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Short Communication

Isolation of bacteria from mechanic workshops' soil environment contaminated with used engine oil

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The study was designed to evaluate the bacterial diversity of soil environment contaminated with used engine oil. Ten mechanic workshops within Enugu metropolis were selected and six soil samples were collected from each site. These were analyzed using Bushnell Haas enrichment medium. Samples were enumerated using ten fold dilutions from 1:10 to 1:100000 from the soil samples. The result showed the isolation of *Bacillus Stearothermophilus* (8.3%) and *Cyanobacteria* (1.7%) from the sites sampled. The number of viable bacterial growth of *B. Stearothermophilus* and *Cyanobacteria* were enumerated and expressed in colony forming units. Agbani had bacteria densities of 5 x 10^4, 1.25 x 10^4 and 6.25 x 10^5 from the three different sites respectively, Asata with 5.10 x 10^4 and 2.5 x 10^4, independence layout with bacterial density of 2.5 x 10^2 and Uwani/Coal camp with 2.5 x 10^4 and 6.25 x 10^5. Amongst the 10 different sites studied, Agbani and Uwani/Coal camp had the highest bacteria density of 6.25 x 10^5.

Key words: Used engine oil, contaminated soil, pollution.

INTRODUCTION

Environmental pollution with petroleum and petroleum products (complex mixture of hydrocarbons) has been recognized as one of the most serious current problems especially when associated with accidental spills on large-scale. If this occurs, hydrocarbons may reach the water table before becoming immobilized in the soil. They spread horizontally on the ground water surface and continue to partition into ground water, soil pore space, air and to the surface of soil particles. The vast range of substrates and metabolites present in hydrocarbon-impacted soils surely provides an environment for the development of a quite complex microbial community (Butier and Mason, 1997). It is used to lubricate the parts of automobile engine in order to keep everything running smoothly (Hagwell et al., 1992).

Used oil was defined by the US Environmental Protection Agency (40CFR Pan 270) as oil that has been refined from crude oil or any synthetic oil; this has been used and as a result of such use is contaminated by chemical impurities which contribute to chronic hazards including mutagenicity and carcinogenicity as well as environmental hazard with global ramifications (Blodgette, 2001). Bioremediation has become an alternative way to remedy oil polluted sites, where the addition of specific microorganism (bacteria, cyanobac-teria, algae, fungi, protozoa) or enhancement of microorganism already present, can improve biodegradation efficiency (Hagwell et al*.,* 1992). These microorganisms can degrade a wide range of target constituents present in oil sludge (Barathi and Vanudevan, 2001; Mishra et al*.,* 2001). A large number of pseudomonas strains capable of degrading polycyclic aromatic hydrocarbons have been isolated from soil (Johnson et al., 1996; Kiyohara et al*.,* 1992). Other petroleum hydrocarbon degraders include *Yokenella* spp., *Alcaligenes* spp., *Roseomanas* spp., *Sreanotrophomanas* spp., *Acinetobacter* spp., *Flavobacter* spp., *cyanobacterium* spp., *capnocytophage* spp., *Moraxella* spp. and *Bacillus* spp. (Antai 1990; Bhattacharya et al., 2002). Other microorganism such as fungi is also capable of degrading the hydrocarbons in engine oil to a certain extent. However, they take longer

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Table 1. Number of isolates from the various mechanic workshops' soil environment contaminated with used engine oil.

Site	Number of samples	Isolate(s)
Asata	18	*Bacillus Stearothermopilus*
Independence Layout	12	*Bacillus Stearothermopilus*
Uwani/Coal camp	12	*Bacillus Stearothermopilus*
Abakpa	12	*Bacillus Stearothermopilus*
Agbani/Trans-Ekulu	12	*Bacillus Stearothermopilus* and *Cyanobacteria*

period of time to grow compared to their bacterial counterparts (Prenafeta-Boldu et al., 2001).

Petroleum products such as engine oil, petrol, diesel and kerosene are used daily in various forms in mechanic workshops. These products tend to harden and change the colour of the soil, which may have untold health hazard on the technicians and artisans. Their soles tend to harden which may alter their movement. This study, thus, was aimed to survey the bacteria present in used engine oil-contaminated soil environment.

MATERIALS AND METHODS

Study site

Study sites were different mechanic workshops in Enugu metropolis. The places were Uwani/Coal Camp, Asata (which included Ogui, College Road and Asata), Independence Layout, TransEkulu/Abakpa and Agbani.

Sample collection

Soil samples were collected from specific location within the workshops that had heavy spillage of used engine oil. The locations had no grasses growing on them and soil samples were collected at three different locations at each workshop. The soils were characterized by hardened surfaces and blackish in colour.

Isolation of degrading agents

The culture media used were Bushnell – Haas medium (Atlas, 1994) which is an enrichment medium for isolation of bacterial degrading organism Tryptose soya milk broth and Nutrient Agar.

Innoculation was done using the enrichment medium of 20 ml in MacCartney bottles into which ten grams of the contaminated soil was added and incubated at 30°C for one week. Samples that were turbid were subcultured into nutrient Agar using Bushnell Haas broth as diluent to observe the morphological characteristics of the isolates.

Bacterial load enumeration

Samples were enumerated by making ten fold dilutions of the soil samples from 1:10 to 1:100000 from broth culture. They were covered or corked and incubated for one week at 30°C in an incubator. From the diluted sample, using a dropper pipette, 0.025 ml of each dilution was dropped on the solid nutrient agar surface. Each inoculum of microorganism developed into a discreet colony. The number of viable micro-organisms in the sample was calculated from the number of colonies formed, the volume of inoculum used by dropper pipette and the dilution factor expressed in colony forming unit.

Identification of isolates

The morphological characteristics of the isolates were identified by gram stain and biochemical reactions (Balow et al., 1992) as well as mortility test. The biochemical reactions include glucose fermentation, catalase production reaction, egg yolk reaction and reaction in tryptose soyabroth.

RESULTS AND DISCUSSION

Sixty soil samples were collected from six different mechanic workshops. The results show a prevalence rate of 10.0% yield of bacteria degrading agents. The isolates are *Bacillus stearothermphilus* (8.3%) and cyanobacteria (1.7%), (Table 1). The bacteria density of *B. stearothermophilus* and cyanobacteria at the various sites were enumerated using ten fold dilutions. The highest yield of bacteria density of 6.25×10^5 colony forming unit was from Agbani and Coal camp while the least of 1.25×10^4 colony forming unit was from Uwani (Table 2).

In motor mechanics workshops there is a constant change in the soil micro-organism as a result of deliberate spillage of used engine oil. These alter the biomass and ecology of the soil such that both microbial communities and grasses can no longer grow on the soil spots. The colour and texture of the soil are affected; this leads to different microbial flora establishment in an attempt to remedy the petroleum product spillage (Bartha and Atlas, 1977). The total heterotrophic bacteria count range from $1.25 \times 10^4 – 6.25 \times 10^5$ in this work. Butier and Mason (1997) indicated that there is an increase in heterotrophic bacteria population in the presence of dispersant agent. Also, Antai (1990) reported two major response to crude oil in which there is an increase in biomass. Although, this disagrees with the work done by Lizarraga-Partida et al. (1982) who observed that petroleum hydrocarbon has little or no effect on the total bacterial heterotrophic environment. *B. Stearothermophilus*

Table 2. Densities of bacteria isolates from the various mechanic workshops' soil environment contaminated with used engine oil.

Site	Bacteria Isolate	Density
Asata	*Bacillus Stearothermopilus*	$2.50 \times 10^4 - 5.10 \times 10^4$
Independence Layout	*Bacillus Stearothermopilus*	2.50×10^4
Uwani/Coal camp	*Bacillus Stearothermopilus*	$2.50 \times 10^4 - 6.25 \times 10^4$
Agbani	*Bacillus Stearothermopilus*	$1.25 \times 10^4 - 6.25 \times 10^4$

with a range of $2.5 \times 10^4 - 6.25 \times 10^5$ was found to reoccur in most sites. This conforms to the work of Ohenhen et al. (2006) with *Bacillus* range of $5.11 \times 10^5 - 5.25 \times 10^5$. *Cyanobacteria* with density of 1.25×10^4 was found to be present in association with oil degrading bacteria (Griffiths et al., 1981).

It has been reported that in most systems, cynobacteria are present in association with oil-degrading bacteria and prevent them from being washed out by immobilizing them in their mucilage. In addition, cynobacteria also supply these bacteria with oxygen produced by photosynthesis and the fixed nitrogen needed for their activity in the degradation process. Cyanobacteria appear to be affected by some constituents of crude oil even at low concentration (Kiyohara et al., 2004). The aromatic compounds have been shown to have more drastic effects than alkanes. These compounds inhibit photo-synthesis and growth, reduce enzyme activity and microbial biomass, and induce changes in cynobacterial species composition (Megharaj et al., 2000). In this study, the low cynobacterial community composition may have affected the colonization of these sites by other microbial populations, thereby allowing low biodegrading activities. However, irregular growth of *B. stearothemophilus* and Cyanobacteria occurred since there was incomplete dispersion of the used engine oil in the media which agree with the work of Griffiths et al. (1981). An important fact is that the medium employed for the isolation of petroleum degrading bacteria may have significant selective effect on bacteria population that was isolated (Mishra et al., 2001). There was an increase in cell number of *B. stearothemophilus* during the degradation process demonstrating the ability of utilizing engine oil as energy source for this organism (Mandril and Lin, 2007).

Studies of community dynamics related to petroleum degrading microbes have the potential to enhance our understanding of the roles played by microbes in the natural genesis of long-term effect of petroleum product pollution and to determine new remediation. From these studies, the used engine oils were not poorly disposed. Therefore, building pit systems, where these used engine oils will be collected and properly treated before releasing them into the soil, to eliminate the hazards of such contaminations, is advocated.

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