BIOREMEDIATION OF SPENT LUBRICATING OIL CONTAMINATED SOIL BY AMENDMENT WITH LIME FERTILIZER

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E. Stephen*, S.L. Obaka and D. Ekeyi
Department of Microbiology, Kogi State University Anyigba, Nigeria
*Corresponding author: Stephen.e@ksu.edu.ng

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Abstract: Bioremediation of spent lubricating oil contaminated soil by amending with 10% lime fertilizer was studied for a period of 56 days. Bacteria and fungi were enumerated using serial dilutions and pour plate method. The bacteria counts ranged from 1.0 x 10^3 to 7.6 x10^3cfu/g in unpolluted soil (UPS), 1.3 x 10^3- 8.8 x 10^3cfu/g in oil polluted soil (PS), 1.0 x 10^3 to 9.2 x 10^3cfu/g in lime amended polluted soil (AMD).Higher counts were observed in AMD than UPS and PS. The fungal counts ranged from 4.0 x 10^3 to 1.1 x 10^3 in UPS, 1.7 x 10^3 to 2.2 x 10^3 in PS, 1.0 x 10^3 to 1.9 x 10^3 in AMD. There was no significant difference in the bacteria and fungi counts at 5% probability level. The organisms isolated were *Staphylococcus* spp, *Micrococcus* spp *Bacillus* spp, *Pseudomonas, Proteus* spp, *Penicillium* spp, *Mucor* spp, *Aspergillus* spp and *Neurospora* spp. The pH (6.30±0.40), moisture (7.60±2.80), nitrate (1.95±1.60) and phosphorus (11.22±2.04) were higher in AMD than UPS and PS. The results of this study indicates that lime fertilizer can be employed for bioremediation of spent lubricating oil polluted soil by increasing the nitrate and phosphorus level of the soil which in turn support the growth of hydrocarbon-utilizing microorganisms.

Keywords: Biodegradation, fertilizer, hydrocarbon, lime, Mucor, nitrate

Introduction

The disposal of spent engine oil into gutters, water drains, open vacant plots and farm is a common practice in Nigeria especially by motor mechanics. This oil, also called spent lubricant or waste engine oil, is usually obtained after servicing and subsequently draining from automobile and generator engines (Anoliefo and Vwioko, 2001).

Spent lubricating oil can also be released into the environment via engine leaks, automobiles and application into rural roads for dust control (ASTDR, 1997). According to Umar *et al*. (2013), spent lubricating oil pollutes the environment when dumped indiscriminately thus affecting the vegetation and microbial flora in the environment. The presence of different types of automobile and machinery has resulted in an increase in the use of lubricating oil. Onuoha *et al*. (2011) reported that oil spills from industries, fuel serving stations, activities in petroleum depots during loading, transportation and auto-mechanic workshops, all combine to contribute to soil contamination.Contamination of soil by hydrocarbon stimulates indigenous microbial populations which are capable of utilizing the hydrocarbons as their carbon and energy source thereby degrading the contaminants. The ability to degrade hydrocarbon substrates is exhibited by a wide variety of bacteria genera (Dally *et al*., 1997; Bogan *et al*., 2003; Malakootian *et al*., 2009; Abdulsalam and Omale, 2009; Abdulsalam *et al*., 2011).

The problems of pollution have led to the exploration of many remedial approaches to effect the cleanup of the polluted soils. Bioremediation is one of such approach which involves the use of microorganisms to detoxify or remove organic and inorganic xenobiotic compounds from the environment. The process relies on microbial enzymatic activities to transform or degrade the contaminants from the environment (Philip and Atlas, 2005). Biostimulation involving lime fertilizer has been reported to reduce soil toxicity (Anyadike *et al*., 2003). In other words, lime can be used to reduce toxic deposition of hydrocarbons in soil. Other material that has been used as biostimulating agents includes manure such as compost (US EPA, 1996), cowpea chaffs (Stephen *et al*., 2013), and poultry litter (Stephen and Temola, 2014). Bacteria consortium has also been reported to augment soil polluted by hydrocarbons (Rahman *et al*., 2002).

Environmental pollution with petroleum and petrochemical products (complex mixture of hydrocarbon) has been recognized as one of the most important silent pollution problem (Stephen *et al.*, 2013). People working in automobile workshop or artisans working on generators are always exposed to spent oil which are potent immunotoxicants and carcinogenic. Accidental leakages from petroleum carrying ships lead to oily layers over the water surface, posing great threat to the aquatic microbiota while leakages from parked automobiles gradually seep into the soils and is easily washed off by surface run-offs into nearby water system. Treatment of hydrocarbon contaminated soil is necessary to protect water supplies, human health and environmental quality (Chang *et al.*, 1996). Owing to the fact that plant derive nutrient for their living from soil and humans depend on plants, it becomes necessary to clean up the hydrocarbon from the soil.

Studies have shown that inorganic manure for instance NPK (15:15:15) or urea or superphosphate have been successfully used in remediating hydrocarbon polluted soil (Anyadike *et al*., 2013; Stephen and Temola, 2014). However, there is dearth of information on the use of lime fertilizer in this area. Anyigba boast of a lime fertilizer company (Confluence Fertilizer Company) and due to the location and availability of the fertilizer which is cheap, and readily available, it is being considered in this study for reclaiming spent lubricating oil polluted soil arising from the indiscriminate dumping of the spent lubricating oil by auto-mechanics and allied artisans on the soil.

Materials and Methods

Sample collection and experimental design

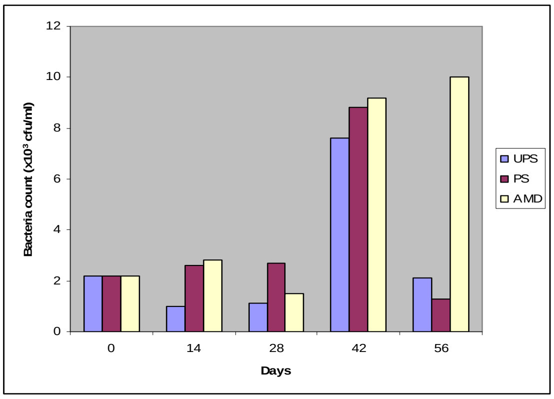
Plot measuring 3 m by 1m was divided into 3 plots each measuring 1 m^2 each. The first plots served as control (without lubricating oil and lime fertilizer). The second contained only spent lubricating oil (ten litres) while the third contained both spent lubricating oil and 1kg of lime fertilizer to achieve 10% amendment level. Sample collection was done every two weeks for a period of 56 days (8 weeks).

Sample analysis

Sampling was conducted bi-weekly for a period of 56 days (8 weeks) to determine the microbiological and physicochemical properties of the soil. The soil samples from the three pots were analyzed microbiologically as described by Public Health England (2014). The pH was determined as described by Thomas (1996). Nitrate was determined by the micro Kjedahl method (AOAC, 2005). The phosphorus content and moisture were determined using the Survey laboratory (1996) method. Descriptive statistics and analysis of variance (ANOVA) was performed using procedure of SPSS version 16 (2007). Experimental precision achieved was reported at $p \leq 0.05$ level.

Result and Discussion

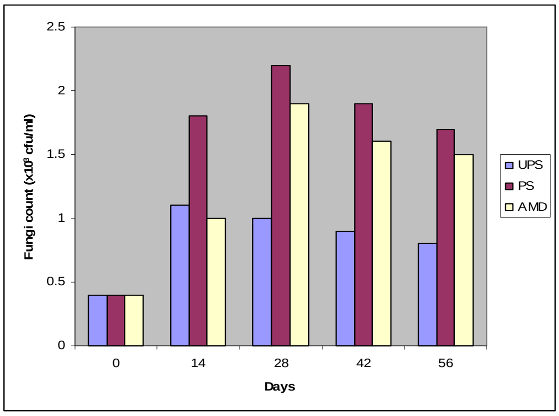
The results of bioremediation studies on spent lubricating oil polluted soil using lime fertilizer shows that the counts of aerobic heterotrophic bacteria in unpolluted soil (UPS) ranged from 1.0 x 10^3 – 7.6 x 10^3cfu/g, 1.3 x 10^3- 8.8 x 10^3cfu/g in oil polluted soil (PS) and 1.0 x 10^3 – 9.2 x 10^3cfu/g in lime amended polluted soil (AMD). Higher counts were observed in AMD at days 42 and 56 compared to UPS and PS (Fig. 1). There were no significant differences (<0.05) in the bacteria counts within the treatments. The differences observed in the bacteria count may be attributed to the consequences of liming which increase the nitrogen and phosphorus contents of the soil. Liming raises soil pH and also eliminate problems associated with acidic soils. This is in agreement with the findings of Okeke and Egbo (2013). These authors observed reduction in total hydrocarbon (THC) content of hydrocarbon polluted soil amended with lime as well as suitable condition for microbial growth compared to an unlimed soil.



UPS = Unpolluted soil, **PS** = Polluted soil, **AMD** = Amended soil

Fig. 1: Total aerobic bacteria count from spent lubricating oil contaminated soil

The fungi counts ranged from 4 x 10^3 – 1.1 x 10^3 cfu/g in UPS , 1.7 x 10^3 – 2.2 x 10^3 cfu/g in PS and 1.0 x 10^3 – 1.9 x 10^3 cfu/g in AMD. The highest fungi count was observed in PS throughout the study. This is in contrast with the observation in bacteria counts. The lowest counts were observed in UPS all through the study period (Fig. 2). The fungal counts generally were lower compared to those of bacteria in all treatments. There was no significant difference between the various soil treatments indicating that liming does not have much effect on fungal growth considering that the highest fungi count was observed in oil polluted soil. This finding is in agreement with Stephen *et al*. (2016). They reported higher population of fungi in mechanic workshop polluted soil than same mechanic workshop soil amended with lime fertilizer. This may be due to the lower acidity in the oil polluted soil compared to the amended soil (Stephen *et al*., 2016). According to Stephen and Temola (2014), fungi thrive in acidic soil than alkaline soil. The lower fungi count observed in the unpolluted soil may be due to the absence of additional phosphorus and nitrate present in the lime fertilizer. The organisms isolated in the course of the study were *Staphylococcus* spp, *Proteus* spp, *Micrococcus* spp, *Bacillus* spp, *Pseudomonas* spp, *Aspergillus* spp, *Mucor* spp, *Penicillium* spp and *Neurospora* spp. These organisms have also been isolated by other researchers including Stephen *et al.* (2013) and recently Stephen *et al*. (2016) from hydrocarbon polluted soils.



UPS = Unpolluted soil, **PS** = Polluted soil, **AMD** = Amended soil

Fig. 2: Total fungi count in spent lubricating oil contaminated soil

Table 1 shows the physicochemical parameters of the soil samples analyzed. The pH ranged from 5.40 ± 0.30 to 6.30 ±0.40. The highest pH was observed in lime amended soil (AMD) while the least pH was observed in unpolluted soil, UPS. There were no significant differences (0>0.05) in the pH between UPS, PS and AMD. Weakly acidic soil was observed during the course of the study. Similar result was obtained by Stephen *et al*. (2016). They adduced the effect of the lime fertilizer as responsible for the weak acidity observed in unamended polluted soil and polluted soil amended with lime.

Table 1: Physicochemical qualities of spent lubricating oil contaminated soil undergoing bioremediation (M+SE)

Parameter	UPS	PS	AMD
pH	5.40 ± 0.30^a	6.00 ± 0.28^a	6.30 ± 0.40^a
Moisture (%)	2.90 ± 0.83^a	3.50 ± 0.80^a	7.60 ± 2.80^a
Phosphorus (%)	8.61 ± 1.22^a	$10.67 \pm 1.67^{a,}$	11.22 ± 2.04^a
Nitrate (%)	0.20 ± 0.04^a	0.41 ± 0.09^a	1.95 ± 1.60^a
Organic matter (%)	1.51 ± 0.33^a	3.50 ± 0.80^a	3.24 ± 0.90^a
Organic carbon (%)	0.88 ± 0.19^a	1.98 ± 0.42^a	1.88 ± 0.52^a

UPS = Unpolluted soil, **PS** = oil polluted soil, **AMD** = Lime amended soil; **a** = means denoted by same superscripts along the same row are not significantly (p<0.05) different. Values are mean of five replicates

The highest moisture content was observed in AMD followed by PS and UPS. It ranged from 2.90 ± 0.83-7.60±2.80%. There was no significant difference (>0.05) in the moisture content between the treatments. The soil moisture content was lower than the values stipulated by the United States Environmental Protection Agency (USEPA) (1989). However, the higher moisture content observed in AMD may be attributed to the effect of the lime fertilizer. According to Atlas and Bartha (1973), application of fertilizer to soil improves the soil water holding capacity, bulk density and nutrients' mobilization for plants. Nitrate content was low in all treatments. It ranged from 0.20±0.04 to 1.95±1.60%. The highest nitrate content was observed in AMD followed by PS. There was no significant difference in the nitrate concentration at 5% probability level. Higher value was recorded in lime amended polluted soil compared to the other two treatments. This is due to the fact that lime contributes to availability of nitrate in nitrate deficient soil (Stephen *et al*., 2016).

Phosphorus content was higher in this study compared to an earlier study by Stephen *et al*. (2016). The phosphorus content ranged from 8.61 ± 1.22 to 11.22 ± 2.04%. There were no significant differences between the treatments at 5% probability level. The higher in phosphorus observed in AMD compared to PS and UPS may be due to the effect of lime fertilizer applied to the soil which has the capability of increasing the solubility and availability of phosphorus. This agrees with the findings of Okeke and Egbo (2013), Stephen *et al*. (2015) and Stephen *et al*. (2016). These authors reported increased phosphorus content hydrocarbon polluted soil amended with lime and organic manure. The highest value of organic carbon was recorded in PS while the least value was observed in UPS. Similar trend in organic carbon was observed in the organic matter content There were no significant differences (<0.05) in the organic carbon and organic matter content between UPS, PS and AMD. Organic carbon and organic matter are required for successful biodegradation. Carbon is a substrate required by microbes and the low organic matter content is an indication that the soil microbes were actively involved in biodegradation of the spent lubricating oil (Okeke and Egbo, 2013).

Conclusion

This study was conducted to investigate the suitability of lime fertilizer in bioremediation of spent lubricating oil polluted soil. Higher microbial populations were observed in spent lubricating oil contaminated soil amended with lime fertilizer than the other two treatments. The pH, moisture content, phosphorus, nitrate, organic matter content was higher in the amended soil compared to the unpolluted soul and the unamended polluted soil. The results indicates that lime fertilizer possess the potential to remediate soil polluted with petroleum products such as petrol, diesel and spent lubricating oil.

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