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Effect of spent engine oil on soil catalase and dehydrogenase activities

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A b s t r a c t. The effect of spent engine oil on soil pH as well as activity of selected enzymes (catalase and dehydrogenase) was studied. The results indicate that spent engine oil caused a slight change in soil pH relative to the control. There was a significant decrease ($p<0.05$) in catalase activity in contrast to a significant increase ($p<0.05$) observed in dehydrogenase activity. On the whole the data suggest that spent engine oil alters soil biochemistry.

K e y w o r d s: catalase, dehydrogenase, soil, engine oil

INTRODUCTION

Spent engine 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). Spent engine oil is a mixture of several different chemicals (Wang *et al*., 2000), including low and high molecular weight (C_{15}-C_{20}) aliphatic hydrocarbons, aromatic hydrocarbons, polychlorinated biphenyls, chlorodibenzofurans, lubricative additives, decomposition products, heavy metal contaminants such as aluminium, chromium, tin, lead, manganese, nickel, and silicon that come from engine parts as they wear down (ATSDR, 1997).

Spent engine oil is a common and toxic environmental contaminant not naturally found in the environment (Dominguez-Rosado and Pichtel, 2004). Large amounts of spent engine oil are liberated into the environment when the motor oil is changed and disposed into gutters, water drains, open vacant plots and farmlands, a common practice by motor mechanics 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 due to engine leaks (Anoliefo and Edegbai, 2000; Osubor and Anoliefo, 2003).

Spent engine oil, when present in the soil, creates an unsatisfactory condition for life in the soil, which is due to the poor aeration it causes in the soil, immobilization of soil nutrients, and lowering of soil pH (Atuanya, 1987). Soil enzymes are biologically produced substances which bind with substrate in streoscopic position that causes changes in the electronic configuration around certain susceptible bonds that culminates in biochemical reactions (Zahir *et al*., 2001). The activity of soil enzymes provides an integrative measure of the biological status of the soil (Li *et al*., 2005).

Dehydrogenases (EC 1.1.1.1) are enzymes which catalyse the removal of hydrogen atom from different metabolites (Nelson and Cox, 2000). Active dehydrogenases are considered to exist in the soil as an integral part of intact cells. They conduct a board range of oxidative activities that are responsible for degradation of soil organic matter (Margesin *et al*., 2000). Soil dehydrogenase activity can reflect changes in the respiratory activity of a given population size in response to changes in the soil environment (Schinner *et al.*, 1996).

Catalase (EC 1.11.1.6) is an iron porphyrin enzyme which catalyses very rapid decomposition of hydrogen peroxide to water and oxygen (Nelson and Cox, 2000). The enzyme is widely present in nature, which accounts for its diverse activities in soil. Catalase activity alongside with the dehydrogenase activity is used to give information on the microbial activities in soil. Both catalase and dehydrogenase activity are very sensitive to heavy metal pollution (Naplekova and Bulavko, 1983; Perez and Gonzalez, 1987; Wilke, 1991). Their values have been suggested to be used as a simple toxicity test (Roger and Li, 1985).

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The objective of this study is to investigate the activity of catalaes and dehydrogeneses in the soil polluted with mineral-based crankcase oil.

MATERIALS AND METHODS

Experimental soil

The sandy soil (sand – 85%, clay – 6%, silt – 8% organic matter – 0.4.% pH 7, and porosity – 37.3%) was collected from a fallow land in Campus ll of Delta State University, Abraka, Nigeria. Soil inoculation was carried out by weighing 1.6 kg of sieved soil sample into six different planting bags. To the first bag of 4 ml of spent engine oil, corresponding to 0.25%, was added and mixed thoroughly by hand. This procedure was repeated for 0.5, 1, 1.5, and 2%. The sixth bag served as control. The pH of the different treated soil samples was determined in distilled water (soil to liquid mixture = 1:3). The mixture was stirred for 5 min and allowed to stand for one hour before measurement using a pH meter with electrode (Jenway 3020).

Preparation of extract for enzyme determination

One hundred ml of phospate 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 supernatant (S1).

Determination of catalase activity

Catalase activity was determined by the method of Cohen *et al*. (1970) where decomposed hydrogen peroxide is measured by reacting it with excess of potassium tetraoxomanganate(VII), $KMnO_4$ and residual $KMnO_4$ is measured spectrophotometrically at 480 nm.

One tenth ml 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 6 N tetraoxosulphate(VI) acid, H_2SO_4 to each of the labelled test tubes containing different concentrations of spent engine oil ranging from 0.25 to 2% and to the blank sample. Also, 7 ml of 0.1 N $KMnO_4$ was added within 30 s and thoroughly mixed.

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

Determination of soil dehydrogenase activity

Dehydrogenase activity was determined using the method described by Tabatabai (1982). Dehydrogenases convert 2,3,5-triphenyl tetrazolium chloride to formazan. The asbsorbance of formazan was read spectrophotometrically at 485 nm. 1 g of sieved soil was placed in test tubes (15 x 100 mm), mixed with 1 ml of 3% aqueous (w/v) 2,3,5-triphenyl tetrazolium chloride and stirred with a glass rod. After 96 h of incubation (27°C) 10 ml of ethanol was added to each test tube and the suspension was vortexed for 30 s. The tubes were then incubated for 1 h to allow suspended soil to settle. The resulting supernatant (5 ml) was carefully transferred to clean test tubes using Pasteur pipettes. Absorbance was read spectrophotometrically at 485 nm. The concentration of formazan was evaluated using extinction coefficient of 15433 Mol cm^{-1} (Dushoff, 1965).

Statistical analysis

The results were expressed as mean ± SEM. All results were compared with respect to the control. Comparisons between the concentrations and control were made by using analysis of variance (ANOVA) and differences at $p<0.05$ were considered as significant.

RESULTS

The effect of spent engine oil on the pH of the soil is shown in Table 1. There was a slight decrease. However, there was no significant difference in pH in any of the treated samples relative to the control.

T a b l e 1. Effect of spent engine oil on soil pH, soil catalase and dehydrogenase activities*

Oil concentration (%)	pH	Catalase activity (K min^{-1})	Dehydrogenase activity (mg g^{-1} dry soil/96 h)
0.00	7.0	8.67 ± 0.02^{b}	4.28 ± 1.73^{b}
0.25	6.9	8.04 ± 0.11^{b}	4.40 ± 0.87^{b}
0.50	6.7	6.75 ± 0.04^{a}	4.67 ± 0.43^{b}
1.00	6.4	4.79 ± 0.06^{a}	7.38 ± 1.22^{a}
1.50	6.2	3.56 ± 0.06^{a}	7.73 ± 1.665^{a}
2.00	6.0	1.57 ± 0.03^{a}	8.68 ± 0.0^{a}

*Results are expressed as mean ± SEM of five determinations, means superscript with different letter differ significantly ($p<0.05$).

Also in Table 1 is the effect of spent engine oil on soil catalase activity presented. There was progressive decrease as the concentration of the spent engine oil increased. There is a significant ($p<0.05$) difference in the activity of the enzyme in soil contaminated with 1-5% of spent engine oil relative to the control.

The effect of spent engine oil on soil dehydrogenase activity is shown in Table 1. There was a progressive increase in the values obtained as the concentrations of the spent engine oil increased and this was found to be significant ($p<0.05$) between (1-2%) oil in soil contamination relative to the control value.

DISCUSSION

Various contaminants such as spent engine oil and heavy metals have been found to alter soil biochemistry, which includes alteration in soil microbial properties, pH, oxygen and nutrient availability (Atuanya, 1987; Brookes, 1995; Odjegba and Sadiq, 2002; Osubor and Anoliefo, 2003). In the present investigation, there was as slight reduction in soil pH which was insignificant compared to the control. Similar observations were made in earlier reports (Atuanya, 1987; Osuji and Nwoye, 2007). Petroleum hydrocarbon mediated decrease in soil pH has been attributed to the production of organic acids by microbial metabolism (Osuji and Nwoye, 2007).

Spent engine oil inhibited soil catalase activity significantly ($p<0.05$). Catalase, urease and dehydrogenases exhibit decrease in their activities after the rate of biodegadation of petroleum has decreased (Frankenberger and Jonhanson, 1982; Janke *et al*., 1992; Van der Waarde *et al*., 1995; Margesin and Schinner, 1997). This could be because catalase being an enzyme its activity is altered by unfavourable conditions, such as hypoxia, unavailability of nutrient and changes in pH.

Spent engine oil caused a significant ($p<0.05$) change in soil dehydrogenase activity. This may be as a result of increase in total microbial respiratory rate. Dehydrogenase activity in soil was a measure of microbial activity and respiration rate (Schinner *et al*., 1996). The increase in dehydrogenase activity can be due to the involvement of specific microorganisms in the metabolism of polyaromatic hydrocarbons (Margesin *et al*., 2000). Oxidoreductases play an important role in energy transformation in the respiration chain and participate in the synthesis of soil humics and in the soil formation process (Gramss *et al*., 1999; Dick, 1997). Similar results were reported by Dominguez-Rosado and Pichtel (2004) who observed that that soil respiration increased initially when spent engine oil was added to soil and initiated by soil dehyrogenaseses.

CONCLUSIONS

1. It is clear that spent engine oil alters enzymatic activities: dehydrogenase and catalase present in the soil.

2. The enzymatic activity provides information on the microbial properties of the soil when exposed to oil pollution.

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