aspergillus flavus*

Parameter	Control1(NC)	Control2(PC)	CD25(g/kg)	CD50(g/kg)	CD75(g/kg)	CD100(g/kg)	CD125(g/kg)
Moisture	5.00 ± 1.03^a	5.30 ± 0.72^a	5.77 ± 0.66^a	5.87 ± 0.71^a	6.70 ± 0.91^{ab}	7.10 ± 1.11^b	7.73 ± 1.54
pH	3.30 ± 0.32^a	3.87 ± 0.75^a	4.97 ± 0.29^b	4.87 ± 0.09^b	5.70 ± 1.00^c	6.73 ± 0.23^d	7.17 ± 0.60
Carbon (%)	6.30 ± 0.65^{bc}	7.67 ± 1.20^c	5.53 ± 0.90^b	5.87 ± 0.70^b	2.63 ± 0.15^a	4.50 ± 0.96^{ab}	2.17 ± 0.34
Nitrogen(%)	3.67 ± 0.72^{ab}	3.20 ± 1.41^{ab}	2.37 ± 0.88^a	2.90 ± 0.49^a	5.67 ± 1.45^b	3.23 ± 0.43^{ab}	5.00 ± 0.58
Potassium (mg/kg)	37.67 ± 3.85^d	21.00 ± 3.79^a	33.00 ± 3.51^c	38.13 ± 6.24^{de}	48.00 ± 9.82^e	27.67 ± 6.44^b	31.00 ± 3.79
Phosphorus (mg/kg)	2.33 ± 0.94^b	0.93 ± 0.41^a	1.70 ± 0.57^{ab}	2.80 ± 0.96^b	5.30 ± 0.61^d	3.37 ± 1.12^{bc}	4.03 ± 1.13
Sand (%)	809.33 ± 2.91^a	808.67 ± 3.53^a	808.33 ± 4.06^a	808.00 ± 3.46^a	808.67 ± 2.60^a	808.67 ± 3.53^a	812.33 ± 2.7 ^{a}
Silt (%)	74.00 ± 5.77^a	75.00 ± 5.69^a	73.83 ± 7.05^a	75.00 ± 7.21^a	74.67 ± 7.06^a	75.33 ± 7.31^a	71.33 ± 9.49
Clay (%)	91.00 ± 3.22^a	90.33 ± 3.48^a	90.33 ± 3.76^a	91.00 ± 2.89^a	91.33 ± 2.33^a	91.33 ± 1.45^a	91.67 ± 2.19

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

DISCUSSION

Influence of treatments on the physicochemical properties of the spent engine oil contaminated soil

The results of the physicochemical properties of the spent engine oil contaminated soil ten months after amendments with different weights of Cow dung showed that there were significant differences among the parameters observed in this study as a result of the activities of the fungi and the treatments on the spent oil contaminated soil. Pots treated with CD75, gave the highest values of moisture content while the lowest value was observed in control 1 (NC).This increase may be attributed to the period (rainy season) when this study was conducted. This observation is similar to the report by Stephen and Egene (2012) who observed increase in moisture content in soil polluted with lubricating oil. They attributed the increase to the time the study was carried out during the rainy season (April - June, 2012). The pH of the spent oil contaminated soil was changed from slightly acidic to slightly alkaline. In support of the finding on pH, Stephen *et al.* (2016) reported that the pH levels of the soil samples increased from acidic to neutral in all soil samples, while Shahida *et al.* (2015) reported pH from 7.02 to 7.5 in different points and slightly alkaline (8.44) in one point of soil contaminated with hydrocarbons. The N, K, and P contents of the spent oil contaminated soil increased after the treatments. This may be as a result of the soil amendment which may have contributed to the degradation of the spent engine oil by the microorganisms. Similar report was presented by Lovely and Cackette (2001) who worked on biodegradation of diesel contaminated soil amended with cowpea chaff and observed significant increase in the nitrogen, potassium, phosphorus and carbon contents in the soil. The investigators attributed the increase to increase in atmospheric nitrogen during the degradation process. The results of the particle size analysis of all the treatments amended with Cow dung showed that there were no significant differences ($p<0.05$) in the amount of sand, clay and silt values of the spent oil contaminated soil.

The addition of organic nutrient initially elevated the nutrient concentration in phosphorus, potassium and nitrogen meaning a positive effect on nutrient concentration. Zavala-Cruz *et al.* (2013) reported similar findings with decreases noticed during the study as microbial activities tend to nullify the initial spike in nutrient concentration. The percentage carbon (C) content in the spent engine oil contaminated soil increased but this was reduced after the application of the treatments. The increase in carbon content might be due to the presence of carbons in the spent engine oil (hydrocarbon), while reduction in organic carbon concentration in this study agrees with the findings of Sandor and Schrader (2012) who reported significant decrease in soil organic carbon at the end of the degradation experiment using compost. The reason for this decrease, they attributed to the loss of organic carbon in the form of carbon dioxide as a source of energy.

Conclusion

The results of this research showed that among the different weights of treatment applied, the pot amended CD75 had significant enhancement on *A. flavus, T. Flavus* and *R. stolonifer*. The increase in organic carbon of the contaminated soil as a result of the addition of SEO to the soil was reduced (degraded) significantly by A. flavus, T. harzianum and R. stolonifer. Mycoremediation that implore the use of organic waste especially CD at CD75 as seen in this study cannot be overemphasize because of its environmental benefits.

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