

Variations in Phosphatase Activity of Crude Oil and Used Crankcase Oil Polluted Agricultural Soil.

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Abstract

A study of the variations in phosphatase activity of crude oil and used crankcase oil polluted agricultural soil was carried out. Eight square metre farmland was cleared and divided into seven lots (A, B₁, B₂, B₃, C₁, C₂, C₃) of 60 cm² each with 30 cm² spaces in between them. Six out of the seven lots were polluted with three different concentrations of bonny light crude oil and used crankcase oil. The results showed a decrease in both alkaline and acid phosphatase activities mainly at the topsoil, with lowest alkaline phosphatase values of 0.6440±0.0026 I.U.L⁻¹ and 0.6440±0.0051 I.U.L⁻¹ for weeks 7 and 8 of 6 dm³ bonny light crude oil and used crankcase oil respectively. Lowest acid phosphatase activities was observed in week 5 of 6 dm³ bonny light crude oil and used crankcase oil with values of 0.0370±0.0039 I.U.L⁻¹ and 0.0305±0.0037 I.U.L⁻¹ respectively.

Key words: Hydrocarbons, Pollution, Soil, Enzyme, Phosphatase.

INTRODUCTION

Petroleum like all fossil fuels primarily consists of complex mixture of molecules called hydrocarbons. Petroleum hydrocarbons are ultimately composed of various portions of alkanes (e.g. methane and ethane), aromatics (e.g. benzene, toluene, ethylene and xylene, collectively known as BTEX), polycyclic aromatic hydrocarbons (PAHs), (e.g. naphthalene, phenanthrene, anthracene and benzo(α)pyrene e.t.c.). In large concentrations, the hydrocarbon molecules that make up crude oil and petroleum products are highly toxic to many organisms, including humans (Alexander, 1994). Industrial activities release substantial amounts of crude oil and its refined products into the environment, as a result of accidents such as storage tank leakage or oil spills during routine transportation and shipping operations (Gonza'Lez *et al.*, 2008). The contaminant load of soil and water is growing steadily each year in parallel with increasing industrialization and energy demand (Wang *et al.*, 1999).

Spilt petroleum derivatives remain persistently in soil and cause significant deterioration of its physical properties (Luthy *et al.*, 1997). Microbial communities exposed to hydrocarbons will become adapted, exhibiting selective enrichment and genetic changes resulting in increased proportions of hydrocarbon-degrading bacteria and bacterial plasmids encoding hydrocarbon catabolic genes (Leahy and Colwell, 1990). This will subsequently lead to increase in production of soil microbial enzymes, which can be used as markers of petroleum hydrocarbon pollution.

Soil enzymatic activity is the driving force behind all biochemical transformations in soil (Gonza'Lez *et al.*, 2008). The activity of soil enzymes provides an integrative measure of the biological status of the soil (Li *et al.*, 2005). The unique regio- and stereospecific properties of microbial enzymes combined with their ability to catalyze reactions in non aqueous media opens up opportunities to exploit enzyme technology in pollution monitoring. Phosphatases catalyze the hydrolysis of organic phosphate esters to ortho-phosphate and thus constitute an important link between biologically unavailable and mineral phosphate pools in the soil (Speir and Ross, 1976). Phosphatases are produced by microorganisms, plant roots and earthworms (Neal, 1973 and Skujins, 1976) and thus are ubiquitous in the soil.

Soil phosphatase activities are sensitive to a number of environmental perturbations such as organic amendments (Spier and McGill, 1979; Bonmati *et al.*, 1985), water logging (Pulford and Tabatabai, 1988), compaction (Dick *et al.*, 1988), fertilizer addition (Spier and McGill, 1979; Pang and Kolenko, 1986), tillage (Doran, 1980), heavy metal inputs (Juma and Tabatabai, 1977; Mathur and Rayment, 1978), and pesticides (Schaffer, 1993). For soil phosphatase activity to be used as an effective indicator of soil quality, the temporal and spatial variability of its activity and the factors controlling the variability must be ascertain. Harrison (1979), observed a seasonal

variation in acid phosphatase activity in forest soil, with 19% of the total annual variation attributed to seasonal effects. This variation is likely to be driven at least in part by the spatial distribution of relatively static soil properties known to influence soil phosphate activity such as soil organic matter, available phosphate content, pH, clay content and that of microorganisms, plant roots and soil fauna, which produces the enzyme.

The location of bacterial alkaline phosphatase in the periplasmic space (which is external to the cell membrane and subject environmental variation than the inner cell), comparatively increases its activity and resistant to inactivation and degradation (Garen and Levinthal, 1960). Although the function of bacterial alkaline phosphatase is still not fully understood, it may serve as a means for the bacteria to generate free phosphate group for uptake and use (Harada *et al.*, 1986). This simple hypothesis is supported by the fact that alkaline phosphatase is usually only produced by the bacteria during phosphate starvation (Harada *et al.*, 1986). However, other possibilities may exist; for instance, the presence of phosphate group usually prevents organic molecules from passing through the membrane, therefore dephosphorylating them may be important for bacterial uptake of organic compound in the wild (Harada *et al.*, 1986). Some complexities of bacterial regulation and metabolism suggest that other more subtle purposes for the enzyme may also play a role for the cell (Garen and Levinthal, 1960). Juma and Tabatabai (1978) reported the dominance of acid phosphatase (also located periplasmic space in bacteria) in acidic soil. However, the sensitivity of phosphatase activity to temporal changes in soil caused by natural and synthetic factors can reflect changes in the respiratory activity of the microbial population in the soil in response to changes in the soil environment. thereby making the enzyme activity a potential indicator of soil quality. The aim of this research is to assess the quality of bonny light crude oil and used crankcase oil polluted soil by monitoring the changes in soil phosphatase activity at different depths.

Materials and Methods

Source of materials

Bonny- light crude oil was obtained from Shell Petroleum Development Company (SPDC) flow station at Egbema, Imo State, Nigeria, while used crankcase oil was obtained from a mechanic workshop at Nekede Mechanic village, Owerri, Imo State, Nigeria. Alkaline and acid phosphatase reagent kits obtained from Randox Laboratories Ltd Antrim, United Kingdom were used for this analyses.

Study area description

The study area for the experiment is located at Federal University of Technology, Owerri, Imo State, Nigeria. The soil belongs to the ferallitic soil. The soil profile is remarkably uniform, approximately the entire area consists of deep uniform sand and loam sand, with a pH of 4.0. There was no record of hydrocarbon pollution on the area.

Experimental design

Eight square metre farmland was cleared and divided into seven lots (A, B₁, B₂, B₃, C₁, C₂, C₃) of 60 cm² each with 30 cm² spaces in between them. These lots were polluted and labelled as follows: Site A (control) is an unpolluted 60 cm² farmland, while sites B₁, B₂ and B₃ are adjacent 60cm² farmlands polluted with 2dm³, 4dm³, 6dm³ of bonny light crude oil respectively. Sites C₁, C₂ and C₃ are adjacent 60cm² farmlands contaminated with 2dm³, 4dm³, 6dm³, of used crankcase oil respectively.

Collection of soil samples

Soil samples were collected by the method of Bashour and Sayegh (2007). Using a sterilized soil auger, samples were collected at 0-5, 15-20 and 25-30 cm depths of sites A, B and C weekly for 8 weeks. The samples were collected in sterilized plastic bags sealed with rubber bands. All samples were labelled with permanent water resistant marker and were taken to the laboratory within one hour of collection for analyses.

Sample preparation.

Soil samples were grounded gently in a mortar for 5 mins and 20.0 mls of distilled water was added to 10.0 grams of the ground sample to release the soil enzymes in a liquid medium. The solution was transferred into a glass separating funnel and allowed to stand for 30 mins at room temperature. A quantity of 3 mls each of the supernatant was used as crude enzyme solution.

Determination of alkaline phosphatase activity.

Alkaline phosphatase activity was determined by the method of King and King (1954) as modified by Walton *et al.*, (1998).

Principle: Alkaline phosphatase (ALP) at pH of 9.8 catalyses the degradation its substrate (phenyl phosphate) to phenol, phosphate and water. The phenol condenses with aminoantipyrine and then oxidized with alkaline ferricyanide to give a red complex, whose intensity at 520nm is proportional to the alkaline phosphatase activity.

Phenyl phosphate $\xrightarrow{\text{Alkaline phosphatase}}$ phenol + phosphate + water.

Procedure:

Three different test tubes were labelled as follows: T (test), S (standard) and B (blank). One millilitre of phosphate buffer was mixed with 1.0 ml of phenyl phosphate, while 1.0 ml of phosphate buffer and 1.0 ml of distilled water were added to the standard and the blank test tubes. The mixtures were allowed to incubate for 5minutes at 37⁰C. Then 0.1 ml of soil enzyme solution was added to the test, while 0.1 ml of distilled water was added to both the standard and the blank. The mixtures were also allowed to incubate for 5minutes at 37⁰C. Then 1.0 ml of alkaline phosphatase and 1.0 ml of distilled water was added to the standard and the blank test tubes respectively. The mixtures were incubated again at 37⁰C for 15 minutes and 0.8 ml of 0.5N NaOH was added to the tubes and the mixture was allowed to cool. Then 1.2 ml of NaHCO₃, 1.0 ml of 4-aminoantipyrine and 1.0 ml of potassium ferricyanide were added to each of the test tubes. The absorbance of the test and standard mixtures were read against the reagent blank after 5 minutes using a 1cm light path cuvette in a Metertech spectronic 20^{D+} digital spectrophotometer.

Calculation:

$$\text{ALP activity. (I.U.L}^{-1}\text{)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc. of standard.}$$

Determination of acid phosphatase activity.

Alkaline phosphatase activity was determined by the method of King and King (1954) as modified by Gibb *et al.*, (1998).

Principle: Acid phosphatase (ALP) at pH of 4.85 catalyses the degradation its substrate (phenyl phosphate) to phenol, phosphate and water. The phenol condenses with aminoantipyrine and then oxidized with alkaline ferricyanide to give a red complex, whose intensity at 520nm is proportional to the acid phosphatase activity.

Phenyl phosphate $\xrightarrow{\text{Acid phosphatase}}$ phenol + phosphate + water.

Procedure:

Three different test tubes were labelled as follows: T (test), S (standard) and B (blank). One millilitre of citrate buffer was mixed with 1.0 ml of phenyl phosphate, while 1.0 ml of citrate buffer and 1.0 ml of distilled water were added to the standard and the blank test tubes. The mixtures were allowed to incubate for 5minutes at 37⁰C. Then 0.1 ml of soil enzyme solution was added to the test, while 0.1 ml of distilled water was added to both the standard and the blank. The mixtures were also allowed to incubate for 5minutes at 37⁰C. Then 1.0 ml of acid phosphatase and 1.0 ml of distilled water was added to the standard and the blank test tubes respectively. The mixtures were incubated again at 37⁰C for 15 minutes and 0.8 ml of 0.5N NaOH was added to the tubes and the mixture was allowed to cool. Then 1.2 ml of NaHCO₃, 1.0 ml of 4-aminoantipyrine and 1.0 ml of potassium ferricyanide were added to each of the test tubes. The absorbance of the test and standard mixtures were read against the reagent blank after 5 minutes using a 1cm light path cuvette in a Metertech spectronic 20^{D+} digital spectrophotometer.

Calculation:

$$\text{Acid phosphatase activity. (I.U.L}^{-1}\text{)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc. of standard.}$$

Results and Discussion

A gross decrease in phosphatase activity mainly at the topsoil was observed in this study. The decrease in phosphatase activity can be attributed to the inability of plants (mostly herbs) to survive under crude oil and

spent engine oil polluted environment may have a reducing effect on the phosphatases produced by plants roots thereby decreasing the phosphatase activity as observed at the topsoil (Figs. 1 and 2). This study agrees with that of Baker, (1982) and Udo and Fayemi (1975), which suggested reasons on how diesel spills on agricultural land generally reduce plant growth. Nwaogu *et al.*, (2008) suggested that the reduced plant growth might range from a direct toxic effect on plants to reduced germination of seeds. Moreover, earthworms cannot thrive in crude oil and engine oil polluted environment and this may contribute to the decrease in both alkaline and acid phosphatase activities as observed in this research.

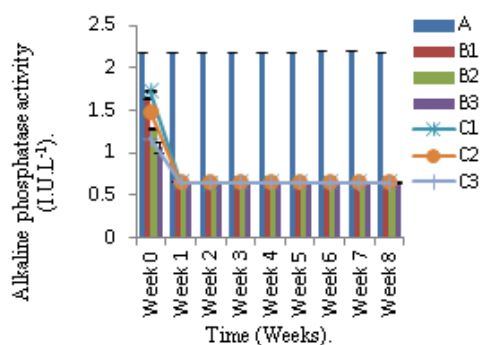


Fig. 1. Effect of crude oil and used crankcase oil on soil alkaline phosphatase at 0-5 cm depth.

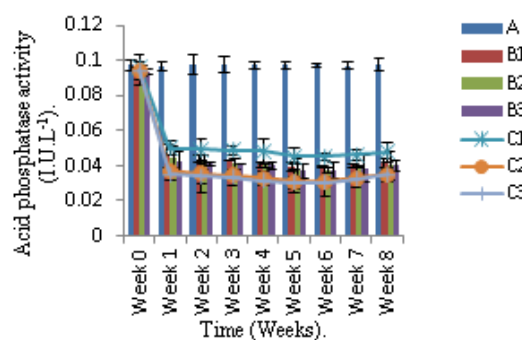


Fig. 2. Effect of crude oil and used crankcase oil on soil acid phosphatase at 0-5 cm depth.

The slight increase observed at 15cm and 30cm depths (Figs. 3 and 5 for alkaline phosphatase and Figs. 4 and 6 for acid phosphatase) are statistically insignificant at $p \leq 0.05$. This may be due to the inability of the petroleum products to penetrate to these depths within the first two weeks of treatment. Phosphatases are produced by plant roots, earthworms and microorganisms (Ramirez-Martinez, 1968; Neal, 1973 and Skujins, 1976). Plant roots may constitute an important source of phosphatases (Martin, 1973) particularly acid phosphatase (Juma and Tabatabai, 1988) in soil, such that differences in spatial distribution of plant communities may account for some of the differences in phosphatase activity (Amado *et al.*, 1997). The root zone for most herbs species are quit shallow (Golet *et al.*, 1993) making this layer a potentially important contributor of root-derived phosphatases to topsoil (Amado *et al.*, 1997).

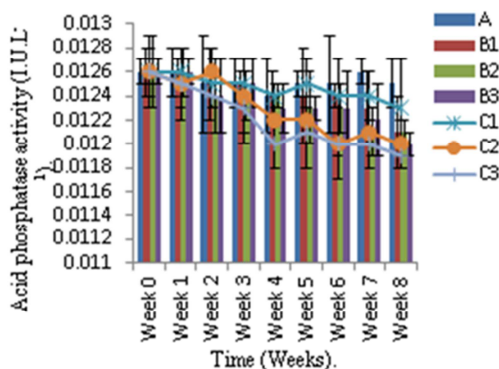


Fig. 3. Effect of crude oil and used crankcase oil on soil alkaline phosphatase at 15-20 cm depth.

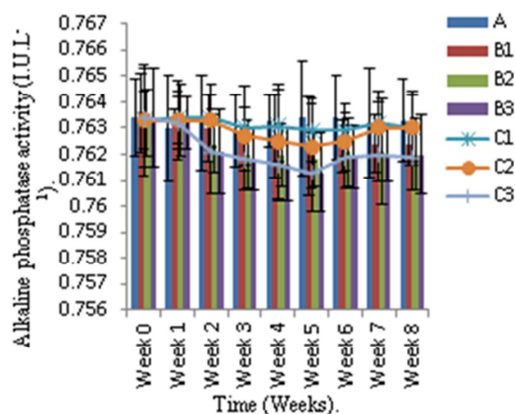


Fig. 4. Effect of crude oil and used crankcase oil on soil acid phosphatase at 15-20 cm depth.

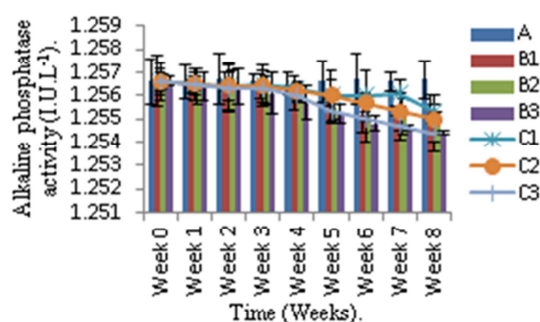


Fig. 5. Effect of crude oil and used crankcase oil on soil alkaline phosphatase at 25-30 cm depth.

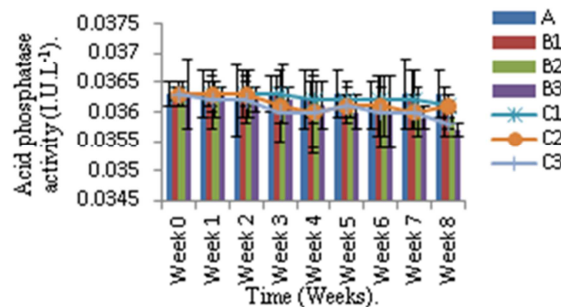


Fig. 6. Effect of crude oil and used crankcase oil on soil acid phosphatase at 25-30 cm depth.

- Key:
- A = Control (an unpolluted 60 cm² farmland)
 - B₁ = 60cm² farmlands polluted with 2dm³ of bonny light crude oil.
 - B₂ = 60cm² farmlands polluted with 4dm³ of bonny light crude oil.
 - B₃ = 60cm² farmlands polluted with 6dm³ of bonny light crude oil.
 - C₁ = 60cm² farmlands contaminated with 2dm³ of used crankcase oil.
 - C₂ = 60cm² farmlands contaminated with 4dm³ of used crankcase oil.
 - C₃ = 60cm² farmlands contaminated with 6dm³ of used crankcase oil.

Akoachere *et al.*, (2008) reported a low heterotrophic bacterial and fungal counts in used crankcase oil contaminated soils. This may have led to a decrease in microbial phosphatase activity, which also contributes to the significant decrease in soil phosphatase activity observed in this study (Figs 1- 6). Odu, (1972) and Amund *et al.*, (1993), showed that soils contaminated with petroleum products have large increase in phosphate content. This study also agrees with the work of Amador and Gorres (2007), which reported that phosphatase production is inhibited by the presence of elevated levels of phosphate in the soil. This may have contributed to the decrease in both acid and alkaline phosphatase activities observed in this research.

Conclusion.

The variations in phosphatase activities as observed in this study may be a useful indicator in monitoring the quantity of crude oil pollution so as to ascertain the soil quality and the activities of soil organism in a crude oil polluted agricultural soil.

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