SOIL LIPASE AND DEHYDROGENASES ACTIVITIES IN SPENT ENGINE OIL POLLUTED ECOSYSTEM

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ABSTRACT

The activities of soil lipase and dehydrogenases as well as soil pH in spent oil pollution were evaluated. Results indicated that spent engine oil inhibited the activity of soil lipase. The inhibition which was in a concentration and time dependent manner was statistically significant ($p<0.05$) when compared between groups. Conversely, the effect of spent engine oil on soil dehydrogenases contrasted sharply with that obtained on soil lipase. The oil stimulated the activity of soil dehydrogenases in a concentration and time dependent manner which was significant ($p<0.05$) when compared between groups. There was increase in acidity as the pH of the oiled soil was progressively reduced which was significant ($p<0.05$) between groups. Overall, these changes altered the entire soil biochemistry and a shift in equilibrium and energy imbalance.

Key words: Soil, spent engine oil, lipase, dehydrogenases, pH.

No: of Figures: 3 **No: of References:26**

INTRODUCTION

Environmental pollution as a consequence of spillage from spent engine oil and petroleum derivatives by anthropogenic means is the major cause of soil toxicity and degradation. Maintenance of ecological equilibrium is a necessity of every natural ecosystem. Any biological disequilibrium as a result of impact of hydrocarbon from spent lubricating oil or any xenobiotics will provoke the insurgence of indigenous microbial communities to biodegrade the foreign compounds and bring the ecosystem to a balance and equilibrium. This is the hallmark of the entire ecosystem function.

Spent engine oil, which is also known as used mineral based crankcase oil, is a brown-to-black liquid produced as a result of high temperature and high mechanical strain on the unused oil (ATSDR, 1997).This petroleum derivative is a mixture of several different chemicals (Wang *et al*., 2000), straight and branched chain aliphatic and aromatic hydrocarbons (ATSDR, 1997), of low and high molecular weights (C_{15} – C_{20}), polychlorinated biphenyls, chlorodibenzofurans, lubricating additives, decomposition products, as well as heavy metals such as aluminium, chromium, tin, lead, manganese, nickel, and silicon some of them come from engine parts as they wear down (ATSDR, 1997).

Hydrocarbons are recalcitrant because of their oily nature and poor water solubility. They persist in nature for long period and cause hazardous effects on ecosystems. Spent engine oil which is a common environmental toxicant not naturally found in the environment (Dominguez-Rosado and Pichtel, 2004) is liberated into the environment when the motor oil is changed and disposed into gutters, water drains, and farmlands, by motor and generator mechanics (Odjegba and Sadiq, 2002). In addition, the oil is also released into the environment from the exhaust system during engine use and engine leakage (Anoliefo and Edegbai, 2000; Osubor and Anoliefo, 2003).

Its contamination on soil ecosystem alters soil biochemistry, immobilizes nutrients and creates oxygen tension (Atuanya, 1987). This limitation in oxygen results to utilization of alternate electron acceptors by indigenous microbial communities which produces an increased reducing environment. The lowered pH adversely affects the physicochemical parameters of the soil.

Soil microbial exudates (the enzymes), which have a central role in the soil environment are used as attractive bio-indicators for monitoring various impacts on the soil. Besides their use in the case of hydrocarbons, soil biological activities have been used as biological indicators of pollution from hydrocarbon, heavy metals and pesticides (Bayer *et al*., 1982). Soil respiration, dehydrogenase and lipase activity as well as microbial counts are the most common indices used to describe microbial activities during hydrocarbon contamination and decontamination (Song and Bartha, 1990; Waarde *et al*., 1995; Scwab and Banks, 1994). The activity of soil enzymes provides an integrative measure of the biological status of the soil (Li *et al*., 2005).

Experimental design

This study was designed for a-forty-two-day investigation in consideration of the volatility and biodegradability of hydrocarbons:

Day- zero
Day- 14
Day- 28 and
Day- 42;

within which, the activities of the aforementioned enzymes were evaluated as a function of time and concentration of the pollutant.

MATERIALS AND METHOD

Project site

Microcosms are a valid and efficient way of examining factors affecting soil biological activities (Bolton *et al*.,1991; Kroer and Coffin, 1992), and this study used soils from three sites dug about 15cm depth from Onitsha in Anambra State of Nigeria to make the microcosms.

Test samples

Spent engine oil was obtained randomly from three different mechanic villages in Onitsha Anambra State of Nigeria and pooled together to obtain a composite sample meant for the analysis.

Determination of pH of spent oil-contaminated soil

Soil inoculation was carried out by weighing 10g of sieved soil sample into six different test tubes. To the first tube, 0.1g of spent engine oil in 10g soil sample corresponding to 1.0%, was added and mixed thoroughly with a steering rod. This procedure was repeated for 1.5, 2.0, 2.5, 3.0 and 3.5%; and into the 7th tube, the control, 20ml of distilled water was added to 10g soil and mixed thoroughly by hand. Into the homogenous slurry formed was immersed pH meter probe (Jenway model) and was allowed to stabilize at 25^{o}C. The pH values were then determined after calibration with buffer solution of pH 7.0 and 4.0.

Determination of the activity of dehydrogenases

The activity of dehydrogenases was determined using the method described by Tabatabai, (1982). Dehydrogenases convert 2,3,5-triphenyltetrazolium chloride (TTC) to formazan. The asbsorbance of formazan was read spectrophotometrically at 485nm.10g of sieved soil was placed into test tubes containing different percent concentrations of spent oil: 1.0, 1.5, 2.0, 2.5, 3.0, 3.5% w/w (oil-soil mixture), and into the blank, distilled water. Then, 5ml of 3% (w/v) aqueous TTC was inoculated into all the tubes mixed and stirred with a glass rod. At the end of 96h incubation at room temperature, 10ml of ethanol was added to each test tube and the suspension was vortexed for 30s. The tubes were then incubated for 1h to allow suspended soil to settle. The resulting supernatant was carefully transferred into clean test tubes, and the absorbance was read spectrophotometrically at 485nm. The concentration of formazan was evaluated using the molar extinction coefficient of dehydrogenase at 15433Mol cm^{-1} Dushoff, (1965); and the activity was determined thereafter

Determination of the activity of lipase

A colorimetric method for determination of soil lipase activity of Schinner *et al*. (1996) was used, where the substrate, p-nitrophenyl butyrate was incubated with the soil samples at 27^{o}C. Soil inoculation

was carried out by weighing 10g of sieved soil sample into six different test tubes. To the first tube, 0.1g of spent engine oil corresponding to 1.0% was added and mixed thoroughly with a steering rod. This procedure was repeated for 1.5, 2.0, 2.5, 3.0 and 3.5%; and into the 7th tube, the control, 20ml of distilled water was added. After 10 minutes incubation at pH 5.4 and centrifugation at 7000g, the resulting supernatant was carefully transferred into clean test tubes and the absorbance of the released p-nitrophenol was determined colorimetrically at 400nm with the molar extinction coefficient of $1.48 \times 10^{-2}M^{-1}cm^{-1}$.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). All results were compared with respect to the control. Comparisons between the concentrations and control were made by using Statistical Package for Social Sciences (SPSS) version 20 and Analysis of Variance (ANOVA). Differences at $P < 0.05$ were considered significant.

RESULTS

The pH of the oiled soil is shown in Fig. 1.0. Relative to the control, there was a progressive reduction in pH values which was statistically significant ($p<0.05$) between groups. The oil impacted soil increased in acidity in a concentration and time dependent manner up to day-28. Spent engine oil stimulated the activity soil dehydrogenase. The increase which was significant ($p<0.05$) when compared between groups was concentration and time dependent as presented in Fig. 2.0.

Spent engine oil inhibited the activity of soil lipase. Comparison between groups showed the inhibition which was statistically significant ($p<0.05$) when compared between groups was in a concentration and time dependent manner as shown in Fig. 3.0.

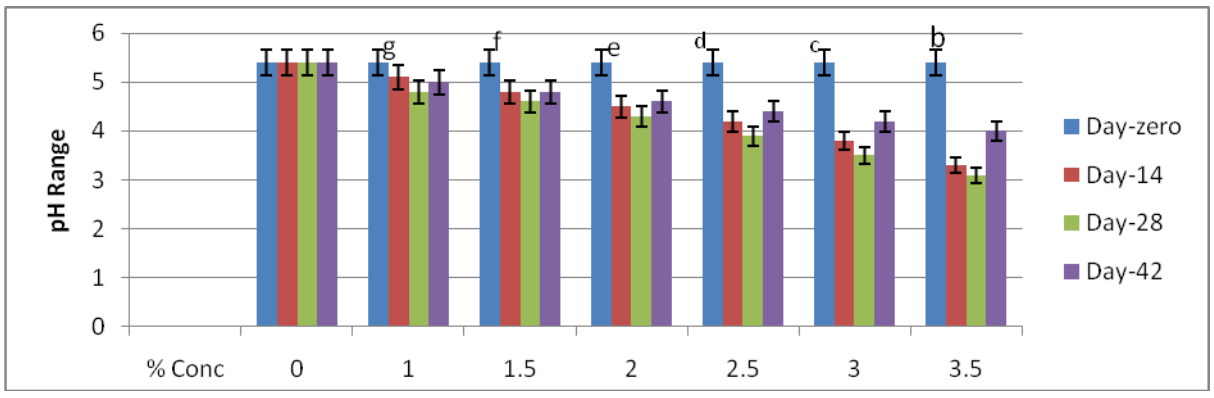

Fig. 1.0: pH of the soil polluted with spent engine oil

Comparison between groups: bars with different letters did not differ significantly ($p<0.05$).

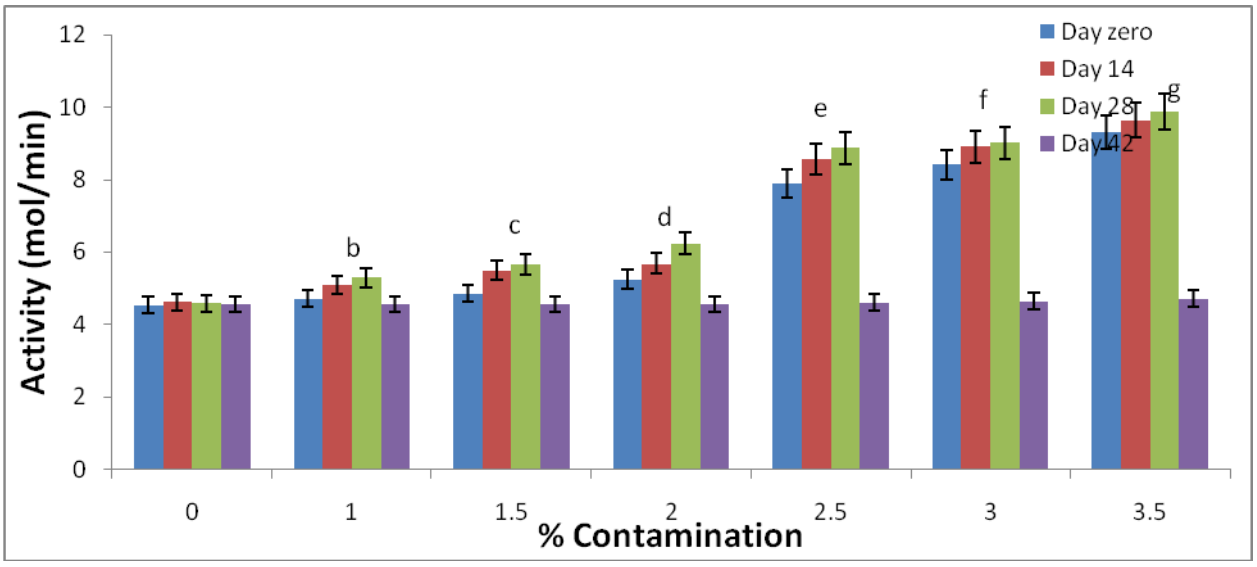

Fig. 2.0: The activity of soil dehydrogenase in the spent oil-polluted soil.

Comparison between groups: bars grouped in different letters differ significantly (p<0.05).

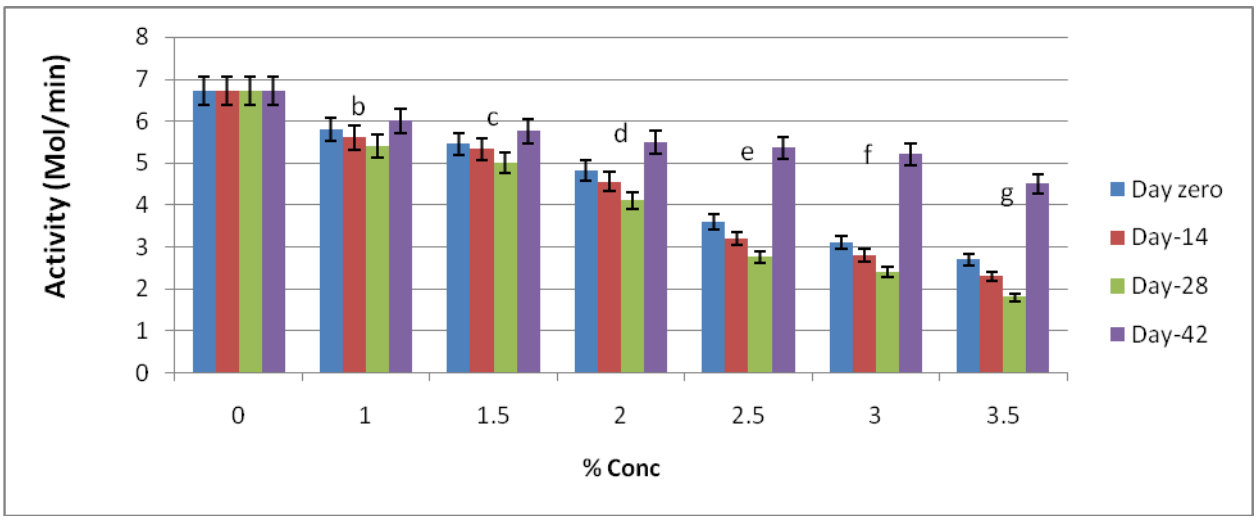

Fig. 3.0: Soil lipase activity in the spent oil-polluted soil

Comparison between groups; bars grouped in different letters differ significantly (p<0.05)

DISCUSSION

Contaminants such as spent engine oil as well as additives (heavy metals) that enter ecosystems have been found to alter the biochemical equilibrium and energy balance of the soil. Their presence causes alteration in soil microbial properties (Atuanya, 1987; Brookes, 1995; Odjegba and Sadiq, 2002; Osubor and Anoliefo, 2003) and their exudates (pH, enzymes) and nutrient mineralization

Microorganisms are known to release extracellular enzymes to mineralize organic compounds to elemental minerals (Nannipieri *et al*., 2001). It is important to underline that these ecto-enzymes attached at the outer microbial cells initiate the hydrolysis and oxidation of high molecular weight substrates such as hydrocarbons to mineral elements (Ladd *et al*., 1996; Nannipieri *et al*., 2001).

For normal metabolism, the ecosystem maintains a certain level of these enzymes which are constitutive in nature no matter the environmental conditions. The incident of a degradable substrate (pollutant) triggers the induction of these enzymes to metabolize the xenobiotic to harmless products or even to a more toxic intermediate; while the enzyme represses and disappears when its substrate is depleted. This type of metabolic control with respect to enzyme induction and repression as a result of introduction of pollutant into microbial cell environment attempts to optimize its internal biochemistry (Onwurah, 2000).

pH which was an important physical property of soil was investigated in this study. Spent oil caused a reduction in soil pH which was significant when compared between groups at various contaminations. Similar observations were made in earlier reports of Achuba and Peretiemo-Clarke (2008), Atuanya, (1987), Osuji and Nwoye, (2007). This increase in acidity could be attributed to the production of organic acids by microbial and enzymatic metabolism. The lowered pH reflected accelerated metabolism and accelerated demand for electron acceptors, thus creating a reducing environment. The increase in acidity which would likely affect mineralization by microorganisms, microbial succession and metabolism, leachability of metals would affect plant growth.

This study investigated two ecto-enzymes involved in the mineralization of hydrocarbons in the spent oiled soil. Spent engine oil caused a significant ($p<0.05$) increase in soil dehydrogenase activity. This is likely to be the result of increase in total microbial respiratory rate. Schinner *et al*. (1996) reported similarly that dehydrogenase activity in soil was a measure of microbial activity and respiration rate. The increase in dehydrogenase activity can be due to the involvement of hydrocarbonclastic organisms in the metabolism of polyaromatic hydrocarbons. Similar results were reported by Achuba and Peretiemo-Clarke (2008), Margesin *et al*. (2000), Dominguez-Rosado and Pichtel (2004) who observed that soil respiration increased when spent engine oil was added and this was initiated by soil dehyrogenases.

Spent oil inhibited the activity of soil lipase significantly. This alteration in the activity could arise from unfavourable conditions such as hypoxia and a reduction in pH which occasioned in the oil-polluted environment indicating that oil biodegradation by microorganisms and metabolic enzymes could lead to production of organic acids. It could also imply that the amino acids at the active sites of soil lipase are irritable to hypoxic conditions and pH decrease, and any condition that creates oxygen tension with a rise in acidic environment adversely affected the activity. It then follows that the aerobic bacterial status / population has a correlation with the activity of the enzyme. This finding is in consonance with the report of Waarde *et al*. (1995), Margesin and Schinner (1999) on the inhibition of the activity of soil lipase following an insult of soil ecosystem with spent oil.

CONCLUSIONS

Spent engine oil caused a shift in equilibrium and energy imbalance in the affected ecosystem. ;It altered the entire soil biochemistry. Microbial activities and

their exudates are veritable indices of spent oil polluted soil. The enzymatic activities of dehydrogenase and lipase in the affected ecosystem provide information on the activities of hydrocarbonclastic organisms.

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