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Original Research Article

Ecosystem Biology of Soil Polluted with Spent Engine Oil

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Abstract

Soil communities, the microorganisms, are the initial and primary recipients of hydrocarbons or any xenobiotics incidented on the soil. This study evaluated the biology of soil organisms, *ex-situ*; the hydrocarbonclastics and non-hydrocarbonclastics, following an exposure to spent engine oil. The result demonstrated that at low and moderate contaminations of 1.5 and 2.5% w/w, hydrocarbons reduced the microbial population from control, 1.28 $x10^9$ cfu, to 3.42 $x10^8$ and 3.06 x 10^8cfu on week one respectively. However, at increased contaminations of 3.5% w/w, hydrocarbons from large oil spills weaken microbial ability to degrade the added hydrocarbons as the prejudicial nature reduced the population to 1.62 x 10^8cfu on week one. Overall, across the weeks, there was insurgence of hydrocarbon-degrading organisms, the hydrocarbonclastics, which on the other hand induced a limitation in microbial diversity. The isolated hydrocarbonclastic microorganisms in the descending order of their biodegradation potentials are: *Pseudomonas aeruginosa*, *Arthrobacter*, *Alealigena*, *Corynebacter*, *Flavo-bacterium*, *Archromobacter*, *Micrococcus*, *Norcardia and Myco-bacterium*. Thus within the ecosystem they devised metabolic diversity with increased catabolic properties primarily aimed at biodegrading the xenobiotics; making them hydrocarbonclastic organisms.

Keywords: hydrocarbonclastics, microorganisms, hydrocarbons.

INTRODUCTION

The soil is the major site of degradative processes in terrestrial ecosystems with an impressive capacity to reduce intractable substances to relatively much simpler compounds. This capacity is derived solely from the microbial exudates (catabolic enzymes) which comprise the decomposer community. Soil communities are highly complex, dominated by bacteria and fungi which in their degradation of organic compounds provide resources and nutrients for plants in the soil. It is the microbial growth and activities in the soil that enrich the soil and thus make it fertile and healthy for agricultural purposes. The significance of enormous amount of microbial biomass in the soil lies not only in their large biochemical capacity, but in the phenomenal diversity of biochemical reactions attributed to the soil microbial population. It is worth noting that soil microbes also interact with other microbial groups. Hence, it is common to find for example, that degradation of plant materials occur readily in the presence of a mixed soil microbial consortium, than it does when one or more groups of the soil microbes are eliminated from the system. Soil microbes exert much influence in controlling the quantities and forms of various chemical elements found in the soil. Most notable are the cycles for carbon, nitrogen, sulphur and phosphorus, all of which are important in soil fertility. The mineralization of organic materials by soil microbes liberates carbon dioxide, ammonium (which is rapidly converted to nitrate by microbes) sulphate, phosphate and inorganic forms of other elements. This is the biochemical basis of nutrient cycling in all the major ecosystems of the world.

Apart from the role of soil microbes in nutrient cycling, organic matter decomposition and pollutant degradation, soil microbes also contribute heavily to the resilience of a disturbed soil ecosystem (Karlen *et al.*, 1992). Thus, the emergence of xenobiotic such as spent engine oil, which disturbed the biochemical equilibrium of the soil ecosystem, triggered the succession of hydrocarbon microbial degraders, the hydrocarbonclastics, (Kaplan and Kitts, 2004), which metabolize the hydrocarbons in the oil and use it as source of carbon and energy. Oxidation by soil microorganisms is the principal mechanism by which oil in the soil is biodegraded (Atlas and Bartha, 1992; Kaplan and Kitts, 2004).

Aerobic bacteria and fungi appear to play the most important role in the oil degradation process (Vanloocke *et al.*, 1975; Llanos, 1976). Following an oil spill, the microbial population in the soil will go through a short period of adaptation or lag phase (Rowell, 1975). Vanloocke *et al.*, (1975) reported that the lag phase often encountered in soil metabolism upon the application of hydrocarbons was caused by the toxicity of low boiling aromatic hydrocarbons. The toxicity aspect was further supported by the work of Walker *et al.*, (1975). On the basis of these reports, it appears that the adaptation time depends to a large extent on the microbial population present in the soil, site and climatic conditions, and the type and amount of oil spilled.

After the microbial population has adapted to the oil, usually microbial numbers and activity will increase. Several researchers have demonstrated increases in microbial activity following the addition of oil to soil by measuring either oxygen uptake or carbon dioxide evolution (in the case of dehydrogenases) from the oil contaminated soil (Gudin and Syratt, 1975; Watts *et al*., 1982). Oil biodegradation in an adequately aerated soil has been found to be closely related to microbial activity. The biodegradation is usually fairly rapid in the beginning and then slows down or declines, probably because of the organic acid produced which created a reducing environment. This biodegradation is largely due to aerobic reactions and thus proceeds fastest in well aerated or oxygenated surface soils. In subsoil, anaerobic conditions usually prevail and in soils where oxygen reserves are low, oil biodegradation will occur much more slowly.

Based on the laboratory and field studies, substantial evidence abound that some constituents of oil are preferentially metabolized by hydrocarbon decomposers, and that the rate of decomposition differs between oil types (Walker *et al.*, 1976; Fedorak and Westlake, 1981). As a vital component of soil ecosystem function, soil microbial community can reflect changes in the functionality of a soil ecosystem. It is on this basis therefore, that soil microbes have been proposed as biological indicators of stress in soil ecosystem insulted with hydrocarbons from spent engine oil (Kaplan and Kitts, 2004).

Research Design

This research was designed for a-forty-two-day investigation in consideration of the volatility and biodegradability of hydrocarbons: day- zero, -14, -28, -42; within which, the microbial biomass was evaluated.

MATERIALS AND METHODS

Sample Collections

Spent engine oil was obtained randomly from three different mechanic villages in Owerri, Imo State, Nigeria and pooled together to obtain a composite sample meant for the analysis. The soil was obtained from Federal University of Technology Owerri, Imo State, Nigeria with an augar inserted about fifteen centimeters into the soil.

Soil Preparation

From the soil samples collected 220Kg was weighed, dried at ambient temperature (25-27ºC), crushed in a porcelain mortar and sieved with cheesecloth to remove tiny stones and other particulate matters. The fractions (10g), after contamination with spent engine oil at different concentrations (1.5, 2.5, and 3.5% w/w) were used for the determination of total microbial biomass and hydrocarbonclastics.

Determination of microbial population in spent engine oil-impacted soil

Sterilization of materials

The Petri dishes were washed with tap water, dried in a dryer at a temperature of 45^{o}C; then oven-dried at 210^{o}C for 2hr. The test tubes, Erlenmeyer flask, pipette tips, crucible, spatula and beakers were autoclaved at a temperature of 120^{o}C and fifteen pounds pressure for 15 min.

Bacterial culture

To sterile water, 10g of soil sample was aseptically introduced into test tubes, tightly capped and vortexed for 5min. Thereafter, 1ml was aseptically transferred into 9ml of sterile distilled water, and ten-fold serial dilutions were carried out. 0.1ml of the solution from the fourth dilution was evenly spread on an already prepared nutrient agar plate and the culture was incubated for a period of 24h. After the incubation period, the total viable count was determined by counting the colony forming units (cfu) and distinct colonies were isolated.

Identification of isolates

The isolates were subjected to the routine bacterial identification procedure using Bergey's Manual of Systematic Bacteriology (Baumann and Schubert, 1994).

RESULT

Table 1: Total microbial population in spent engine oil-impacted soil (x 100)

% Contamination	**Week 0ne (cfu/g)**	Week Two **(cfu/g)**	Week Four **(cfu/g)**	Week six **(cfu/g)**
Control 1.28×10^{9}				
1.5	3.42 x10^{8}	3.98 x10^{8}	4.20 x10^{8}	3.71 x10^{8}
2.5	3.06 x10^{8}	3.29 x10^{8}	3.60 x10^{8}	3.40 x10^{8}
3.5	1.62 x10^{8}	2.00 x10^{8}	2.68 x10^{8}	2.72 x10^{8}

The prejudicial nature of crude oil reduced the biomass as the concentration increased on week one. Thereafter, there was insurgence of hydrocarbonclastic organisms as presented in Table 1.

Microbial isolates in the crude oil-impacted soil
Bacterial strains: *Pseudomonas aeroginosa*, *Flavobacteria*, *Nocardia*, *Corynebacteria*, *Mycobacteria*, *Micrococcus sp*, *Rhodococcus*, *Streptomyces*, *Bacillus sp*, *Arthrobacter* and *Cyanobacteria*.

Fungal strains: *Fusarium sp*, *Aspergillus niger*, *Candida sp*, and *Penicillium.*

DISCUSSION

Following an oil spill, the microbial population in the soil passed through a short period of adaptation or lag phase, a reduction in biomass and a limitation in microbial diversity from 1.28×10^9 to 1.62×10^8 cfu/g in week one at 3.5% contamination. The lag phase encountered in this study upon the application of spent oil may be attributed to the toxicity of hydrocarbons (Ebulue *et al*., 2017). Walker *et al.*, (1975) reported similarly the microbial lag phase following the introduction of hydrocarbon from oil. They attributed it to the toxicity of the later where they concluded that the time lag was equivalent to the time required for the active oil-degrading microbial population to grow and synthesize the enzymes required for oil decomposition. Thus, this initial decrease in the microbial population in the soil sample contaminated with spent engine oil supports the report that spent oil is prejudicial to soil ecosystem. This development may be attributed to the fact that the oil elicited its acute toxicity effects on some strains of microorganisms.

Following the insult, some microbial strains which could not withstand this toxicity were eliminated, while the hydrocarbonclastic strains survived it. However, at increased concentrations overtime, there was increase in microbial population from 1.62×10^8 to 2.68×10^8 cfu/g which cuts across weeks -one to -four at 1.5 – 3.5% contamination. The implication in this upsurge was attributable to the hydrocarbon-degrading organisms (the hydrocarbonclastics), which use hydrocarbon as source of carbon and energy thereby increasing the biomass. The significance of this insult has been shown to enhance microbial growth in the affected soil due to increase in the availability of degradable substrate, and as such microbial biomass and activity are generally much higher in the spent engine oil-impacted soil than in the bulk soil. It is this enhanced microbial activity as a consequence of the degradable substrate that constitutes the hallmark of remediation of the oil polluted soil.

Overall, the result implicated that at increased contamination, hydrocarbons increased the abundance of hydrocarbon-degrading microorganisms (the hydrocarbonclastics), but on the other hand, induced a limitation in microbial diversity; an effect that slows soil organic matter mineralization and associated nutrient remineralization (Ebulue *et al*., 2017).

The rate of biodegradation of spent engine oil by hydrocarbonclastic organisms isolated from oil-impacted soil showed that the biodegraders which are *Pseudomonas aeruginosa, Micrococcus varians, Azotobacter vinelandii and Bacillus subtilis* have different abilities in the breakdown and utilization of the oil. *Pseudomonas aeruginosa* had the highest growth in the sterilized soil supplemented with the highest percentage concentration of the spent oil. This was followed by *Micrococcus varians*, *Azotobacter vinelandii and then Bacillus subtilis.* The degrading bacteria used these pollutants as a new carbon and energy sources. The results of this study are similar to that reported by Margesin *et al*., (2000) which indicated that the count of degrading bacteria increased with addition of oil substances. Additionally, it is not surprising that *Pseudomonas aeruginosa* exhibited the highest growth. This is because not only that it was isolated frequently from oil-contaminated soils (crude or spent), but also because it is known to possess a more competent and active hydrocarbon-degrading enzymes than other biodegraders as reported by Onwurah (2000).

CONCLUSION

Overall, at increased contamination, hydrocarbons in the oil-impacted soil increased the abundance of hydrocarbon-degrading microorganisms (the hydrocarbonclastics), but on the other hand, induced a limitation in microbial diversity; an effect that slows soil organic matter mineralization and associated nutrient re-mineralization (Ebulue *et al*., 2017). It is this enhanced microbial activity as a consequence of the degradable substrate that constitutes the hallmark of remediation of the oil-polluted soil.

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