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Comparative Activities of Soil Enzymes from Polluted Sites in Egbema, Imo State, Nigeria

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Abstract

The levels of activities of dehydrogenase, hydrogen peroxidase, catalase and lipase of different soil strata from hydrocarbon polluted site (HCPS), domestic waste polluted site (DWPS) and palm oil mill effluent polluted site (POMEPS) as well as unpolluted site were evaluated. Soil samples, namely, subsoil from 50 cm below soil surface, and topsoil were collected from different locations in Egbema urban area in Ohaji/Egbema local government area of Imo state, Nigeria. Soil enzymes activities were measured using spectrophotometric and titrimetric methods. Dehydrogenase activities of topsoil and subsoil from POMEPS were significantly ($p<0.05$) higher than those from unpolluted site. Comparative analysis of soil enzyme activity showed that topsoil and subsoil from HCPS gave the highest hydrogen peroxidase activity of $8.48 \pm 0.01 \times 10^{-3}$ U/g and $7.52 \pm 0.02 \times 10^{-3}$ U/g respectively. Topsoil from POMEPS gave the highest lipase activity of 1.90 ± 0.02 U/g and was followed by lipase activity of subsoil from DWPS=1.85 ± 0.02 U/g. Catalase activity of the subsoil was significantly ($p<0.05$) higher than that of the topsoil from all soil sites. The overall variability in enzymes activities of soil strata from different polluted sites defined the pattern of soil contamination, which could serve as biomarkers for ascertaining level of soil pollution as well as monitorial indices for bioremediation.

Keywords: Catalase; dehydrogenase; hydrogen peroxidase; lipase; polluted soil

Introduction

Soil is an important component of all terrestrial ecosystems as well as a main source of production in agriculture and forestry. Soil substances are in dynamic state in that there are continual interactions among soil minerals, organic matters and organisms, which influence the physicochemical and biological properties of terrestrial systems [1]. Furthermore, soil ecosystem is an extremely heterogeneous microenvironment where physical, chemical and biological processes occur simultaneously among numerous inorganic and organic components and living organisms. Among this variety of soil components, soil enzymes exert predominant influence on the ecosystem because they (a) catalyze all the biochemical transformations occurring in soil (b) guarantee the exchange of materials among the biotic and abiotic portions and the soil ecosystem (c) allow the growth, survival and activity of soil living organisms [2].

Soil contamination or soil pollution is caused by the presence of xenobiotics or organic matters that can alter natural soil environmental indices. It is typically caused by industrial activity [3], agricultural chemicals [4], or improper disposal of waste. Soil pollution can elicit extreme changes in soil properties [5] and therefore, empirical evaluation of these changes is essential for soil management [6]. However, the effect of soil pollution on enzymes activities is, for the most part, complicated due to the complexity of microbial community in soils. The response of different enzyme to the same pollutant may vary greatly and the same enzyme may respond differently to different pollutant [7].

Soil enzymes have significant effects on general soil biology, and particularly, growth and nutrient uptake by plants in ecosystems. Indeed, all biochemical transformations taking place in soil are dependent on the presence of enzymes. All soil types are composed of different intracellular and extracellular enzymatic components, produced by microbial organisms (bacteria, fungi), or derived from animal and plant sources such as plant roots, lysed plant residues, digestive tracts of small animals etc. [**8].**

Soil enzymes activities are very sensitive to both natural and anthropogenic disturbances and show a quick response to the induced changes [9-11]. Accordingly, in view of increasing trend in environmental pollution, it is of utmost concern to evaluate extreme alterations in soil enzymes activities, which could provide insights into the indices required for monitoring incidence of soil pollution and bioremediation as well as possibly improving soil fertility [12]. The present study provided comparative assessments on variations in activities of different soil enzymes such as dehydrogenase, hydrogen peroxidase, catalase and lipase in soil samples exposed to different pollutants. Since enzymes activities are linked with several ecosystems processes such as soil formation, organic matter transformation and bioremediation activities, the outcome of the present study will provide the comparative impacts of different soil pollutants on soil enzymes that facilitate the dynamic properties of all soil types.

Materials and Methods

Collection of soil samples and study sites

Soil samples were collected during the wet season of July, 2015 from different locations in Egbema urban area in Ohaji/Egbema local government area of Imo State. Soil samples from two different soil

strata, namely, subsoil from 50 cm below soil surface, and topsoil were collected from hydrocarbon polluted site (HCPS) in Uada farm settlement. Four soil samples were collected in the same manner from palm oil mill effluent polluted site (POMEPS) and domestic waste polluted site (DWPS) both in Mmahu community. Egbema is situated at the northern apex area of the lower Niger Delta, between Latitudes 5°21'-5°41' N and Longitudes 6°37'-6°49' E. Likewise, unpolluted samples of subsoil from 50 cm below soil surface, and topsoil were collected from Federal University of Technology, Owerri (CONTROL site), which was regarded as an unpolluted site following preliminary tests.

Preparation of soil samples

Soil samples for enzyme assay were stored in sterile polyethylene bags at cold temperature of below 15° C to avoid the loss of moisture, inhibition of microbial activities and enzymatic reactions, after which large soil aggregates and pebbles were removed using spatula. The samples were kept as much as possible in their original conditions in order to maintain the properties and identities of samples at all stages of sample preparation.

Measurement of soil enzymes activities

Measurement of soil enzymes activities, namely, dehydrogenase, hydrogen peroxidase, lipase and catalase activities were carried out using standard methods. Soil enzymes activities were expressed in enzyme unit per gram wet weight of the soil samples (U/g).

Dehydrogenase activity

Soil dehydrogenase activity was measured using the methods previously described by Casida, et al. [13]. Five grams of soil sample was mixed with 10 ml of 0.2% aqueous solution of triphenyl tetrazolium chloride (TTC) in a specimen bottles. Ten millilitre of 0.1 N Tris buffer solution (pH=7.6) was added and the bottles were sealed and incubated at 37° C for 6 h. The reduced triphenyl formazan (RTF) formed was extracted using 10 ml of CH_3OH. The extract was centrifuged at 3000 rev/min for 10 min. The absorbance of the supernatant was measured at maximum wavelength (λmax)=485 nm using CH_3OH as blank. The concentration of RTF was evaluated using extinction co-efficient; Σ=15433 Mol cm^{-1}.

Hydrogen peroxidase activity

Soil hydrogen peroxidase activity was measured using titrimetric methods according to Alef and Nannipieri [14]. To extract soil hydrogen peroxidase, 5.0 g of the soil sample was added into an Erlenmeyer flasks and 20 ml of CH_3OH was added and allowed to stand for 30 min after vigorous shaking. The soil suspension was filter using Whatman No 1 filter paper to obtain the filtrate (enzyme extract). Five millilitres of the extract was pipette into a conical flask. The extract was acidified by adding 1.0 ml of 2.0% H_2SO_4. Methylene blue indicator (0.2 ml) was added and the content of the flask was titrated against 0.01 N $KMnO_4$. The end point was indicated when the solution turns light purple.

Lipase activity

The titration method according to Ugochukwu, et al. [15] was used to assay soil lipase activity. Lipase activity was measured by titrating fatty acid liberated by the hydrolysis of triacylglycerol (TAG) with 0.1 M NaOH using 0.1% alcoholic phenolphthalein as indicator. Reactivity of sample was quenched after 5 min by the addition of 2.0 ml of C_2H_5OH to the assay mixture.

Catalase activity

Assay of soil catalase activity was according to the methods described by Cohen, et al. [16]. Ten grams of soil sample was weighed into a beaker and 20 ml of phosphate buffer (pH=7.0) was added and stirred. The content was allowed to stand for 10 min and centrifuged at 3000 rev/min for 10 min. One millilitre of the supernatant was introduced into a test tube containing 5.0 ml of 0.01% H_2O_2. Thereafter, 7.0 ml of 1.0 N $KMnO_4$ was added within 30 sec and thoroughly mixed. Enzymatic reactions were initiated by adding 1.0 ml of 6.0 NH_2SO_4. The absorbance of the enzyme mixture was measured at λmax=480 nm.

Statistical analyses

The data from soil analyses were subjected to one way ANOVA to test the level of significance among three different polluted soil samples as well as the unpolluted soil samples using SPSS Statistics 20.0 software.

Results and Discussion

Soil dehydrogenase activity varied within the range of 7.47 ± 0.04-0.1 ± 0.01 U/g, of which topsoil from POMEPS gave the highest enzyme activity (7.47 ± 0.04 U/g) followed by topsoil from DWPS (4.32 ± 0.02 U/g), topsoil from HCPS (3.43 ± 0.02 U/g) and topsoil from CONTROL site (0.10 ± 0.001 U/g) (Figure 1). Additionally, Figure 1 showed that the subsoil from POMEPS also gave the highest dehydrogenase activity of 5.82 ± 0.02 U/g followed by subsoil from DWPS (5.043 ± 0.02 U/g), subsoil from CONTROL site (1.94 ± 0.01 U/g) and subsoil from HCPS (1.30 ± 0.01 U/g). Comparative analyses showed that dehydrogenase activity of topsoil from HCPS, DWPS and POMEPS were significantly higher ($p<0.05$) than that from the CONTROL site.

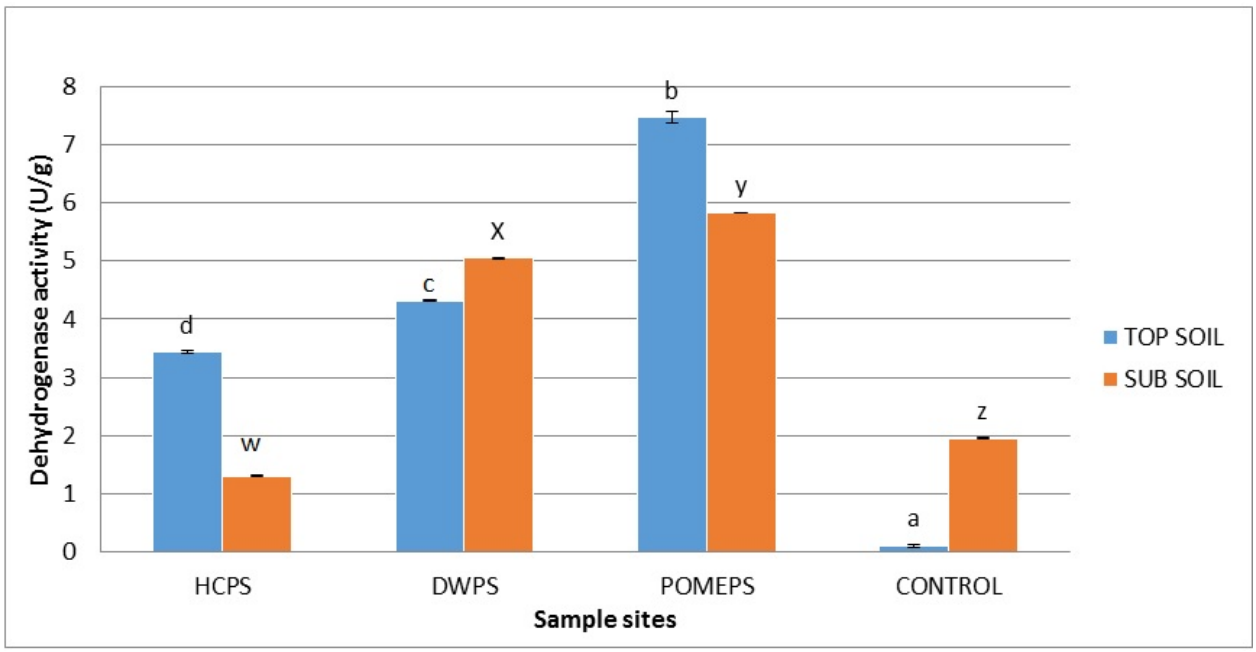

Figure 1: Dehydrogenases activity of topsoil and subsoil of polluted and unpolluted sites.

HCPS: Hydrocarbon polluted site, DWPS: Domestic waste polluted site, POMEPS: Palm oil mill effluent polluted site, CONTROL: CONTROL site. Means denoted by the same letter are not significantly different at $p>0.05$.

Figure 2 showed significant ($p<0.05$) alterations in hydrogen peroxidase activity from the various sample sites. Hydrogen peroxidase activities of the topsoil and subsoil were within the range of $6.64 \pm 0.03 \times 10^{-3}$ to $8.48 \pm 0.04 \times 10^{-3}$ U/g, of which HCPS gave the highest enzyme activity, whereas hydrogen peroxidase activity of subsoil from DWPS exhibited the lowest enzyme activity (Figure 2). Hydrogen peroxidases catalyze the oxidation of variety of organic and inorganic compounds [17] which could be a useful index in ascertaining the progress of bioremediation exercise.

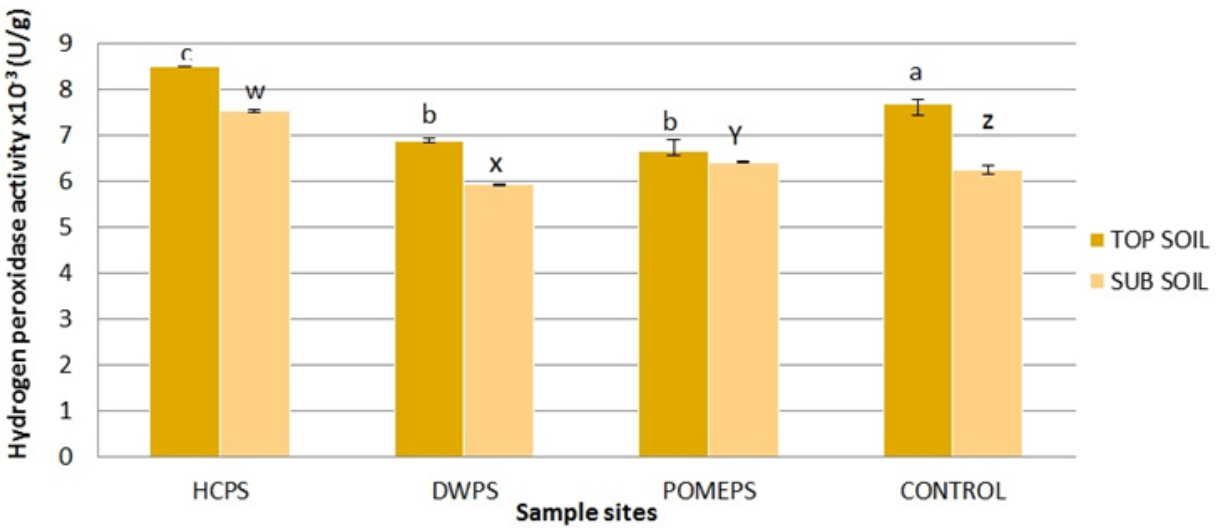

Figure 2: Hydrogen peroxidase activity of topsoil and subsoil of polluted and unpolluted sites.

HCPS: Hydrocarbon polluted site, DWPS: Domestic waste polluted site, POMEPS: Palm oil mill effluent polluted site, CONTROL: CONTROL site. Means denoted by the same letter are not significantly different at $p>0.05$.

The lipase activity of topsoil from CONTROL site was 1.36 ± 0.02 U/g, whereas lipase activities of topsoil from HCPS, DWPS and POMES were as followings: 1.25 ± 0.01 U/g, 1.15 ± 0.01 U/g and 1.90 ± 0.02 U/g, respectively (Figure 3). In addition, lipase activities of subsoil from polluted and unpolluted sites were in the following increasing order: DWPS=1.85 ± 0.02 U/g>CONTROL site=1.20 ± 0.01 U/g; HCPS=1.20 ± 0.01 U/g>POMEPS=1.00 ± 0.01 U/g. However, lipase activity of subsoil from HCPS, CONTROL site and POMEPS exhibited no significant difference at $p<0.05$.

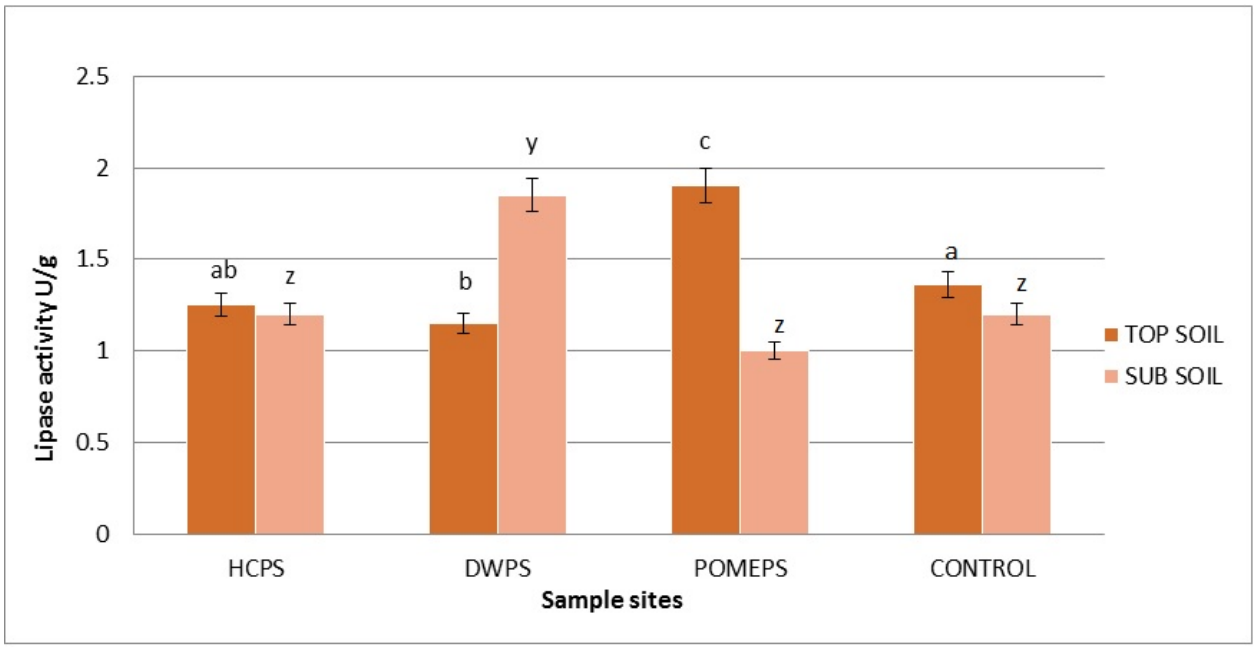

Figure 3: Lipase activity of topsoil and subsoil of polluted and unpolluted sites.

HCPS: Hydrocarbon polluted site, DWPS: Domestic waste polluted site, POMEPS: Palm oil mill effluent polluted site, CONTROL: CONTROL site. Means denoted by the same letter are not significantly different at $p>0.05$.

Figure 4 showed that the levels of soil catalase activities of subsoil from polluted and unpolluted sites were significant higher ($p<0.05$) than those of corresponding subsoil samples. Conversely, soil catalase activities of topsoil from HCPS, DWPS and POMES exhibited no significant difference ($p>0.05$) but were significantly lower ($p<0.05$) than that from the CONTROL site (Figure 4). Overview of Figure 4 showed that soil catalase activity of the topsoil from HCPS, DWPS, POMES and CONTROL site was within a relatively narrow range of 95.17 ± 0.19-97.55 ± 0.23 U/g, whereas those of the subsoil from HCPS, DWPS, POMES and CONTROL site was within the range of 96.68 ± 0.13-102.31 ± 0.32 U/g. The comparatively high level of subsoil catalase activity from HCPS appeared to suggest presence of high quantity of biodegradable substrates in this soil type as previously reported [18]. Additionally Yu, et al. [19] had proposed that soil catalase activity increased with corresponding increase in concentrations of hydrocarbon pollutants, which was in concord with the present findings.

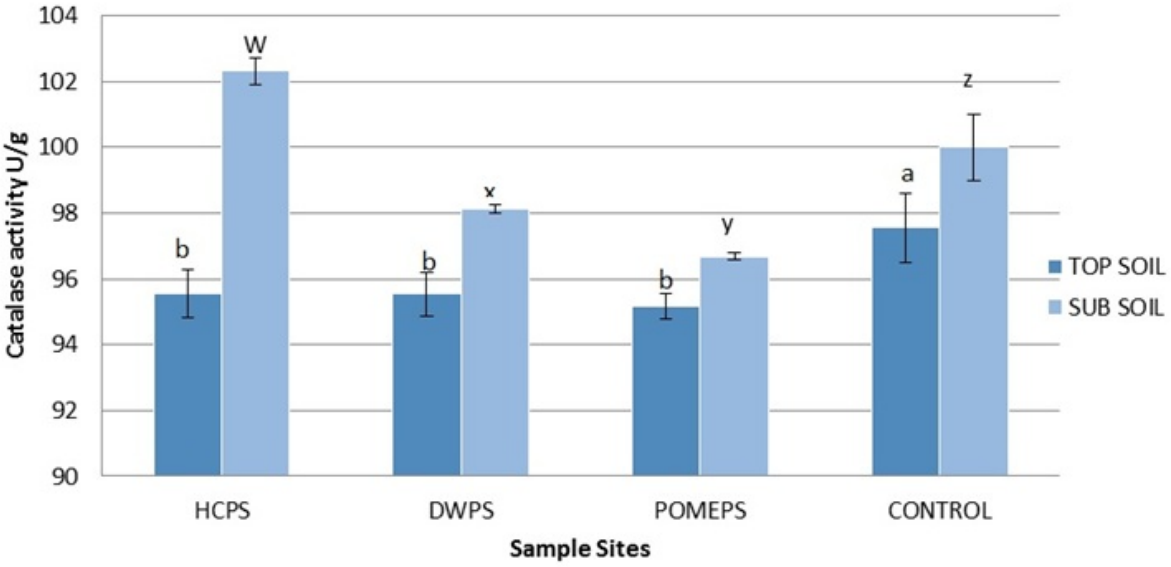

Figure 4: Catalase activity of topsoil and subsoil of polluted and unpolluted sites.

HCPS: Hydrocarbon polluted site, DWPS: Domestic waste polluted site, POMEPS: Palm oil mill effluent polluted site, CONTROL: CONTROL site. Means denoted by the same letter are not significantly different at $p>0.05$.

Furthermore, subsoil dehydrogenase activity of DWPS and POMEPS were significantly higher ($p<0.05$) than that of the CONTROL site; whereas subsoil dehydrogenase activity of HCPS was significantly lower ($p<0.05$) than that of the CONTROL site. Previous studies reported evidence of increased dehydrogenase activity of soil samples from POMES and HCPS [20,21] and in soils polluted with pulp and paper mill effluents [22]. Conversely, low level of soil dehydrogenase activity indicated incidences of low dose pesticides and fly ash pollutions [23,24]. The raised level of soil dehydrogenase activity appeared to suggest corresponding increase in total microbial respiratory rate since the level of dehydrogenase activity in soil was a reflection of microbial population and respiratory rate [12,25-27]. Previous reports showed that raised level of soil dehydrogenase activity enhanced biodegradation of palm oil mill effluent (POME) [28].

Conclusion

It is imperative to understand the possible roles of soil enzymes in the maintenance of soil fertility and management of the ecosystem. Alteration in soil enzyme activity is one of numerous strategies researchers use to evaluate the progress of bioremediation. Overall, increases in soil enzymes activities were indications of corresponding impact of pollutant on the soil ecosystems. However, the present study showed that lipase activity of subsoil and topsoil from HCPS as well as lipase activity of topsoil from DWPS were comparable with that of soil samples from the CONTROL site. These were obvious indications that soil lipase activity from the above mentioned soil polluted sites may not serve as useful biomarkers for evaluation of soil pollution and bioremediation. Nevertheless, overall variability in enzymes activities of soil strata from different polluted sites, for the most part, defined the pattern of soil contamination, which could serve as biomarkers for ascertaining level of soil pollution as well as monitorial indices for bioremediation.

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