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Enzyme–based Assay for Toxicological Evaluation of Soil Ecosystem Polluted with Spent Engine Oil

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Authors' contributions

This work was carried out in collaboration between all authors. Author INO designed and supervised the study and author MME a postgraduate student wrote the protocol. The first draft of the manuscript was written by author OO. Authors ACU and OO managed the literature search and author MME carried out the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aim: In this study, the experiment was designed to investigate the effect of contamination of soil ecosystem with spent engine oil at various concentrations.
Design: Soil samples were obtained from zoological garden University of Nigeria Nsukka while spent engine oil was obtained from the Mechanic Village, Nsukka. Test tubes labelled 1- 7 containing various percentages of spent engine oil 1.0, 1.5, 2.0, 2.5, 3.0, 3.5% w/w (oil-soil mixture); and into the 7th tube, the control contained only the soil sample. The study was designed for thirty-five-days (0, 14, 28 and 35 day) at various degrees of pollution by spent oil.
Results: The result showed that spent engine oil stimulated the activity of soil dehydrogenase in a concentration and time dependent manner: from (4.72 ± 0.015) mol/min at 1.0% contamination to

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(9.30 ± 0.021) mol/min at 3.5% contamination on day-zero; and from (5.29 ± 0.032) mol/min at 1.0% contamination to (9.78 ± 0.040) mol/min at 3.5% contamination on day-28; the activity of soil catalase was inhibited from (0.195 ± 0.005) mol/min at 1.0% contamination to (0.054 ± 0.004) mol/min at 3.5% contamination on day-zero; and from (0.18 ± 0.004) mol/min at 1.0% contamination to (0.042 ± 0.002) mol/min at 3.5% contamination on day-28. The moisture content increased from (6.4 ± 0.01) at 1.0% contamination to (24.24 ± 0.0) at 3.5% contamination on day-zero; and from (4.56 ± 0.056) at 1.0% contamination to (19.31 ± 0.0212) at 3.5% contamination on day-28. Similarly, there was an increase in total petroleum hydrocarbon (TPH) from (0.03 ± 0.0) to (0.86 ± 0.0) that cuts across days-zero to -28 at concentrations (1.0-3.5%) contamination. At increased concentrations (3.5% w/w) of contamination, hydrocarbons increased the abundance of hydrocarbon-degrading microorganisms from 3.26 $x10^7$ ± 0.02 cfu on day-zero to 6.55 x 10^8 ± 0.04 cfu on day-28; but on the other hand, induced a limitation on microbial diversity.

Conclusion: The concentration of the hydrocarbonclastic bacteria in the spent engine oil-contaminated soil correlated with the enzyme induction activity. These effects which altered the entire soil biochemistry could disrupt ecosystem dynamics by slowing soil organic matter mineralization and associated nutrient re-mineralization.

Keywords: Contamination; dehydrogenase; microorganisms; mineralization; hydrocarbonclastic; biodegradation.

1. INTRODUCTION

Contamination of the existing and potential agricultural lands is the major problem associated with the processing, distribution, usage and disposal of crude, refined and spent petroleum products. Such contaminants are the limiting factors to soil fertility and hence crop productivity because they are toxic to soil organisms and to plants [1,2] and can transfer to surface and ground waters, thus threatening the human health [3]. These pollutants, although they are biodegradable, create an unsatisfactory condition for life in the soil. They can bio-accumulate in food chains where they disrupt biochemical or physiological activities of many organisms [4]. Due to poor aeration, they cause immobilization of soil nutrients and lowering of soil pH [5]. They can display potential carcinogenic and mutagenic activities in the soil [4,6]; alter the succession of microorganisms [7], which is directly associated with the induction and activities of soil enzymes [2,8]. In general, it can be said that apart from some quantitative changes which occur under such conditions, the CFU increased [1,8] and the activities of soil enzymes are altered (increased or decreased). This, however, does not lead to any improvement in the soil fertility; thus the response of plants to oil pollution is unambiguously negative [9,10]. This is mainly due to the destructive influence of the oil on the soil structure and soil air, with loss of soil mineral nutrients such as potassium, sodium, calcium, magnesium, nitrogen, sulphate, phosphate, nitrate and this leads to exposure of the soil ecosystem to leaching and erosion [11].

When oil is spilled onshore or near shore, the soil and other components of the terrestrial ecosystem are inevitably affected. In an environment that is completely aquatic, the oil sometimes floats on water surface, where it is disposed to shorelines by wind and wave actions, invariably affecting the soil. In an environment that is completely terrestrial, the penetration and spread of the oil is a function of the nature of the topography of the soil environment. The soil is a prime factor in agricultural productivity and socio-economic activities; therefore, any threat or substantial impairment to the soil usually affects the people's livelihood and galvanizes into public outcry [12].

These deleterious effects have made it mandatory to have a counter measure for the spent engine oil (hydrocarbon) pollution in the environment. Bioremediation of the petroleum hydrocarbon-contaminated soil environment is a potentially important application of environmental biotechnology. In this approach, microorganisms are utilized under some specified conditions to ameliorate the negative effects in a cost-effective and environmentally friendly approach. The main strategies in bioremediation of oil spills, which include bio-stimulation, nutrient application, bio-augmentation, microbial seeding with competent or adapted hydrocarbonoclastic bacteria or their consortium, and genetically engineered microbes, have been utilized. Although the promise of bioremediation is yet to be realized, innovative areas in environmental biotechnology for oil spill clean-up have been addressed [4].

The soil is a prime factor in agricultural productivity and socio-economic activities. It is one of the most dynamic sites of biological interactions in nature. It is a site where both the biotic and abiotic components of the ecosystem interact freely to support, sustained and create products or by-products of metabolic processes.

One central problem in toxicity testing of soil is measuring the responses of the living community to an impact. The soil has a complex community, not readily partitioned into easily counted populations. Thus, studies of toxic stress on soil have measured some community-level or ecosystem-level parameters.

Very direct methods have been used, often using soils *in situ*. An example is total bacterial numbers showing a sigmoidal response to metal concentrations in the soil around a brass foundry, since 'dose' declines with distance [13].

Soil enzymatic activities which have a central role in the soil environment are used as attractive bio-indicators for monitoring various impacts on the soil. Dehydrogenases are enzymes that catalyse the removal of hydrogen atoms from substrates [14]. Active dehydrogenases are considered to exist in the soil as the integral part of intact cells. They conduct a broad range of oxidative activities that are responsible for degradation of soil organic matter [15,16,17]. Soil dehydrogenases activity can reflect changes in the respiratory activity of a given population size in response to changes in the soil environment [18].

Catalases are iron porphyrin and antioxidant enzymes that catalyse very rapid decomposition of hydrogen peroxide to water and oxygen [14]. The enzyme is widely present in nature, which accounts for its diverse activities in the soil.

Both catalase and dehydrogenase are used as biomarkers for microbial activities in the soil as they are very sensitive to pollution by hydrocarbons, heavy metals and pesticides [19,20]. According to Kiss [19] and Przystas et al. [21], the activity of soil enzymes is the most appropriate measure of soil biological activity; because it is the basis for soil metabolism, as it decides the speed and direction of metabolic transformations that occur in the soil. Their values have been suggested to be used as simple toxicity test in oil pollution [22].

Joseph and Salau [23] reported that increase in levels of oil spillage and increasing levels of forest loss negatively affect agricultural production or productivity in the Niger Delta.

Although, the knowledge of the effects of spent engine oil on different soil enzyme activities is still poor; the aim of this work, is to determine the effect of spent engine oil contamination on soil dehydrogenase and catalase activities.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Project site

Soil sample were taken at 15cm depth from Botanical garden of the Department of Botany, Faculty of Biological Sciences, University of Nigeria Nsukka located on the coordinates: 6°51′24″N 7°23′45″E. Soil sample was obtained from the above mentioned site using hand trowel at the depth of 5-10 cm while spent engine oil was obtained from the Mechanic Village, Nsukka. Test tubes labelled 1-7 containing various percentages of spent engine oil 1.0, 1.5, 2.0, 2.5, 3.0, 3.5% w/w (oil-soil mixture); and into the 7th tube, the control contained only the soil sample.

2.2 Methodology

2.2.1 Soil analysis

The sandy soil from the Botanical Garden of UNN was manually sieved using 0.5 mm to remove tiny stones and other particulate matters.

2.2.1.1 Determination of soil pH

20 ml of distilled water was added to 10 g soil and mixed thoroughly by hand. Into the homogenous water slurry formed was immersed pH meter probe (Jenway model) and was allowed to stabilize at 25℃. The pH values were then determined after calibration with buffer solution at pH 7.0 and 4.0.

2.2.1.2 Determination of soil temperature

Test tubes were labelled 1-7. Into these, the following concentrations of spent engine oil were introduced: 1.0, 1.5, 2.0, 2.5, 3.0, 3.5% w/w (oil-soil mixture) while 20 ml of distilled water was added to was added to 10 g of soil in test tube 7 acting as the control. The temperature of each sample in the tube was determined in ℃ with thermometer.

2.2.1.3 Determination of moisture content

Soils contaminated with different percent concentrations of spent engine oil was introduced into six petri dishes (1.0, 1.5, 2.0, 2.5, 3.0, 3.5% w/w (oil-soil mixture) while the 7th dish (control) only contained 10 g of soil. All the dishes, including the control were flooded with 10 ml of water. The weight of the flooded soil was determined. After 24h oven drying at 80^0C, the percentage of water evaporated was calculated by difference:

$$\%\, Water = \frac{(x-y)}{x} x\, 100$$

2.2.2 Assay methods for oil pollution

The science of ecotoxicology has revealed that the effects of pollutants in natural ecosystems are diverse, complex, and often unpredictable. Dissatisfaction is increasing due to lack of enough ecological standard ecotoxicological tests and a realization that extrapolation from laboratory findings to real-world situations is often impractical [24].

2.2.3 Effects of spent oil pollution on soil pH, temperature and moisture content

The influence of soil pH on oil decomposition was evaluated by McGill and Nyborg [25] who found that oil decomposition was slow under acidic soil conditions. Rowell [26] reported that under acidic conditions microbial activity was slower and fungi tend to predominate over bacteria. Bacteria are probably the most important group at least in the early stages of oil decomposition and are favoured over fungi by neutral to slightly alkaline pH values. Vanloocke et al. [27] reported that in the neutral pH region (6- 7.5), bacteria such as *Actinomycetes* as well as fungi can be active. Dibble and Bartha [28] found that oil sludge biodegradation was optimal at a soil pH of 7.5 to 7.8. Studies conducted in Alberta also indicated that optimal oil biodegradation occurred at a near neutral pH [25,29].

2.2.4 Determination of total petroleum hydrocarbon (TPH)

Total petroleum hydrocarbon content was determined gravimetrically by the method of Odu et al. [30] to provide an estimate of the available total hydrocarbon at the time. Toluene in 20 ml aliquot was added into six test tubes containing different concentrations of spent engine oil 1.0, 1.5, 2.0, 2.5, 3.0, 3.5% w/w (oil-soil mixture) as well as and into the 7th tube, the control. After manually shaking for 30 min by hand, the liquid phase of the extract was measured spectrophotometrically at 420 nm. The TPH in the soil was estimated with reference to the standard curve derived from fresh spent oil diluted with toluene using the equation y = 1.094x; where y = absorbance and x = concentration.

2.2.5 Determination of microbial population in the oil-impacted soil

2.2.5.1 Sterilization of materials

All equipment was sterilized by autoclaving.

2.2.6 Bacterial culture

Soil samples of 10 g each was added aseptically to 7 ml sterile water in six test tubes vortexed at 4000 rpm for 5 min. Thereafter, 1 ml was aseptically transferred into 9 ml of sterile distilled water, and ten- fold serial dilution was carried out. 0.1 ml 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 24 h. After the incubation period, the total viable count was determined by counting the colony forming units (CFU) and distinct colonies were isolated.

2.2.7 Identification of isolates

The isolates were subjected to the routine bacterial identification procedure with Bergey's Manual of Systematic Bacteriology [31].

2.2.8 Gram staining

The stock cultures were activated by cultivation on a sterile spent engine oil agar media and incubated at a temperature of 37^oC for 72 h. Thereafter, a smear was made on a glass slide and covered with few drops of primary stain, crystal violet. After a minute of exposure to the staining solution, the slide was washed with plenty of water. The smear was treated with few drops of Lugol's iodine (mordant) and allowed to react for a minute; Lugol's iodine as a mordant. The slide was again washed with water and then decolorized in acetone for 30 sec. Thereafter, it was washed with plenty of water without any delay. The smear was finally treated with few drops of counter stain (Safranin) and washed again with plenty of water after a minute. The

slide was then blotted dry and immersion oil was added and viewed under the microscope with an objective lens of X 100.

2.2.9 Preparation of extract for enzyme determination

One hundred (100) ml of phosphate buffer, pH 7.4, was added to 10 g of soil and homogenized gently. The soil suspension was filtered using cheesecloth. The filtrate was centrifuged at maximum speed of 7000 g for 10 min to obtain the supernatant [32].

2.2.10 Determination of the activity of soil catalase

Catalase activity was determined by the method of Cohen et al. [33], where decomposed hydrogen peroxide was measured by treating it with excess of potassium tetraoxomanganate (VII), ($KMnO_4$) and residual $KMnO_4$ was measured spectrophotometrically at 480 nm.

One hundred microliter (100 µl) of the supernatant was introduced into differently labelled test tubes containing 0.5 ml of 2 Mmol hydrogen peroxide and a blank containing 0.5 ml of distilled water. Enzymatic reactions were initiated by adding sequentially, at the same fixed interval, 1ml of 6N tetraoxosulphate (VI) acid, H_2SO_4 to each of the labelled test tubes containing different percent concentrations of spent engine oil 1.0, 1.5, 2.0, 2.5, 3.0, 3.5% w/w (oil-soil mixture); and also to the blank. And 7 ml of 0.1N $KMnO_4$ was added within 30 sec. and thoroughly mixed.

Spectrophotometer standard was prepared by adding 7 ml of 0.1N $KMnO_4$ to a mixture of 5.5 ml of 0.05N phosphate buffer, pH 7 and 1 ml of 6N H_2SO_4. The spectrophotometer was then zeroed with distilled water before taking absorbance readings at 480 nm.

The concentration of catalase was determined using the Beer-Lambert's law, A = ECL with the molar extinction coefficient of catalase at 4.02 mol cm^{-1}; and the activity was determined thereafter.

2.2.11 Determination of the activity of dehydrogenase

The activity of dehydrogenase was determined using the method described by Tabatabai [33]. Dehydrogenases convert 2, 3, 5-triphenyl tetrazolium chloride (TTC) to formazan. The absorbance of formazan was read spectrophotometrically at 485 nm.

Samples of 10 g of sieved soil was placed into test tubes containing different percent concentrations 1.0, 1.5, 2.0, 2.5, 3.0, 3.5% w/w (oil-soil mixture), and into the blank, distilled water. Then, 5 ml of 3% (w/v) aqueous 2, 3, 5-triphenyl tetrazolium chloride was added into all the tubes, mixed and stirred with a glass rod. After 96h of incubation at 27℃, 10 ml of 95% ethanol was added to each test tube and the suspension was vortexed for 30s. The tubes were then incubated for 1 h to allow suspended soil to settle. The resulting supernatant was carefully transferred into clean test tubes, and the absorbance was read spectrophotometrically at 485 nm. The concentration of formazan was evaluated using the molar extinction coefficient of dehydrogenase at 15 mol cm^{-1} [34] and the activity was determined thereafter.

2.3 Statistical Analysis

The results were presented as means of replicates for different concentrations. One-way ANOVA was used for testing significance at *P*=.05 degree of freedom. All statistical analysis was carried out using SPSS version 16.0 statistical package.

3. RESULTS

3.1 Soil Analysis

The physicochemical analyses of the soil revealed a mixture of sandy, clay and loam soil. Textural characteristics of the soil indicated high percentage of coarse clay and a combination of silt and fine sand. The pH was 7.1.

3.2 Effect of Spent Engine Oil on Soil pH

The pH of the oiled soil is shown in Fig. 1. Relative to the control, there was a progressive reduction in pH values as the concentration of the spent engine oil and its duration of contact increased. Thus, the soil was acidic. The acidity increased from day-zero up to day 14 and day 28; beyond which, there was a decline; i.e., pH values began to increase.

3.3 Effect of Spent Engine Oil on Soil Temperature

Following the oil pollution, there was increase in temperature of the soil. Relative to the control,

the soil temperature increased in a concentration and time dependent manner. The temperature increased across days 14 and 28 and declined thereafter on day 35 as shown in Fig. 2.

3.4 Moisture Content of the Oil-impacted Soil

The oiled soil was characterized with high moisture content relative to the control, at each concentration; there was increase in moisture content which declined with time as shown in Fig. 3.

3.5 Total Petroleum Hydrocarbon (TPH) of the Oil-polluted Soil

Following the contamination, there was a synergistic increase in total petroleum hydrocarbon (TPH). This increase in TPH declined with time as presented in Fig. 4.

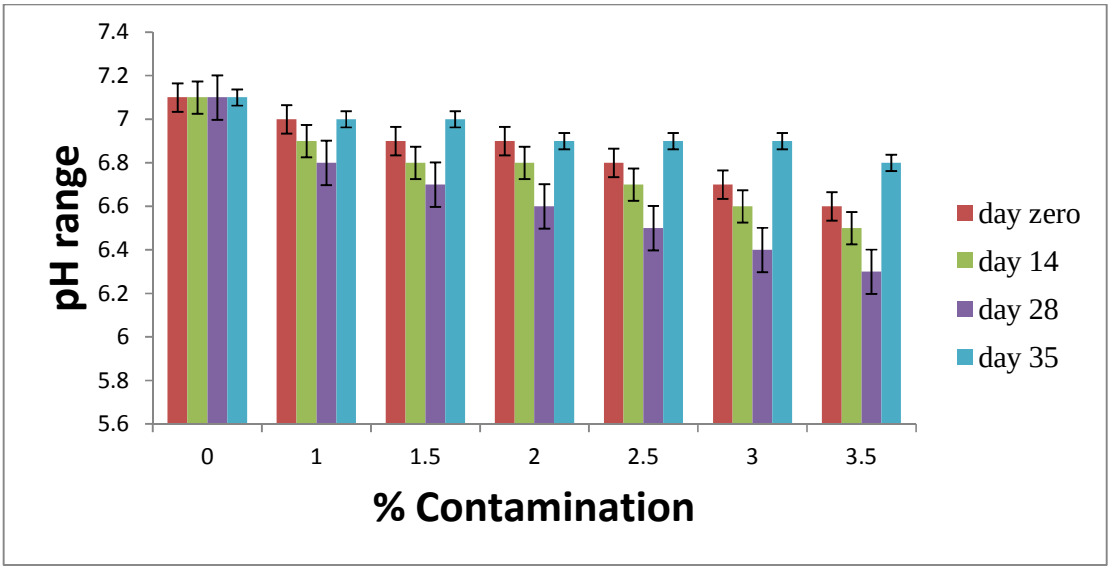

Fig. 1. pH of the soil polluted with spent engine oil

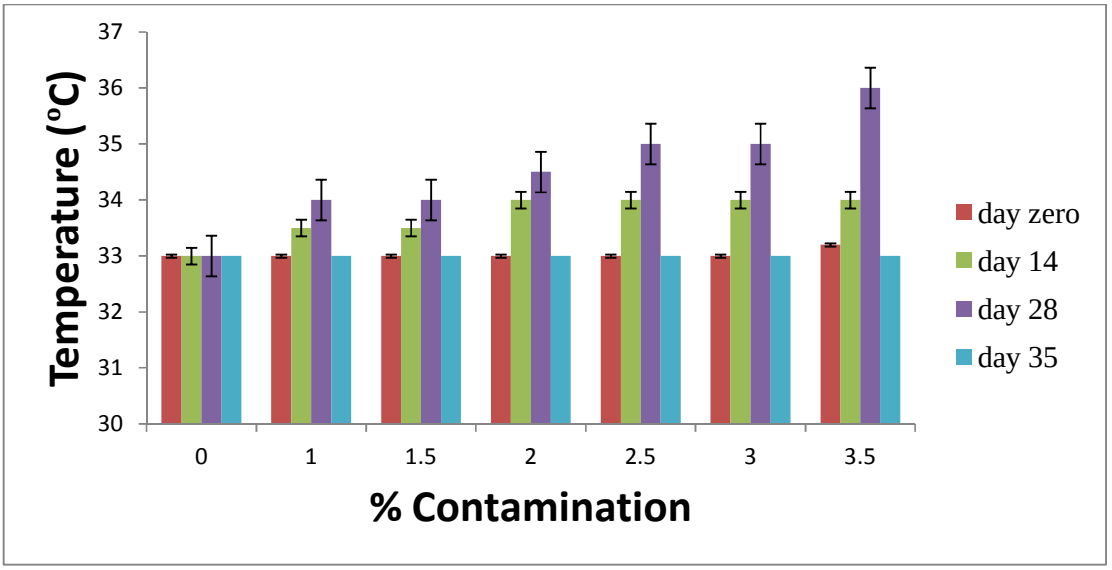

Fig. 2. Temperature of the soil polluted with spent engine oil

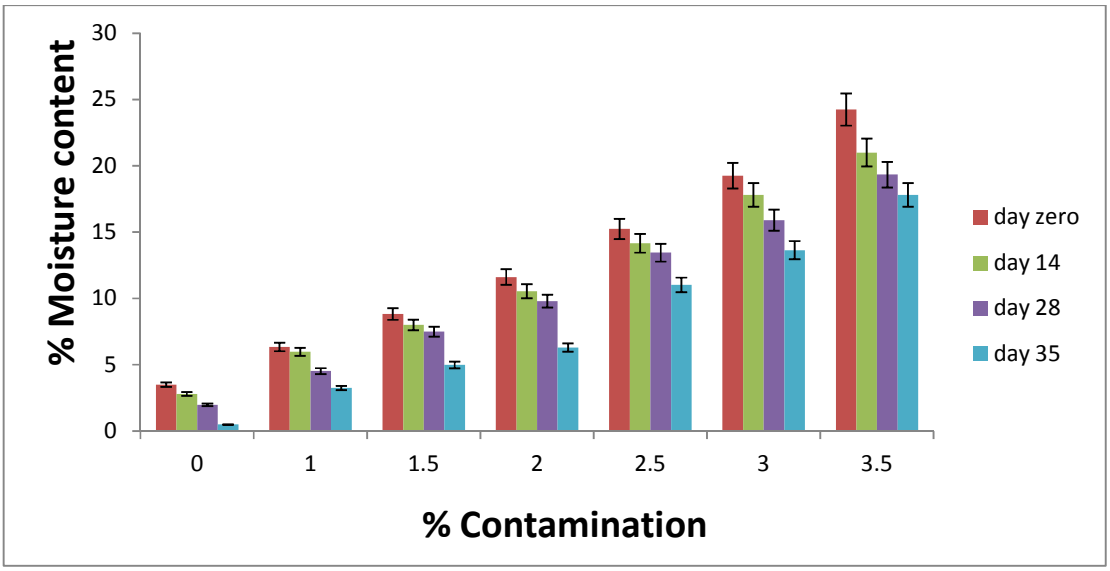

Fig. 3. Moisture content of the oil-impacted soil

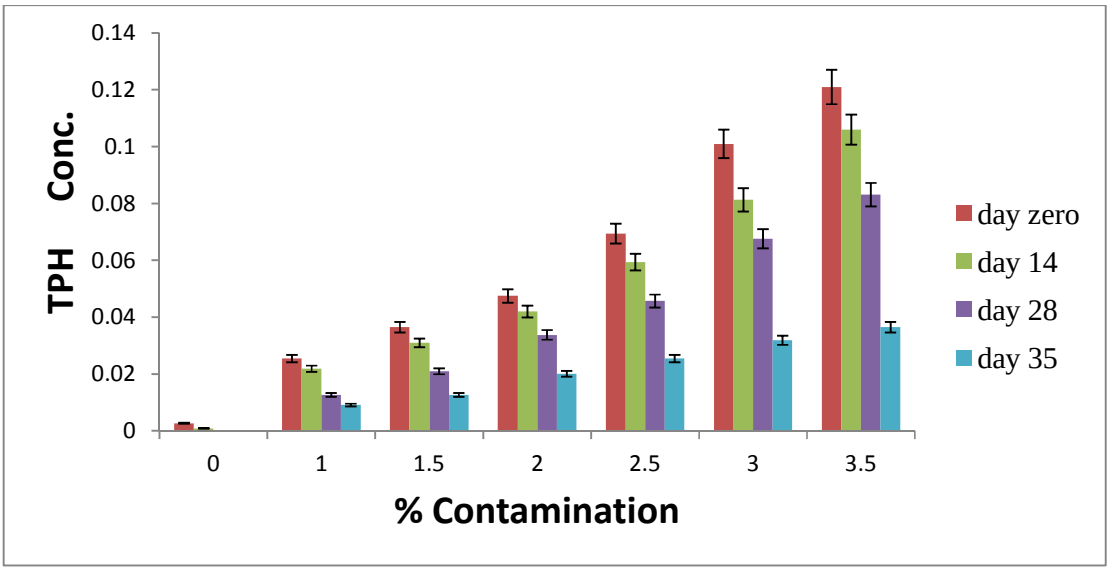

Fig. 4. Total petroleum hydrocarbon (TPH) of the oil-polluted soil

3.6 Microbial Population of the Oil-impacted Soil

The mean total aerobic and anaerobic viable and culturable bacterial count present in the soil sample from the Botany garden of University of Nigeria, Nsukka was 1.22 x 10^9 cfu/g. The total aerobic and anaerobic bacterial count observed in spent engine oil-contaminated soil sample on days; zero, 14, 28, and 35 are presented on Table 1. There was a decline in total microbial count following oil pollution on day- zero. However, on days 14 and 28 there was significant (*P*=.05) increase in cfu which thereafter began to decline on day 35.

3.7 Effect of Spent Engine Oil on the Activity of Soil Catalase

Spent engine oil inhibited the activity of soil catalase in a concentration and time dependent manner. Relative to the control, there was a progressive decrease in the activity as the concentration of the spent engine oil and

duration of contact increased. This was observed on days zero, 14, and 28. However, by day 35, the activity returned to normal as shown in Fig. 5.

3.8 Effect of Spent Engine Oil on the Activity of Soil Dehydrogenase

The effect of spent engine oil on soil dehydrogenase contrasted sharply with that obtained in soil catalase. The oil stimulated the activity of soil dehydrogenase in a concentration and time dependent manner as presented in Fig. 6. Relative to the control, there was a progressive increase in the activity as the concentration of the spent engine oil and duration of contact increased. The increase in activity progressed up to day-28, and declined thereafter.

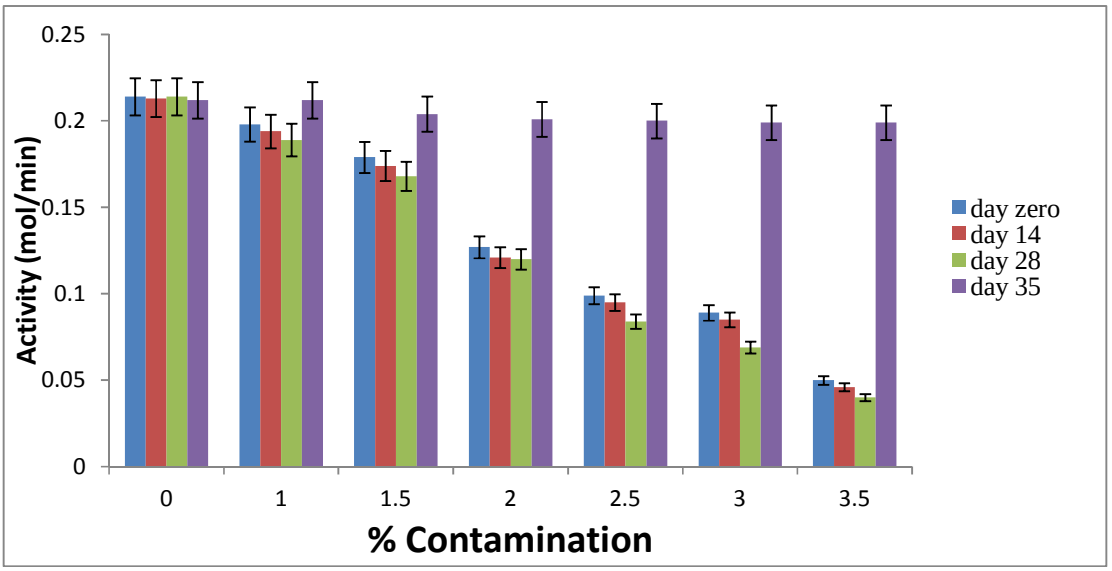

Fig. 5. The activity of soil catalase in the spent oil-impacted soil

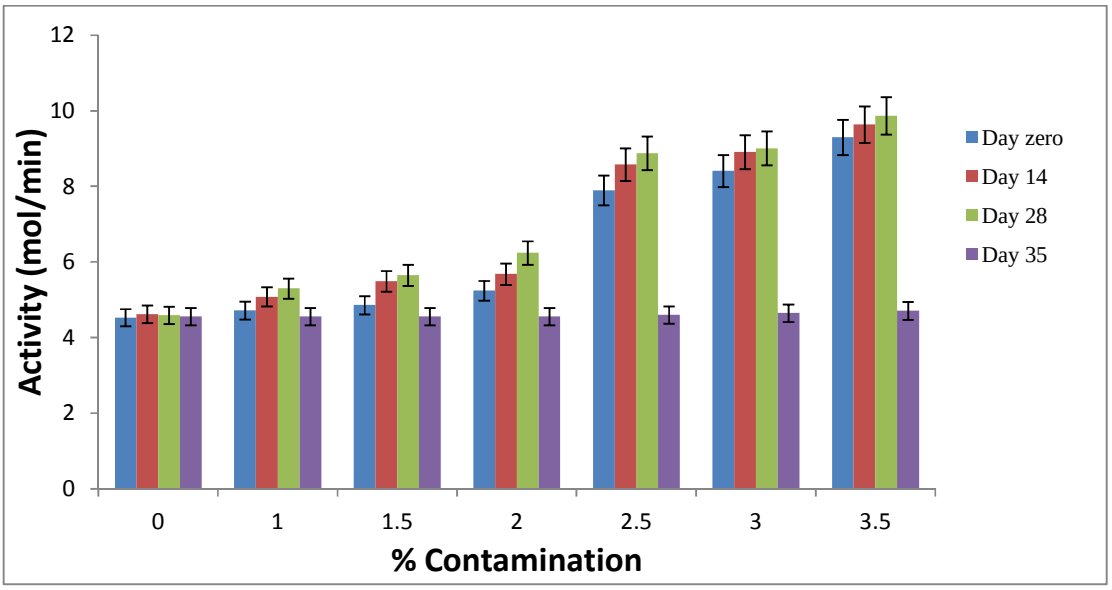

Fig. 6. The activity of soil dehydrogenase in the spent oil-polluted soil

Table 1. Microbial population of the oil-impacted soil (× 100)

% contamination	Day zero (CFU/g)	Day 14 (CFU/g)	Day 28 (CFU/g)	Day 35 (CFU/g)
0.0	$1.22^{a} \times 10^{9}$	$1.22^{a} \times 10^{9}$	$1.22^{a} \times 10^{9}$	$1.22^{a} \times 10^{9}$
1.0	$3.55^{b} \times 10^{8}$	$3.68^{b} \times 10^{8}$	$3.80^{b} \times 10^{8}$	$2.00^{ab} \times 10^{8}$
1.5	$3.05^{b} \times 10^{8}$	$3.80^{b} \times 10^{8}$	$3.99^{b} \times 10^{8}$	$2.22^{bb} \times 10^{8}$
2.0	$4.11^{b} \times 10^{7}$	$3.92^{b} \times 10^{8}$	$4.76^{b} \times 10^{8}$	$2.38^{bb} \times 10^{8}$
2.5	$3.98^{b} \times 10^{7}$	$4.01^{b} \times 10^{8}$	$5.01^{b} \times 10^{8}$	$2.60^{bb} \times 10^{8}$
3.0	$3.80^{b} \times 10^{7}$	$4.25^{b} \times 10^{8}$	$5.44^{b} \times 10^{8}$	$2.89^{bb} \times 10^{8}$
3.5	$3.26^{b} \times 10^{7}$	$4.55^{b} \times 10^{8}$	$6.55^{b} \times 10^{8}$	$3.04^{ba} \times 10^{8}$

Numbers with the same superscripts shows non-significant results (P=.05) while those with the same double superscripts are significant (P=.05) both within and between groups

4. DISCUSSION

Petroleum exploration and exploitation activities by the oil industries are the major cause of environmental degradation in the Niger Delta area of Nigeria. The contamination of the natural environment with petroleum derived products such as polycyclic aromatic hydrocarbon (PAH) poses an extremely serious problem. Following an impact on the natural soil ecosystem with hydrocarbons from petroleum-derived product (such as spent engine oil), soil microbial hydrocarbon degrader is enhanced. The growth of these microorganisms which are hydrocarbonclastics, is primarily to degrade and metabolize the xenobiotic. These microbial hydrocarbon degraders use these pollutants as carbon source and energy, and thereby return the biological equilibrium to normalcy. Simultaneously, this upsurge in microbial count is directly associated with induction of soil enzymes and increase in their activities. However, with time, the counts of heterotrophic bacteria began to decrease as a result of depletion of the pollutant. This automatically reflects in the enzyme induction and activity. Similarly, the impact of spent engine oil on soil ecosystem was evaluated using two important enzymes, catalase and dehydrogenase. Soil contaminated with spent engine oil decreased the activity of catalase in a concentration dependent manner while the spent engine oil enhanced the activity of dehydrogenase, also in a concentration dependent manner. The incorporation of these two enzymes into a diagnostic kit is reminiscent of ELISA. The activity of catalase and dehydrogenase 35 days practically changed especially between 28 and 35 days due to possible nutrient depletion and reduced carbon source from the inoculated spent engine oil.

From this study, the positive correlation between the pH of the soil and the amount of spent engine oil added may be an implication that spent oil pollution led to a reduction in soil pH. This may be attributable to microbial metabolism of the hydrocarbon present in the oil, which consequently gave rise to the production of organic acids that resulted to the increase in the acidity of the soil ecosystem. This is supported by the report of Osuji and Nwoye [35] and Osam et al. [36]. The increase in soil acidity affects plant growth, microbial succession and metabolism. Soil pH governs the rate and extent of microbial degradation of the added petroleum hydrocarbons [27,37]. The influence of soil pH on oil decomposition was also evaluated by McGill and Nyborg [25] who found that oil decomposition was slow under acidic soil conditions. Rowell [26] reported that under acidic conditions, microbial activity was slower and fungi tend to predominate over bacteria. Bacteria are probably the most important group at least in the early stages of oil decomposition. Our results indicated that lowered pH can cause degradation of hydrocarbon. Vanloocke et al. [27] reported similarly that in the pH region 6.0-7.5, bacteria as well as *Actinomycetes* and fungi can be active. Dibble and Bartha [28] found that oil sludge biodegradation was optimal at a soil pH of 7.5 to 7.8. Moreso, not only does pH determine the growth rates and enzymatic potentials of the organisms, it also governs the type of organisms concerned in the carbon cycle [38].

The rise in temperature of the oil-impacted may be as a result of various biochemical reactions taking place in the affected soil. Akubugwo et al. [39] reported similar increase in temperature where the degradation of the mangrove of the soil ecosystem exposed to oil spillage, and the blackness of the oil attracts the intensity of sun light thereby contributing to a rise in temperature.

The resulted increase in moisture in this study may be attributed to the formation of oil scum on the soil surface. The oil film prevents aeration and water infiltration into the subsoil layers. Lack

of water and oxygen could be detrimental to life in the soil as it creates oxygen tension and hinders gaseous diffusion which probably aided the persistence of oil and moisture on the surface. The result of the physicochemical properties of the affected soil is in consonance with what Dibble and Bartha [28,36,40] reported for oil spills on soils in Niger Delta. They reported low permeability value for hydrocarbon-contaminated soils than in uncontaminated areas.

Following an oil spill, the microbial population in the soil passed through a short period of adaptation or lag phase from 1.22×10^9 to 3.55×10^8 CFU on day-zero. The lag phase encountered in this study upon the application of spent oil may be due to the presence of low molecular weight aromatic hydrocarbons. Walker et al. [41] and Rowell [26] reported similarly the microbial lag phase following the introduction of hydrocarbon from oil and 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 populations to grow and synthesize the enzymes required for oil decomposition. Thus, a decrease in the bacterial population in the soil sample contamination with spent engine oil supports the report that spent engine oil is prejudicial to soil ecosystem. This development may be attributed to the fact that spent engine oil elicited its acute toxicity effects on some strains of microorganism. Following the damage on the soil community, some bacterial strains which could not withstand this toxicity were eliminated; some became extinct; while the hydrocarbonclastic strains survived it. However, at increased concentrations, there was increase in bacterial population from 3.68×10^8 to 6.55×10^8cfu. The implication of this increase may be due to the ability of these microorganisms to adapt to the unfavourable conditions and multiplied along with the hydrocarbonclastic strains, thereby increasing the biomass. 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 on microbial diversity.

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 and Bacillus subtilis* differs in their abilities to breakdown and utilized the spent 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 and then Bacillus subtilis.* The degrading bacteria used these pollutants as a new carbon and energy source. The result of this study is similar to that reported by Margesin et al. [16] about degrading and heterotrophic bacterial population changes, which indicated that the count of degrading bacteria increased with addition of oil substances. However, results indicated that with time, the counts of degrading bacteria are approximately equal to heterotrophics which commonly can be due to compatibility of heterotrophic organisms with soil condition. Moreso, it is not surprising that *Pseudomonas aeruginosa* exhibited the highest growth. Since it was isolated frequently from soil-contaminated-spent engine oil and also because it was known to possess a more competent and active hydrocarbon degrading enzymes than other biodegraders as reported by Walker et al. [42] and Onwurah [43]. Ijah and Okonga [44] also came to a similar view that *Pseudomonas aeruginosa* is known to be fast growing and capable of degrading a wide variety of organic compounds.

In our findings, the biodegradation process which was monitored by the weight loss of spent engine oil introduced into the soil revealed that *Pseudomonas* within the first 7 days of incubation caused an average weight loss of 0.6 g spent oil per day, and this decreased to 0.5 g day^{-1} by the 14^{th} day. This further decreased to an average of 0.4 g by the 28^{th} day. Unlike *Pseudomonas aeruginosa*, the average weight loss of spent oil per day for *Micrococcus varians* increased from 0.4 to 0.5 g day^{-1} within the first 7 days of incubation, and thereafter fluctuated between 0.3 to 0.5 g. This same trend was also found in *Bacillus subtilis* which had the lowest rate of biodegradation. This result is replete with the work of Ekpo and Umoh [45]. It has also been reported that *Pseudomonas* species, because of their ability to degrade a wide range of pollutants, exhibited an increased rate of removal of the pollutant, trichloroethylene (TCE) from ground water [46]. In this study, spent engine oil did not only reduce the total aerobic bacterial count of the fresh soil sample in a concentration and time dependent manner; soil catalase suffered a similar fate. Thus, there was a progressive decline in the enzyme activity as the concentration and contact time of the contaminant increased.

5. CONCLUSION

In conclusion, spent engine oil which permeates the soil has adverse influence on the soil environment; causing a possible upset in the biochemical equilibrium of the soil measured as dehydrogenase and catalase activities. The study demonstrated that the actual effect of spent engine oil on the biochemical activities of the soil depended largely on the degree of contamination and duration of contact with this petroleum derivative. The concentration of the hydrocarbonclastic bacteria in the spent oil-contaminated soil correlated with the degree of hydrocarbon input, contact time, enzyme induction and its activity. The result indicated that soil enzyme activities are biomarkers and attractive indicators of soil ecosystem polluted with spent engine oil. They are the most appropriate measure of soil biological activities, and the basis for soil metabolism; as they decide the speed and direction of metabolic transformations that occur in the soil. The activities of soil enzymes provide an integrative measure of soil health and their values could be used as simple toxicity test in spent oil polluted environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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