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MICROBIOLOGICAL AND PHYSICOCHEMICAL PROPERTIES OF DIESEL SIMULATED SOIL

[1*] E. Stephen, A. S. Usman, M.O. Okolo, E.A. Akogu[2] and O.P. Abioye[3]
Department of Microbiology, Kogi State University, Anyingba, Kogi State, Nigeria.
Department of Science Laboratory Technology, Kogi State Polytechnic, Lokoja, Nigeria
Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria.
*Corresponding author: psychsea07@gmail. Com, +234-8036347036

ABSTRACT
Microbiological and physicochemical properties of diesel simulated soil (11 % v/w) was studied for a period of 8 weeks. The results revealed lower counts in bacteria and fungi in diesel polluted soil (DPS) compared to oil free soil (OFS). There were significant differences ($P<0.05$) in both bacteria and fungi counts obtained from diesel free soil and diesel polluted soil. Ten organisms were isolated in the course of this study. These were *Aspergillus flavus, A.niger, Bacillus cereus, Candida tropicalis, Micrococcus luteus, Pseudomonas aeruginosa, Proteus mirabilis, Staphyloccoccus aureus, Geotrichum candidum* and *Rhizopus nigricans*. Diesel pollution increased the organic carbon, organic matter and nitrogen contents of the soil. There were no significant differences in pH, moisture, organic carbon, organic matter and nitrogen at 5% probability level. The result obtained indicated that diesel pollution had altered the microbial and physicochemical properties of the soil.
Keywords: oil free soil, diesel polluted soil, physicochemical properties

INTRODUCTION

There has been a rapid increase in population worldwide resulting in increased demand for hydrocarbon products such as diesel, petrol, kerosene, pesticides and industrial chemicals (Chakrabarty *et al*., 1998). Although many of the chemicals are utilized or destroyed, a high percentage is released into the air, water and soil representing a potential environmental hazard (Alexander, 1995; Stephen *et al*., 2011). Processing and distribution of petroleum hydrocarbons as well as the use of petroleum products lead to contamination of soil (Ayotamuno *et al*., 2006). Changes in soil properties due to contamination with petroleum- derived substances can lead to water and oxygen deficits as well as shortage of available forms of nitrogen and phosphorus (Wyszkowska and Kucharski, 2000).

Hydrocarbon spill is a serious threat to the ecology (Schafer *et al.*, 2009). According to Dorn *et al*. (1998), hydrocarbon contains substances that are toxic to the flora and fauna found in the ecosystem. Diesel contains low molecular weight compounds and high proportion of saturated hydrocarbons that are usually more toxic than long chained hydrocarbons. Diesel pollution is on the increase in Nigeria, as well as other developing countries (Stephen *et al*., 2011). The increase use of diesel generators has increased the consumption which invariably results in diesel contamination of soil and water bodies when such generators are serviced, and the oil is discarded carelessly in the environment. This often results in distortion of soil physical, biological and chemical properties. This study, therefore, was undertaken to assess the effect of diesel oil on soil microorganisms and the physicochemical properties of the oil contaminated soil.

MATERIALS AND METHODS

Sample collection

The experimental design consisted of six perforated earthenware pots containing 9 kg of soil each. Three pots served as the control

(Diesel free soil, DFS) while the other three pots were simulated with one litre of diesel each (Diesel polluted soil, DPS) to achieve an 11.11% pollution level. The soil samples were collected bi-weekly for a period of 8 weeks (May –June, 2012) and homogenized before analysis. The pots were perforated to increase aeration and to avoid water logging.

Laboratory/ statistical methods

The pH of the soil was determined at ambient temperature using glass electrode pH and conductivity meter (Hannia, Italy) in 1:1 water to soil ratio. Nitrogen was determined by the micro Kjedahl method (Ibitoye, 2006) while phosphorus was determined as described by Murphy and Riley (1962). The ignition method of Akinsanmi (1975) was used to determine the organic matter content, while the dry weight method was used to determine the moisture content. Microbiological analysis was carried out following the procedure described by Harrigan and McCane (1990). Frequency and occurrence of organisms was determined using the approach of Dung and Stephen (2010). Descriptive statistics and paired T-test was performed using SPSS version 16. Experimental precision achieved was reported at $p \leq 0.05$ levels.

RESULTS

Figure 1 shows the total viable count of bacteria obtained from the soil samples analysed. The count in diesel free soil (DFS) ranged from 1.1x 10^7 to 4.2 x10^7 cfu/g and 8.0 x10^6-2.6x10^7 cfu/g for diesel polluted soil (DPS). DFS had higher counts than DPS. There were significant differences (P>0.05) in the bacterial counts obtained from DFS and DPS. Figure 2 shows the fungal count obtained from the soil samples- DFS and DPS. The counts ranged from 3.0 x10^6 to 13.0x10^6 cfu/g for DFS, 1.0 x 10^6 – 7.0 x10^6 cfu/g for DPS. The fungi counts were higher in DFS than DPS. There were significant differences (P>0.05) in the fungal counts obtained from DFS and DPS. Table 1 show the frequency and occurrence of microorganisms isolated in this study. The bacteria were *Bacillus cereus, Micrococcus luteus, Pseudomonas aeruginosa, Proteus mirabilis* and *Staphylococcus aureus* while the fungi include *Aspergillus flavus, A. niger, Candida tropicalis, Geotrichum candidum* and *Rhizopus nigricans. Aspergillus flavus* had the highest frequency of occurrence (14.58%) followed by *A. niger* and *Pseudomonas aeruginosa* (12.50%). The least frequency of occurrence was observed in *Geotrichum candidum* (6.25%).

Table 2 show the mean physicochemical properties of the soil samples analysed. The pH of all the samples tends towards alkalinity. The values obtained for pH, organic carbon, organic matter content, nitrogen and moisture were higher in diesel polluted soil than in diesel free soil while phosphorus concentration was higher in diesel free soil than in diesel polluted soil. There were no significant differences (P>0.05) in the pH, organic carbon, organic matter content, nitrogen and moisture between the diesel free soil (DFS) and diesel polluted soil (DPS). However, a significant difference was observed in the phosphorus concentration between diesel free soil (DFS) and diesel polluted soil (DPS).

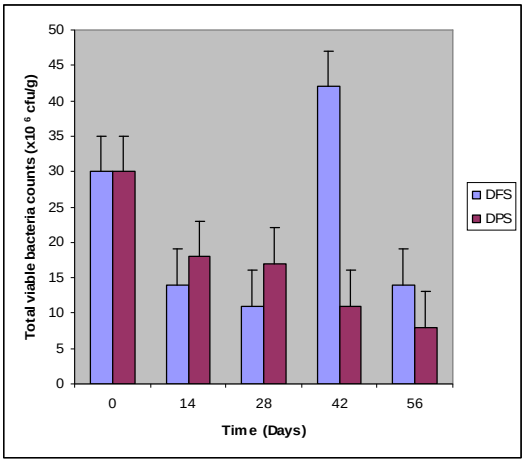

Figure 1: Total viable bacteria counts obtained from soil samples analysed

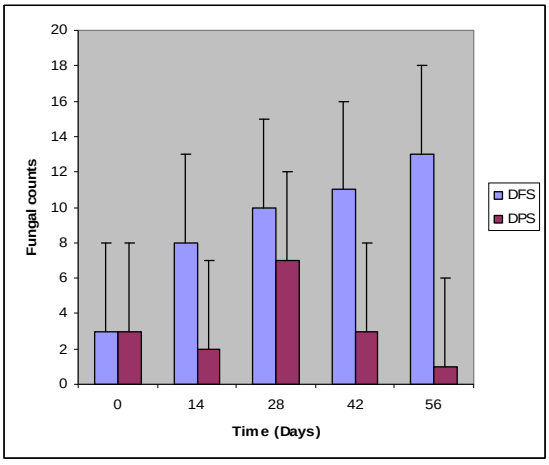

Figure 2: Fungi counts obtained from soil samples analysed

Table 1: Frequency and occurrence of microorganisms in soil samples analysed

Isolates	DFS	DPS	Total	Percentage
Aspergillus flavus	4	3	7	14.58
A. niger	3	3	6	12.50
Bacillus cereus	3	2	5	10.41
Candida tropicalis	3	2	5	10.41
Geotrichum candidum	2	1	3	6.25
Micrococcus luteus	2	2	4	8.33
Proteus mirabilis	2	2	4	8.33
Pseudomonas aeruginosa	4	2	6	12.50
Rhizopus nigricans	2	2	4	8.33
Staphylococcus aureus	2	2	4	8.33
Total			48	≈100

DFS: Diesel free soil, DPS: Diesel polluted soil

Table 2: Physicochemical characteristics of soil sample analysed (mean ± standard error of mean).

	DFS	DPS
pH	7.14±0.18	7.34±0.08
Organic Carbon (%)	1.14±0.08	1.43±0.30
Organic matter (%)	2.01±0.14	2.47±0.52
Nitogen (%)	0.25±0.02	0.32±0.06
Moisture (%)	17.60±3.40	21.57±1.87
Phosphorus (ppm)	7.83±0.39 [a,]	7.44±0.36 [b]

[a,b]: means denoted by different superscripts along the same row are significantly ($p<0.05$) different.

DISCUSSION

Higher bacteria and fungal counts were observed in Diesel free soil (DFS) compared to Diesel Polluted Soil (DPS). This could be attributed to non- exposure of DFS to Diesel which did not distort the biological and physical properties of the soil. This is in agreement with Leahy and Colwell (1990) and Stephen *et al*. (2011). These researchers reported higher bacteria counts in unpolluted soil than polluted soil. The low microbial counts in DPS may be due to inability of some organisms to withstand the high concentration of the diesel (11.11% pollution level) in the environment. This finding is similar to that of Bossert and Bartha (1994) who observed low microbial counts in polluted soil and attributed it to harmful metabolites and toxic compounds in the environment as a result of petroleum degradation. Ten organisms were isolated in the course of the study. These were *Bacillus cereus, Micrococcus luteus, Pseudomonas aeruginosa, Proteus mirabilis, Staphyloccoccus aureus, Aspergillus flavus, A.niger, Candida tropicalis, Geotrichum candidum* and *Rhizopus nigricans.* These organisms have been isolated elsewhere from hydrocarbon polluted soil (Ijah and Abioye, 2003; Okereke *et al*., 2007; Stephen *et al*., 2011;Stephen and Egene, 2012). Ijah and Abioye (2003) reported the predominance of *Bacillus spp* in kerosene spilled site in Minna,

Niger State, Nigeria while Okereke *et al*. (2007) reported consistent isolation of *Pseudomonas, Bacillus spp and Staphylococcus spp* in oil spill sites in Egema, Imo State, Nigeria. According to Chikere *et al*. (2009), hydrocarbon polluted soils are dominated by Gram negative bacteria such as *Pseudomonas spp* and *Proteus spp*. *Aspergillus flavus* and *A. niger* have been reported as fungi that degrade hydrocarbons. Kuiper *et al*. (2004), Okereke *et al*. (2007), Nkweenleng *et al*. (2008) and Chikere *et al*. (2009) reported the crude oil degradative abilities of *A. flavus* and *A. niger* isolated from oil spilled sites. The pH of the soil samples tend towards neutrality. This range of pH has been reported by Bossert and Bartha (1994) and Stephen and Egene (2012) to favour the degradation of diesel oil. Similar pH values were observed by Stephen *et al*. (2011) and Stephen and Egene (2012) from diesel and waste lubricating oil polluted soils.

The higher organic carbon and organic matter contents observed in DPS compared to DFS may be as a result of the addition of the diesel to the soil. Similar result was reported by Stephen *et al*. (2013) in spent lubricating oil polluted soil subjected to phytoremediation. Organic carbon serves as source of energy for the metabolism of the diesel from the soil.

Nitrogen content of DPS was slightly higher than DFS. This agrees with the findings of Stephen and Ijah (2011) and Stephen and Egene (2012). These investigators reported increase nitrogen contents of soil polluted by hydrocarbons. The reason could be due to higher organic matter content in the diesel polluted soil.

The moisture content in DFS was lower than DPS. This observation may be due to low permeability and infiltration of water in the diesel polluted soil compared to the diesel free soil (Edema *et al*., 2011).

The phosphorus concentration was higher in DFS than DPS. This observation is contrary to that of Stephen and Egene (2012) who reported higher phosphorus content in spent lubricating oil polluted soil compared to oil free soil and attributed it to the presence of the oil. The higher phosphorus content in DFS relative to DPS may be due to the existence of reduced condition in the soil that made phosphorus soluble and brought some into solution (Ayotamuno *et al*., 2006).

CONCLUSION

The results of this study show that diesel polluted soils had high organic carbon, organic matter, nitrogen and moisture than diesel free soil. This is an indication that pollution of the environment with diesel accidentally or due to human activities can enter the food chain and seriously affects animal and human health; distort the microbial population and consequently the soil physicochemical properties. Hence, there is a need to enlighten the public against the danger posed by indiscriminate disposal of diesel oil in the environment.

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