Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 2, No. 4, p. 10-14, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Investigation on the long term effects of palm oil mill effluent pollution on soil catalase activity and dehydrogenase activity of soil micro organisms

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Received: 26 January 2012
Revised: 02 April 2012
Accepted: 04 April 2012

Key words: Palm oil effluent, pollution, catalase activity, dehydrogenase activity, soil micro organism.

Abstract

The long term effect of palm oil mill effluent pollution on soil catalase activity and dehydrogenase activity of soil micro organisms was investigated using 2, 3, 5 – triphenyltetrazolium chloride (TTC) dehydrogenase activity inhibition test. Samples from long term palm oil effluent-polluted soil were used as test while soil samples without palm oil effluent pollution served as the control. Results revealed that there was a significant ($p>0.05$) decrease in soil catalase activity and dehydrogenase activity of micro organisms, found in the chronic palm oil mill effluent – polluted soil samples when compared to the control. The results indicated that palm oil effluent contain some toxic substances which at toxic levels inhibited soil catalase activity as well as dehydrogenase activity of soil micro organisms thus affecting soil physiology and soil fertility. It is, thus, expedient to prevent toxic, prolonged palm oil effluent-pollution on our agricultural soils.

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Introduction

The fact that the effect of chronic palm oil mill effluent discharged on our agricultural soil has not been given the proper attention it deserves, may be due to lack of knowledge of its effect. (Elendu, 1998; Zakaria, 2002). Its impacts on the inhibition of soil microbial dehydrogenase activity affects soil fertility. Dehydrogenase activity in soil is a measure of microbial activity and respiration rate (Schinner *et al.,* 1996).

Dehydrogenase is one of the most frequently used enzymes for test of soil micro organisms because its activity is very sensitive to organic, inorganic and heavy metal pollution (Wilke, 1991). Changes in soil dehydrogenase activity indicate the amount of micro organisms present in the soil (Kobus, 1995).

Soil catalase activity shows a significant correlation with organic carbon content, soil microbial biomass, oxygen consumption, carbon dioxide evolution and dehydrogenase activity (Margesin *et al.*, 2000).Also,

palm oil mill effluent is the final mixed waste that stream from the sterilization, clarification and hydrocyclone process in a typical palm oil mill. Palm oil mill effluent is a blackish slurry with obnoxious odour that affect agricultural soil at toxic level (Lim, 1987). Palm oil effluent with biological oxygen demand (BOD) less than 5000ml/L is beneficial to soil and some crops but, BOD above 5000 ml/L is detrimental to soil and micro organisms (Achuba and Peretiemo Clark, 2008).

In Nigeria, palm oil is processed industrially and locally throughout the palm belt, covering the Niger Delta area. In rural communities, individuals and co-operatives societies set up local palm oil mills, in most cases, palm oil mill effluent from these mills is often discharged directly untreated into nearby agricultural lands. Tubonimi *et al.,* (2007) studied the impact of palm oil effluent on water quality on Olaya lake in Niger Delta, Nigeria. They found out that palm oil mill effluent contains high levels of nutrients and heavy metals. Its low acidic pH is ecologically harmful to soil and water as it increases the acidity of soil and water (Tubonimi *et al.,* 2007). This study sought to investigate the effect of chronic palm oil effluent-pollution on soil catalase activity as well as soil microbial dehydrogenase activity. Palm oil effluent pollution is a common environmental challenge in the Niger Delta area, but no adequate attention has been given to it like petroleum hydrocarbon pollution.

Materials and methods

Soil sample collection

Palm oil mill effluent-polluted soil samples used for the study were collected at a depth of 0-30 cm from a discharge point near a palm oil processing mill in Umuchima village, in Ihiagwa, Owerri, Nigeria. Soil samples, collected at the same depth from a normal garden soil from the same village, 1km away from the discharge point, served as the control. Micro organisms (bacteria) were isolated from the two sets of soil samples using the method as described by Holt *et al.,* (1994)

Assay of catalase activity

Catalase activity of the soil samples was assayed using the method as described by Rani *et al.*, (2004).

Screen test for 2, 3, 5-triphenyl tetrazolium chloride (TTC) reduction (dehydrogenase activity).

On a colony of each microbial isolates from the two sets of soil samples were each grown on nutrient agar, a drop of 1:1 mixture of aqueous solution of TTC (0.4% w/v) to glucose (2% w/v) was placed. The plates were incubated at room temperature for 1hr. Production of red coloured formazon was indicative of TTC reduction.

Determination of the effect of palm oil effluent on soil micro organisms by total dehydrogenase assay

Dehydrogenase assay method as described by Praveen – Kumar (2003) was adopted for the study. The dehydrogenase activity was determined using 2, 3, 5-triphenyl tetrazolium chloride (TTC) as the

artificial electron acceptor, which was reduced to the red coloured triphenyl formazon (TPF). The assay was done in 5ml volumes of nutrient broth-glucose TTC medium in separate 20ml screw-capped test tubes. One gram of palm oil mill effluent-polluted soil and normal garden soil was each inoculated with microbial isolates in triplicate glass tubes containing 2.5ml phosphate buffer (pH 6.8), nutrient broth-glucose medium at room temperature (28 ± 2°C) for 30 minutes. Thereafter, 0.1ml of 1% w/v TTC in deionzed distilled water was added in each glass tube. The final concentrations of nutrient broth, glucose and TTC in the medium were 2, 2 and 0.25 mg/ml respectively. The control consisted of microbial isolates without palm oil mill effluent. The reaction mixtures were incubated statically at room temperature (28 ± 2°C) for 16 hours. The TPF produced was extracted in 5ml of amyl alcohol and determined spectrophotometrically at 500nm (λ max). The amount of formazon produced was determined from a standard dose response curve (0 – 20 mg/ml TPF in amyl alcohol). Dehydrogenase activity in the test soil samples was calculated relative to the control.

Data analysis

Data generated from catalase assay were expressed as means ± standard deviation (S.D) and analyzed using one way analysis variance (ANOVA) with $p<0.05$ taken to be significant. The degree of enzyme activity (EA) inhibition was determined relative to control. On the basis of measured absorbance as shown in equation below

$$EA\ (\%\ of\ control) = \frac{TA}{TC} \times \frac{100}{1}$$

Where, CA is the absorbance of tripheny formazon produced in the control (without palm oil effluent pollution), TA the absorbance of triphenyl formazon produced in the test (with palm oil effluent pollution).

Three replicate tests were carried out on each set of experiment. The data were plotted in terms of soil microbial enzyme activity on y-axis versus the control and test on x-axis with means and standard deviations (n = 3) shown as data points and bars respectively.

Results

The results for catalase activity (Fig 1) show that soil catalase activity from palm oil effluent – polluted soil decreased significantly ($p<0.05$) when compared to the control.

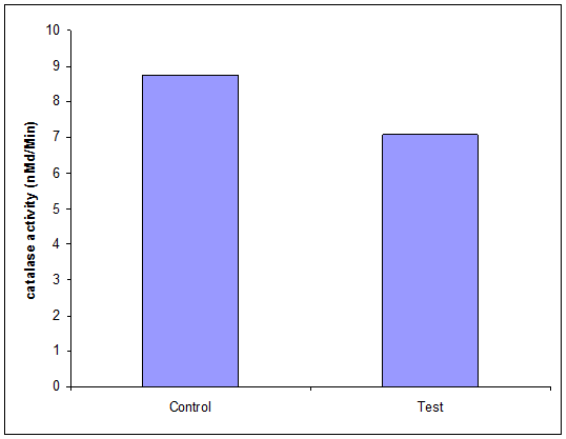

Fig. 1. Effect of palm oil effluent on soil catalase activity.

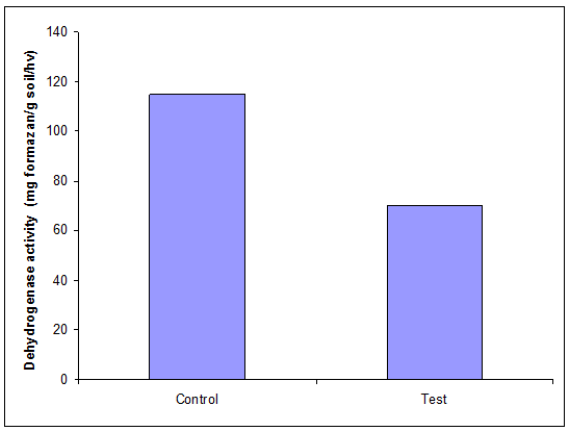

Fig. 2. Effect of palm oil effluent on the dehydrogenase activity of soil micro organisms.

The results obtained for the dehydrogenase activity on soil micro-organism (Fig 2) show that there was a significant ($p<0.05$) decrease in the dehydrogenase activity of micro organisms from palm oil effluent – polluted soil when compared to the control.

Discussion

Results obtained in this study show that chronic palm oil effluent pollution altered soil catalase activity. The soil catalase activity significantly ($p<0.05$) decreased from 8.75 ± 0.62 (nMol/min) in the control to 7.07 ± 0.50 (nMol/min) in palm oil effluent – polluted soil (Fig 1). Chronic exposure of soil to palm oil effluent would bring changes in soil conditions such as pH, hypoxia as well as reduction in the number and activities of soil micro-organisms (Maila and Cloete, 2005). These changes make the soil which was previously fertile before exposure to the effluent pollution to lose its productive potentials (Maila and Cloete, 2005).

When soil enzyme activity is negatively affected by effluent discharges, plants, man and other animals that depend directly and indirectly on it are also adversely affected (Osuji and Nwoye, 2007)

Environmental sustainability depends largely on a sustainable soil ecosystem (Adriano *et al.,* 1998). When soil is polluted, the physiochemical properties are affected which may decrease its productive potentials (Osuji and Nwoye, 2007).

The results obtained for dehydrogeanse activity for micro organisms from palm oil effluent – polluted soil indicate that there was a significant ($p<0.05$) decrease in their dehydrogenase activities when compared to the control (Figure 2). The significant decrease in activity is attributed to the chronic exposure of the soil micro organisms to palm oil effluent which exposed the organisms' dehydrogenase systems to more work, in the oxidation of organic matter contained in the effluent-polluted soil. This result agrees with the reports of Garcia – Gil *et al,* (2000), who worked on long term effects of municipal solid waste compost application on soil enzyme activities and microbial biomass, and Drucker *et al*, (1979); Kizilkaya *et al*, (2004) who also worked on the effect of heavy metal contamination on soil microbial biomass and activity.

The dehydrogenase enzyme systems apparently fulfill a significant role in the oxidation of organic matter, as they transfer electron from substrates to acceptors.

Many specific dehydrogenase systems are involved in the dehydrogenase of soil, these systems are an integral part of the micro organism (Von Mersi and Schinner, 1991). The most widely used substrate for dehydrogenase assay is 2, 3, 5- triphenyl tetrazolium chloride (TTC), which produces red coloured water insoluble tripheny formazon. The apparent redox potential of TTC is about -0.08v, which makes it act as an acceptor for many dehydrogenases. The use of total dehydrogenase assay is recognized as a useful indicator of the overall measure of microbial metabolism (Tabataba, 1982).

Conclusion and Recommendation

Our investigation revealed that long term exposure of soil to palm oil effluent, adversely affected soil catalase activity as well as dehydrogenase activity of soil micro organisms.Consequently, those involved in palm oil processing using oil mills, should be enlightened on the danger inherent in indiscriminate discharge of the untreated effluent on our agricultural soil, as it adversely affects soil fertility and productivity. Effective enlightenment can be achieved through sustained dissemination of information in this regard, using the media and trained agricultural officers.

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