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Comparative Assessment of the Activities of Acid and Alkaline Phosphatases in Spent Crankcase Oil-Polluted Soil Ecosystem

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Abstract

Assessment of the level of contamination from possible impact of spent crankcase oil on soil ecosystem is imperative for the determination of environmental acceptability. This study investigated this impact *ex-situ* using a culture-dependent approach to evaluate the total microbial counts; biochemical and physicochemical tools to determine the activities of their exudates (acid and alkaline phosphatases), total petroleum hydrocarbon (TPH) and pH as indices. The experiment demonstrated that at 1.5 – 3.5% contamination across days-zero to -28, spent crankcase oil stimulated significantly ($p<0.05$) the activity of alkaline phosphatase in a concentration and time dependent manner from 4.0 ± 0.03 to 8.0 ± 0.00Katal. Acid phosphatase suffered inhibition significantly ($p<0.05$) from 6.0 ± 0.05 to 2.8 ± 0.01Katal. The contamination significantly ($p<0.05$) increased the total petroleum hydrocarbon (TPH) across all the days relative to control and this lowered the pH from 5.9 ± 0.00 to 4.8 ± 0.00. An initial reduction in microbial biomass from 1.32 x 10^9 ± 0.00 to 3.48 x10^8 ± 0.00 cfu/g on week one, and induction of hydrocarbon-degrading organisms, (the hydrocarbonclastics), to 3.7 x 10^8 ± 0.10cfu/g at 1.5 – 3.5% contamination overtime correlated with enzyme induction, and activity. Ecosystem dynamics and mineralization were impaired and disrupted and the entire soil biochemistry was altered.

Keywords: Spent crankcase oil, acid phosphatase, alkaline phosphatase, microorganisms, pH, TPH.

1.0: INTRODUCTION

Environmental pollution as a consequence of impact from spent crankcase oil, crude oil, petroleum-derived products, as well as polycyclic aromatic hydrocarbons (PAHs) and volatile aromatics (benzene, toluene, ethylbenzene, xylene) collectively indicated as BTEX causes soil ecosystem toxicity and degradation. Ecosystem being a community of living organisms in conjunction with non-living components of the environment that are interactive is adversely affected from this impact. These products deteriorate soil, air, and water ecosystems (Wang and Bartha, 1990; Sztompka, 1999). The soil is very essential for human existence for various reasons; a prime factor in agricultural productivity and socio-economic activities; therefore, any threat or substantial impairment usually affects the people's livelihood and galvanizes into public outcry. However, several abuses from anthropogenic means has made the soil to be the first line of recipient of oil pollution such as petroleum (crude oil) and petroleum-by-products (spent crankcase oil), dumping of wastes and other contaminating substances (Osam, 2011; Nwaugo *et al*., 2006).

Spent crankcase oil, which is also known as used mineral-based crankcase oil, is a brown-to-black liquid produced when new mineral-based crankcase oil is subjected to high temperature and high mechanical strain (ATSDR, 1997). It is a mixture of several different chemicals such as low and high molecular weight (C_{15} – C_{20}) aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, decomposition products, heavy metal contaminants such as aluminum, chromium, tin, lead, manganese, nickel and silicon that come from engine parts as they tear and wear down (ATSDR, 1997). Spent crankcase oil being a common and toxic environmental contaminant; though biodegradable, is not found in natural environment (Dominguez-Rosado and Pichtel, 2004). It is introduced into the environment from the exhaust system during engine use, engine leakage or when the engine oil is changed and disposed into gutters, water drains, farmlands; a common practice of motor and generator mechanics (Anoliefo and Edegbai, 2000; Osubor and Anoliefo, 2003).

Used oil is recognized as a carcinogenic risk to man. The main carcinogenic substances in used oil are polycyclic aromatic hydrocarbons (PAHs) with 3–7 rings, such as benzopyrene, benzantracene, and chrystene (Randeles *et al*., 1991).

One way of demonstrating whether an area is contaminated or polluted with crude or spent crankcase oil is by estimating the total hydrocarbon content of the impacted soil. Records of hydrocarbon content occasionally taken enhance our ability to ascertain the extent of contamination, especially by comparing with data from pristine areas or available baseline data from regulatory bodies. Empirical records of the hydrocarbon content are therefore of great monitoring importance for effective management of an ecosystem impacted with spent crankcase oil.

These contaminants, though biodegradable are the limiting factors to soil fertility and hence crop productivity, as they create an unsatisfactory condition for life in the soil because they are toxic to soil organisms and to plants (Delille and Pelletier, 2002; Wyszkowska *et al*., 2002). They can bio-accumulate in food chains where they disrupt biochemical or physiological activities of many organisms (Onwurah *et al.*, 2007); due to poor aeration they cause on the soil, also cause nutrient immobilzation and lowering of soil pH (Atuanya, 1987; Achuba, 2008). They have the potentials to trigger carcinogenic and mutagenic activities within the soil (Krahl *et al*., 2002; Onwurah *et al*., 2007); alter the succession of microorganisms (Kaplan and Kitts, 2004), which is directly associated with the induction and activities of soil enzymes (Wyszkowska *et al*., 2002; Wyszkowska and Kucharski, 2004) and synthesis of soil adenosine triphosphate (ATP).

Soil enzymatic activities which have a central role in the soil environment are used as attractive bio-indicators for monitoring various impacts on soil (Bayer *et al*., 1982). By their activities they transform toxic petroleum products into harmless substances. They catalyze important metabolic processes including the decomposition of organic inputs and the detoxification of xenobiotics. Besides their use in the case of hydrocarbons, soil enzymatic activities have been used as biological indicators of soil ecosystem polluted with heavy metals or pesticides (Bayer *et al*., 1982). The activities of acid and alkaline phosphatases and total microbial counts are the most common indices used to describe microbial activities during oil pollution on soil ecosystem (Song and Bartha, 1990; Scwab and Banks, 1994). Their activities provide an integrative measure of the biological status of the soil (Li *et al*., 2005).

Phosphatases (acid and alkaline) catalyze the hydrolysis of ester–phosphate bonds, sodium p-nitrophenyl phosphate, leading to the release of phosphate (P), which can be taken up by plants or microorganisms (Quiquampoix and Mousain, 2005). It has been shown that the activities of phosphatases (like those of many hydrolases) depend largely on soil properties such as, soil organism interactions, plant cover, leachate inputs and the presence of inhibitors and activators (Speir and Ross, 1978). Phosphatases catalyze the hydrolysis of both esters and anhydrides of phosphoric acid, and according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NCIUBMB), they can be classified as phosphoric monoester hydrolases or phosphomonoesterases (EC 3.1.3.2), phosphoric diester hydrolases or phosphodiesterases (EC 3.1.4.17).

Impact assessment which is a systematic and repetitive collection and analysis of data that can be used to determine the quality of the environments as they are or would be was conducted to estimate the biological response to spent crankcase oil and the response was extrapolated from its effect on microbial communities. The most obvious effect of pollutants' exposure to microbial communities is direct toxicity which results to rapid death. The extent of loss in microbial activity/ biomass and alteration in their exudates (enzymes, ATP and pH) can be used to assess the toxicity of that pollutant in the environment. Thus hydrocarbons increase the abundance of hydrocarbon degrading microorganisms, but on the other hand induce a limitation in microbial diversity (Ebulue *et al*., 2017). The consequent loss in mineral elements as they are washed away and become bio-unavailable to plants could lead to a reduction in crop yield, thus the response of plants to oil pollution is unambiguously negative (Chaineau *et al*., 1997; Salanitro *et al*., 1997). The cation exchange capacity (CEC) which is the ability of the soil particles to hold cations is lost and replaced by phytotoxic elements such as aluminum and manganese ions. This effect which altered the entire soil biochemistry could disrupt ecosystem dynamics by slowing soil organic matter mineralization and associated nutrient re-mineralization (Ebulue *et al*., 2017).

1.1: Aim and Objectives

To comparatively determine the activities of soil microbial exudates, enzymes (acid and alkaline phosphatases) of the spent crankcase oil impacted soil. The objectives are:

i. To assess the impact of spent crankcase oil on soil pH.
ii. To determine the total petroleum hydrocarbon (TPH) content of the spent crankcase oil-impacted soil.
iii. To assess the total microbial biomass and hydrocarbonclastic organisms of the oil impacted soil.

1.2: Research Design

This research was designed for contamination at 1.5, 2.5 and 3.5% w/w for a-thirty-five-day investigation, Day- zero, Day- 14, Day- 28 and Day- 35; in

consideration of the volatility and biodegradability of hydrocarbons within which, the aforementioned parameters were determined.

2.0: Materials; Sample Collections

The soil sample was obtained from the premises of Federal University of Technology, Owerri, Imo State, Nigeria, with an auger inserted about fifteen centimeters into the soil; while the spent crankcase oil was obtained from a mechanic village.

2.1: Soil Preparation

Ten grams (10g) of soil sample was contaminated with spent crankcase oil at different concentrations (1.5, 2.5, and 3.5% w/w) for the determination of the aforementioned parameters.

2.2: METHODS

2.2.1: Determination of pH of spent crankcase oil-contaminated soil

The method of Ebulue (2020) was used to evaluate the pH, where Bench pH Meter 3510 was used for easy read-out of pH after soil inoculation with spent oil at different degrees of contamination.

2.2.2: Determination of total petroleum hydrocarbon (TPH)

Principle: It is based on the estimation of the total petroleum hydrocarbon (TPH) in the soil with reference to the standard curve derived from fresh unused crankcase oil diluted with toluene using the equation y = 1.094x; where y = absorbance and x = concentration.

Procedure: Total petroleum hydrocarbon content was determined gravimetrically by the method of Odu *et al*., (1989), to provide an estimate of the available total hydrocarbon with time and the liquid phase of the extract were measured spectrophotometrically at 420nm. The total petroleum hydrocarbon (TPH) in the soil was estimated with reference to the standard curve using the equation y = 1.094x; where y = absorbance and x = concentration.

2.2.3: Determination of microbial population in the spent crankcase 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.

2.2.4: Determination of acid and alkaline phosphatase activities

Principle: The method of Tabatabai and Bremner (1969) was used, which involves colorimetric estimation of the p-nitrophenol released by phosphatase activity when the oil-contaminated soil was incubated with sodium p-nitrophenyl phosphate solution and toluene in 0.1N modified universal buffer at pH 6.5 for acid phophatase, and pH 11 for alkaline phosphatase.

Protocol: The phosphatase activity determination method consisted of incubation of reactive mixture containing 10g soil samples: 1.5, 2.5 and 3.5% w/w (oil-soil mixture) with the substrate, 5ml of 0.1N sodium p-nitrophenyl phosphate. The method of Tabatabai and Bremner (1969) was used, which involves colorimetric estimation of the p-nitrophenol released by phosphatase activity when the oil-contaminated soil was incubated with sodium p-nitrophenyl phosphate solution and toluene in 0.1N modified universal buffer at pH 6.5 for acid phophatase, and pH 11 for alkaline phosphatase.

Soil inoculation was carried out by weighing 10g of sieved soil sample into four different test tubes. To the first tube, 0.1g of spent crankcase oil (corresponding to 1.0%), was added and mixed thoroughly with a steering rod. This procedure was repeated for 1.5, 2.5 and 3.5%; and into the 4th tube, the control, 20ml of distilled water was added. After one hour (1hr) incubation process at 27^{o}C, the intense yellow color due to the released p-nitrophenol was measured spectrophotometrically at 410nm with the molar extinction coefficient of 1.6 x 10^{7} M^{-1} cm^{-1} for acid phosphatase; and at 405nm with the molar extinction coefficient of 1.8 x10^{4} M^{-1} cm^{-1} for alkaline phosphatase and the activity was determined thereafter as follows using Beer-Lambert's law; A = ECL where C = A/EL. And Activity = Katal.

2.3: Statistical Analysis

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

3.0: RESULTS

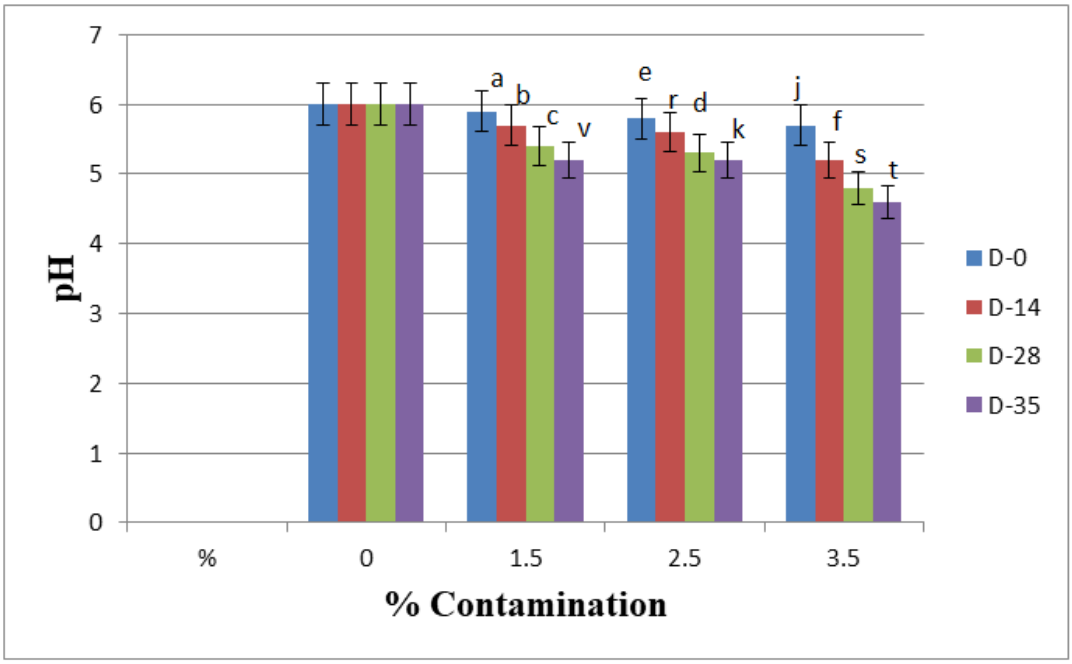

Fig 1.0: pH of the soil polluted with spent crankcase oil

Comparison between groups: Bars with different letters differ significantly ($p<0.05$).

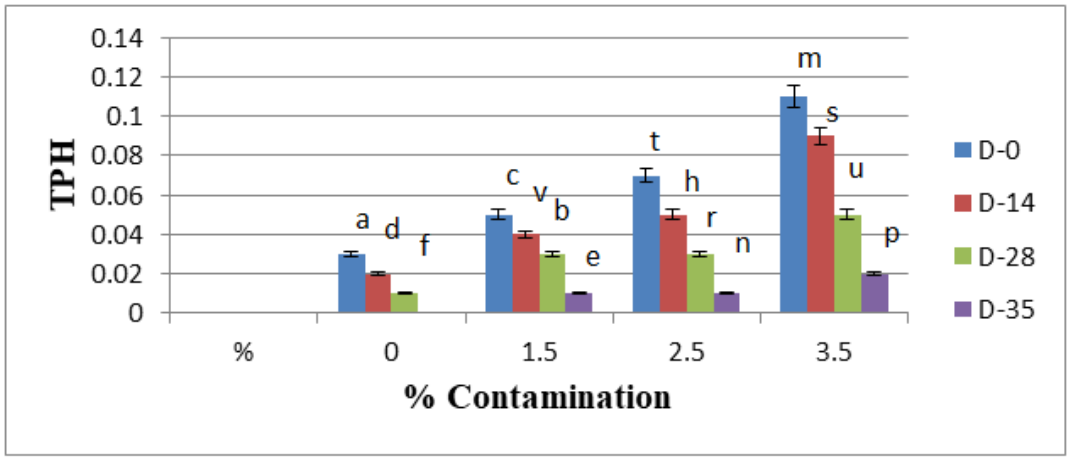

Fig 2.0: Total petroleum hydrocarbon (TPH) of the spent crankcase oil-polluted soil

Comparison between groups: Bars with different letters differ significantly ($p<0.05$).

Table 1.0: Total microbial population in the spent crankcase oil-impacted soil (x 100)

% Contamination	Week 0ne (cfu/g)	Week Two (cfu/g)	Week Four (cfu/g)	Week six (cfu/g)
Control 1.32×10^9				
1.5	3.48×10^8	3.66×10^8	4.0×10^8	3.80×10^8
2.5	3.08×10^8	3.4×10^8	4.2×10^8	4.0×10^8
3.5	2.6×10^8	3.0×10^8	3.7×10^8	3.5×10^8

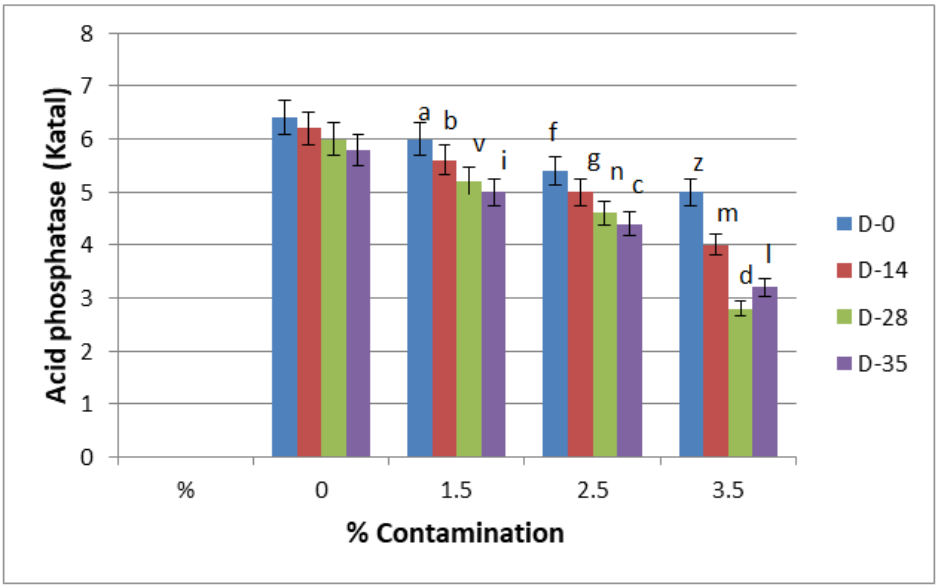

Fig 3.0: Activity of acid phospatase in the spent crankcase oil-polluted soil

Comparison between groups: Bars with different letters differ significantly ($p<0.05$).

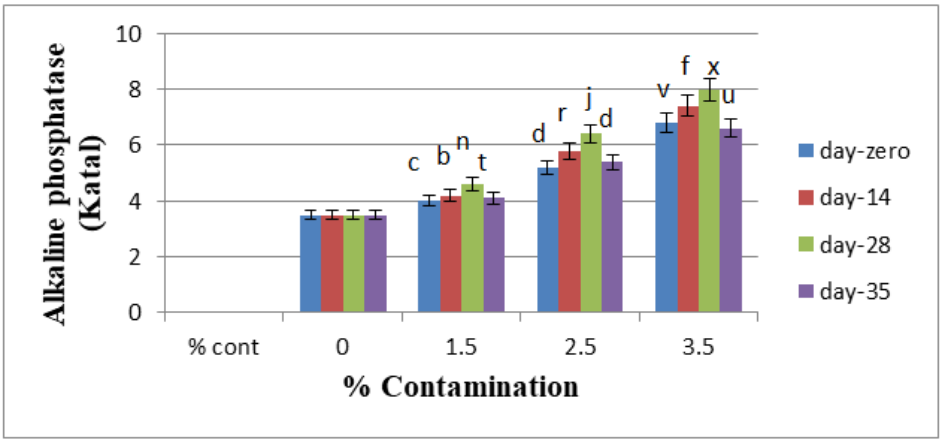

Fig 4.0: Activity of alkaline phosphatase in the spent crankcase oil-polluted soil

Comparison between groups: Bars with different letters differ significantly ($p<0.05$)

4.0: DISCUSSION

Utilization of chemical contaminants incidented on the soil by different microbial communities as sources of carbon and energy ameliorates a wide range of contaminants from oily waste such as petroleum-by-products and polyaromatic hydrocarbons. The prevailing environmental conditions are among the most important limiting factors for optimum utilization which culminates in bioremediation.

Contamination of the natural environment with petroleum-derived compounds poses an extremely serious problem. In this study, spent crankcase oil has shown to have adverse effects on the soil ecosystem. It adversely affected the physical status of the soil, the pH and alteration of soil hydrogen ion concentration. It also affected the microbial community which is the sole producer of soil enzymes. Above all, spent crankcase oil altered the entire soil biochemistry. These findings are in tandem with the previous works of Wyszkowska and Kucharski (2005), Jidere and Akamigbo (2009), Ebere *et al.*, (2011), Ebulue and Ebulue (2022) and Ebulue (2022).

Soil pH which is an important physical property refers to acidity or alkalinity, which is a measure of the concentration of hydrogen ions [H^+]. It is defined by the equation: pH = – Log [H^+]. Soil with a large [H^+] is acidic (i.e., low pH). This acidity would increase the solubility of elements which increases their mobility, lability and probability of leaching into ground water; while when the [H^+] is low, the soil is basic (i.e., high pH), and as a result, cations would be on the particle exchange sites causing lower probability of leachability (Zhang *et al.*, 2007) From this investigation, the positive correlation between the pH of the soil and the amount of spent

crankcase oil added may be an implication that spent oil pollution led to a reduction in soil pH.

Statistically, comparison between groups showed that spent crankcase oil caused a significant reduction in soil pH ($p< 0.05$) on days-14 and -28. The reduction in pH was in a concentration and time dependent manner from 5.9 ± 0.0 to 4.8± 0.0 which cuts across days-zero to -28 at concentrations 1.0 – 3.5%. The lowered pH reflected accelerated metabolism and accelerated demand for electron acceptors thus creating a reducing environment. This could be attributable to microbial metabolism of the hydrocarbon present in the spent crankcase contaminated soil, which consequently gave rise to the production of organic acids that resulted to the increase in the acidity of the affected ecosystem. This is replete with the report of Osuji and Nwoye (2007), Osam *et al.*, (2013). This increase in acidity would likely affect plant growth, microbial succession and metabolism and leachability of metals.

Soil microorganisms are very important to plants for many reasons; one of which is that, plants need them to obtain nutrients from the soil. This is because, for a plant to obtain nutrients from the soil, the nutrient must be soluble and in the immediate environment of the root (the rhizosphere). Following an oil spill, the microbial population in the soil passed through a short period of adaptation or lag phase and a limitation in microbial diversity from $1.32 \times 10^9 \pm 0.00$ to $3.48 \times 10^8 \pm 0.01$ 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 crankcase 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 $3.48 \times 10^8 \pm 0.01$to $3.7 \times 10^8 \pm 0.00$ cfu/g which cuts across weeks -one to -four at 1.0 – 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 crankcase 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 re-mineralization (Ebulue *et al.*, 2017).

In this study, spent crankcase oil did not only reduce the total aerobic bacterial count of the fresh soil sample in a concentration and time dependent manner; (Ebulue *et al.*, (2017), acid phosphatase activity suffered the same fate. This may not be surprising because the activity of the enzyme in the spent crankcase oil-polluted soil was gradually inhibited from 6.0 ± 0.05 to 2.8 ± 0.01Katal which was significant ($p < 0.05$) that cuts across days-zero to -28 at concentrations 1.0 – 3.5%. Thus, there was a progressive decline in the enzyme activity as the concentration and contact time of the contaminant increased. This alteration in the activities of the aforementioned enzyme 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 site of acid phosphatase are irritable to hypoxic and pH increases, 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), Ebulue (2022), Achuba and Peretiemo-Clarke (2008) on the inhibition of the activity of soil catalase, lipase and acid phosphatase following an insult of soil ecosystem with hydrocarbon.

On the other hand, our investigation revealed that spent crankcase oil increased the activity of alkaline phosphatase significantly ($p < 0.05$) in a concentration and time dependent manner, from 4.0 ± 0.03 to 8.00 ± 0.00Katal which cuts across days-zero to -28 at concentrations 1.0 – 3.5%. This stimulatory effect of the spent crankcase oil on the activity of the aforesaid enzyme which was stronger as the rate of contamination and duration of contact increased was not an indication of a fertile soil. This finding is in harmony with the work of Achuba and Peretiemo-Clarke (2008) on the activity of enzyme in spent oil pollution.

5.0: CONCLUSION

From this research it has become evident that spent crankcase oil adversely affects soil ecosystem. The increasing acidity from this impact is prejudicial to our farm lands. Awareness is therefore needed in disposing the used oil.

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