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

Chioma Y Ezirim1*, Paul C Chikezie2, Kizito M Iheanacho1 and Ngwu R Nwachukwu1

1Department of Biochemistry, Federal University of Technology, Owerri, Nigeria
2Department of Biochemistry, Imo State University, Owerri, Nigeria

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 Uda farm settlement. Four soil samples were collected in the same manner from palm oil mill effluent polluted site (POMPEPS) 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 (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 CH₃OH. 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 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% H₂SO₄. Methylene blue indicator (0.2 ml) was added and the content of the flask was titrated against 0.01 N KMnO₄. 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 C₂H₅OH 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% H₂O₂. Thereafter, 7.0 ml of 1.0 N KMnO₄ was added within 30 sec and thoroughly mixed. Enzymatic reactions were initiated by adding 1.0 ml of 6.0 NH₄SO₄. 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 POMPEPS 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 POMPEPS 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 POMPEPS 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 ± 0.03x10^{-3} to 8.48 ± 0.04x10^{-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.
The lipase activity of topsoil from CONTROL site was 1.36 ± 0.02 U/g, whereas lipase activities of topsoil from HCPS, DWPS and POMEPS 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 significantly 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.

References

1. http://www.fao.org/docrep/009/a0100e/a0100e0d.htm.
2. Gianferda L, Roa MA, Mora M (2011) Handbook of Soil Sciences. In: Huang PM, Li Y, Sumner ME, Huang ME, Li PM, et al. (eds) Enzymatic activity as influenced by soil mineral and humid colloids and its impact on biogeochemical processes. (2ndedn), CRC Press. pp: 1-24.
3. Nagaraju M, Narasimha G, Rangaswamy V (2009) Impact of sugar industry effluents on soil cellulase activity. Int Biodeterior Biodegradat 2: 1088-1092.
4. Raju MN, Venkateswarlu K (2014) Effect of repeated applications of buprofezin and acephate on soil cellulases, amylase, and invertase. Environ Monit Assess 186: 6319-6325.
5. Raju MN, Narasimha G, Rangaswamy V (2017) Soil Enzymes: Influence of sugar industry effluents on soil enzyme activities. Spring Brief Environ Sci 7: 51.
6. Das SK, Varma A (2011) Role of enzymes in maintaining soil health. In: Shukla G, Varma A (eds) Soil enzymology, Soil biology 22: 25-42.
7. He ZL, Yang XE, Baligar VC (2003) Microbiological and biochemical indexing systems for assessing acid soil quality. Adv Agronomy 78: 89-138.
8. Rao MA, Scelza R, Gianfредa L (2014) Soil enzymes. In: Gianfредa L, Rao MA (eds) Enzymes in agricultural science. Omics Group eBooks, USA. pp: 10-24.
9. Dick RP (1997) Soil enzyme activities as integrative indicators of soil health. In: Pankhurst CE, Doube BM, Gupta VSR (eds) Biological Indicators of Soil Health. CAB International. New York. pp: 121-156.
10. Wyszkowska J, Wyszkowski M (2010) Activity of soil dehydrogenases, urease, and acid and alkaline phosphatases in soil polluted with petroleum. J Toxicol Environ Health 73: 1202–1204.

11. Wolinska A, Stepniewska Z, Anna P (2015) The effect of environmental factors on total soil DNA content and dehydrogenase activity. Arch Biol Sci 67: 493-501.

12. Wolinska A, Kuzniar A, Szafranek-Nakonieczna A, Jastrzebska N, Roguska E, et al. (2016) Biological activity of autochthonic bacterial community in oil-contaminated soil. Water Air Soil Pollut 227: 130.

13. Casida LE Jr, Klein DA, Thomas S (1964) Soil dehydrogenase activity. Soil Sci 98: 371-376.

14. Alef K, Nannipieri P (1995) Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London pp: 576.

15. Ugochukwu KC, Agha NC, Jude-anthony ON (2008) Lipase activities of microbial isolates from soil contaminated with crude oil after bioremediation. Afr J Biotechnol 7: 2881-2884.

16. Cohen G, Dembiec D, Marcus J (1970) Measurement of catalase activity in tissue extracts. Anal Biochem 34: 30-38.

17. Hamid M, Rehman K (2009) Potential applications of peroxidases. Food Chem 115: 1177-1186.

18. Tejada M, Heras CD, Kent M (2007) Changes in the quality indices during ice storage of farmed Senegalese sole (Solea senegalensis). Eur Food Res Technol 225: 225-232.

19. Yu FQ (2008) Effects of Mycorrhiza Symbiosis on the enzymes activities in the Rhizosphere. University of Inner Mongolia Agriculture. People's Republic of China.

20. Achuba Fl, Peretemo-Clarke BO (2008) Effect of spent engine oil on soil catalase and dehydrogenase activities. Int Agrophys 22: 1 4.

21. Chinyere CG, Iroha AE, Amadike UE (2013) Effects of altering palm oil and cassava mill effluent pH before dumping on dumpsite soils physicochemical parameters and selected enzymes activities. J Biodivers Environ Sci 3: 46-58.

22. Siddaramappa R, Wright RJ, Coddling EE, Gao G, Gregory W, et al. (1994) Evaluation of coal combustion by products as soil liming materials: their influence on soil pH and enzyme activities. Biol Fertil Soils 17: 167-172.

23. Baruah M, Mishra RR (1986) Effect of herbicides butachlor, 2,4-d and oxyfluorfen on enzyme activities and CO2 evolution in submerged paddy field soil. Plant Soil 96: 287-291.

24. Pitchel JR, Hayes JM (1990) Influence of fly ash on soil microbial activity and populations. J Environ Qual 19: 593-597.

25. Tabatabai MA (1982) Soil enzymes. In: Page AL, Miller RH, Keeney DR, Baker DE, Rhoades JD, et al. (eds) Methods of soil analysis, Part 2, Chemical and microbiological properties. (2ndedn), American Society of Agronomy–Soil Science Society of America, Madison, WI. pp: 903-947.

26. Schinner F, Ohlinger R, Kandeler E, Margesin R (1996) Methods in soil biology. Springer, Berlin, Germany.

27. Margesin R, Walder G, Schinner F (2000) The impact of hydrocarbon remediation (diesel oil and polycyclic aromatic hydrocarbons) on enzyme activities and microbial properties of soil. Acta Biotechnol 20: 313-333.

28. Nwaogu LA, Agha NC, Iheijirika CE (2012) Investigation on the long term effects of palm oil mill effluent pollution on soil catalase activity and dehydrogenase activity of soil microorganisms. J Biodivers Environ Sci 2: 10-14.