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Research Article

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Dehydrogenase Enzyme Assay in an Oil Polluted Soil Environment

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Abstract

Soil pollution resulting from oil exploration, exploitation and illegal bunkering activities introduce pollutants and hampers the activities of soil microbiota. Soil dehydrogenase assay stands out as one of the major test of soil pollution.Soil samples were collected from different automobile workshops in UmuobaUratta in Owerri North Local Government Area of Imo state, Nigeria. The levels of activities of dehydrogenase of fifty (50) soil samples from hydrocarbon polluted and unpolluted sites were evaluated. Soil enzymes activities were measured using spectrophotometric methods at absorbance of 485nm (A_{485}). Dehydrogenase activities (DHA) of the soil samples were significantly ($p<0.05$) higher than those from unpolluted site. Comparative analysis of soil enzyme activity showed that soil samples from pollution site 5 (UR3), had the highest dehydrogenase activity with a value of 1.699mg/ml and the control sample had the least activity with a value of 0.711mg/ml. The overall variability in enzymes activities of soil samples from different polluted sites show that petroleum products (spent engine oil) alter soil biochemistry.

Keywords: Dehydrogenase assay, spent oil, soil pollution microbial activities

Introduction

Industrialization and urbanization have resulted to frequent oil spills and discharge of waste products into the environment (Achi, 2003; Tolulope, 2004). Frequent cases of loss of soil microorganisms, inadequate crop yield and loss of farmland, mutants and recalcitrant disease found in human and animals as well as contamination of underground water can be attributed to soil contamination by hydrocarbons which results in the reduction of soil activities carried out by microorganisms (Achuba and Peretiemo, 2008).Such activities include; nitrogen cycle, degradation of organic matter, maintenance of soil health, fertility and productivity.

It has been documented that some soil bacteria and fungi with the aid of their enzymes utilize petroleum hydrocarbons as a carbon source. Bioremediation of oil contaminated soils have broad prospects because of its low cost, no secondary pollution and *in situ* processing (Misshra and Jyot, 2001; Stryer and Berg, 2002). Dehydrogenase among other enzymes play significant roles in energy conversion and overall processes of materials (Gu *et al*., 2009). They also inhibit the effect of pollutants and dangerous

hydrocarbons in the soil and are used as an indicator of overall soil microbial activity, because they occur intracellular in microbial cells.

Soil enzymes analysis helps to establish correlation with soil fertilization, microbial activity, biochemical cycling of various elements in soil, degree of pollution (by heavy metals), oil spillage, and to assess succession stage of an ecosystem. Therefore, measurements of enzyme activity in degraded soils have useful function in examining impacts of environmental change or management on soil enzyme activities (Subhani *et al*., 2001; Kiss *et al*., 2005).

This study reports on the dehydrogenase enzyme activity (DHA) in an oil polluted soil environment.

Materials and Methods

The study area covers Umuoba Uratta in Owerri North Local Government Area, Imo State in the South-Eastern Nigeria. The area is concentrated with automobile mechanics. The soil in the environment is frequently contamination with petroleum product such as spent engine oil discharged into the environment. Large amounts of this waste petroleum product is disposed into gutters, water drains, undeveloped plots and farmlands around the area causing environmental degradation and attendant health effect.

Sample Collection

Soil samples for analysis were randomly collected on site from the surface (0-15cm depth) using alcohol-disinfected trowels, into sterile polyethylene bags (Ziploc) for enzyme analysis. A total of fifty (50) samples were collected from the study areas while control samples were collected from an area with no hydrocarbon activities. Samples were taken to the laboratory in sealed ice packs for enzyme analysis within twenty four (24) hours.

Assay of Soil Dehydrogenase Activity

All the reagents for the assay was prepared from analytical grade chemicals (Sigma). 3% TTC was prepared from 3g of 2, 3, 5-triphenyl tetrazolium chloride (TTC) diluted in 97ml of water and sterilized by autoclaving. 20% glucose was prepared by dissolving 20g of glucose in 80ml of sterile water, and sterilized by autoclaving.

Preparation of Formazan Standard Curve

Graded concentration of Triphenyl formazan (TPF) was prepared to obtain 0-300µg/ml optical density (OD) values of the graded standards obtained at A_{485}.A standard curve was obtained (Fg.1) by plotting OD against concentration of formazan. The values of the formazan in soil DHA assay were extrapolated from the standard curve.

Preparation of Soil Samples and Analysis

Composite soil samples from impacted and control sites were air-dried, ground, sieved (2mm) and stored at room temperature (28 ± 2^{O}C) for 24hours.

Dehydrogenase activity was determined using the modification of the method described by Tabatabai (1982; 1994), Abdelmagid and Tabatabai (1987), Brzezinska *et al*. (2001) and Karaca *et al*.(2011). Dehydrogenases convert 2, 3, 5-triphenyl tetrazolium chloride to formazan. The absorbance of formazan was read spectrophotometrically at 485 nm. Sieved soil (1 g) was placed in test tubes (15 × 100 mm), mixed with 1 ml of 3% aqueous (w/v) 2, 3, 5-triphenyl tetrazolium chloride, 2ml of sterile water and 1ml of 20% glucose and stirred with a glass rod. After 72 h of incubation (27˚C), 5 ml of butanol was added to each test tube and the suspension was vortexed for 30 s. The tubes were then centrifuged at 400rpm for 5 minutes to separate the suspended soil from the formazan formed. The resulting supernatant (5 ml) was carefully transferred to clean test tubes using Pasteur pipettes. Absorbance (A) was read spectrophotometrically at 485 nm. Values of the formazan plot were extrapolated from a standard formazan response curve.

Results

Fig.1 shows the standard plot for the determination of the amount of formazan formed after soil dehydrogenase activity assay. The graph indicated an R^2 value of 0.9997 and a linear model of
Absorbance = 0.8875 (formazan) – 0.0333

The amount of formazan was calculated for each optical densities obtained in values are shown in Table 4.1.

Table 1: The values of formazan formed for optical densities obtained.

NJ1	NJ2	UR1	UR2	UR3	CT
2.031887	1.448225	1.542873	1.511324	2.051042	0.616676
1.554141	1.389634	1.591324	1.572169	1.528225	0.098366
1.458366	1.727662	0.490479	1.580056	1.144	0.559211
2.089352	2.094986	1.333296	1.542873	1.473014	1.000901
0.489352	1.755831	1.474141	0.953577	1.58231	0.403718
1.90907	1.261183	0.208789	1.116958	1.661183	0.398085
0.624563	1.694986	1.362592	1.238648	2.079211	1.512451
0.811606	0.226817	1.173296	1.272451	2.073577	1.095549

Keys: NJ1 = pollution site 1; NJ2 = pollution site 2; UR1= pollution site 3; UR2= pollution site 4; UR3= pollution site 5; CT = control site

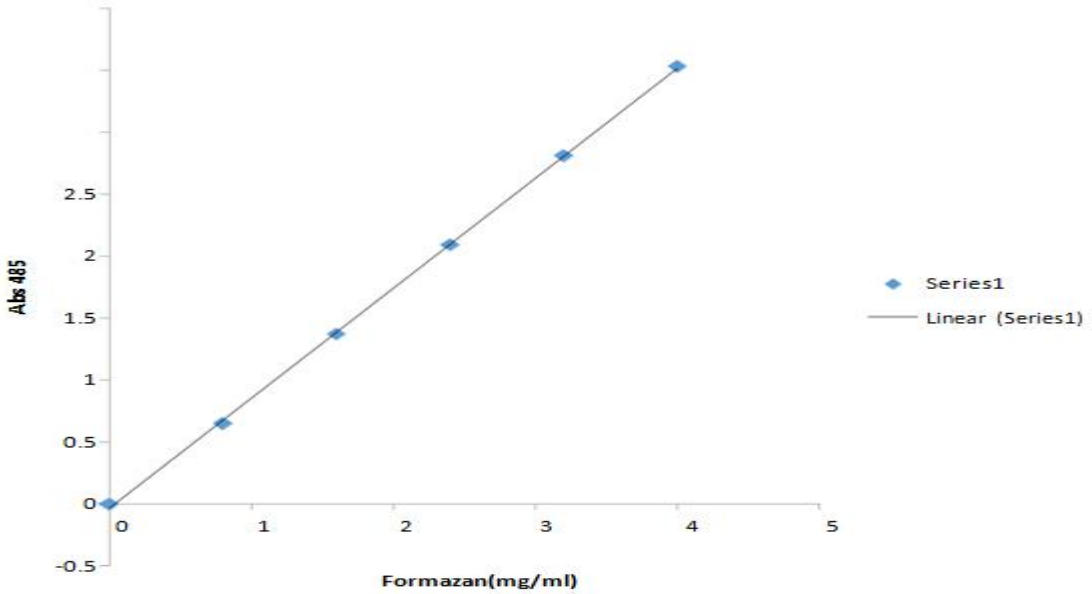

Fig. 1: Standard plot for the determination of amount of formazan formed.

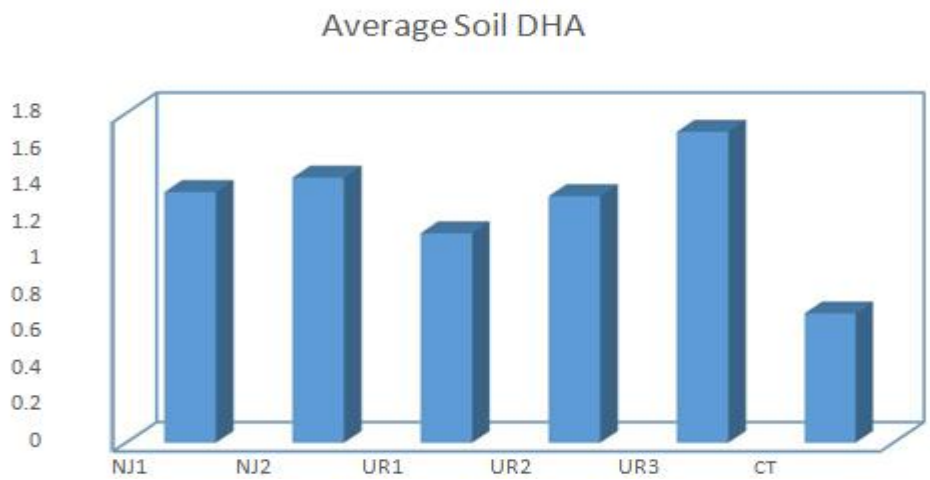

Fig. 2: Average soil dehydrogenase activity.

Fig. 2 shows the average soil DHA of samples collected from five (5) locations and a control sample. Results shows that UR3 had the highest DHA with a value of 1.699mg/ml the control sample (CT) was the least DHA value of 0.711mg/ml.

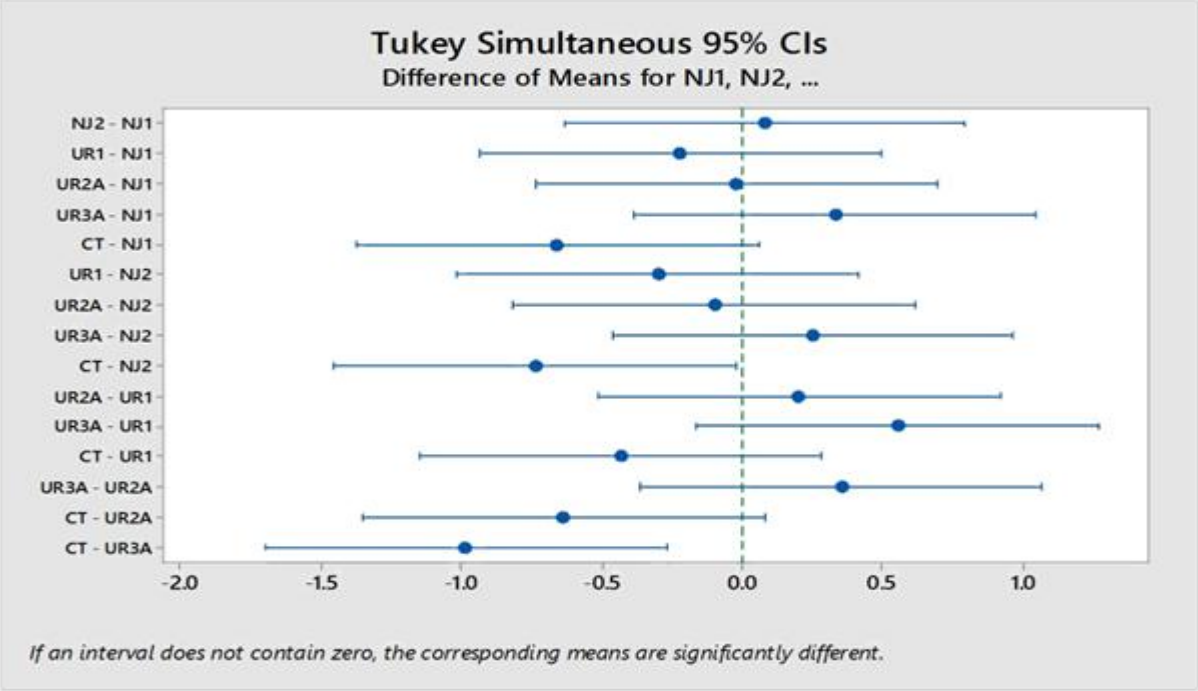

Fig. 3: Differences that occurred with the soil samples.

Fig. 3 indicates the differences that occurred within samples using Tukey Simultaneous Test.

Data from Table 1 was subjected to ANOVA and Tukey Pairwise test to ascertain the difference in the means and significance of the data. ANOVA results shows it that values in the table were significantly different from each other at $P < 0.05$ with P value of 0.005. Also, Tukey Pairwise test shows that samples UR3, NJ2, NJ2, UR2 and UR1 were significantly indifferent from each other. However, differences were significant for the samples NJ2 and UR3 when compared to the control sample.

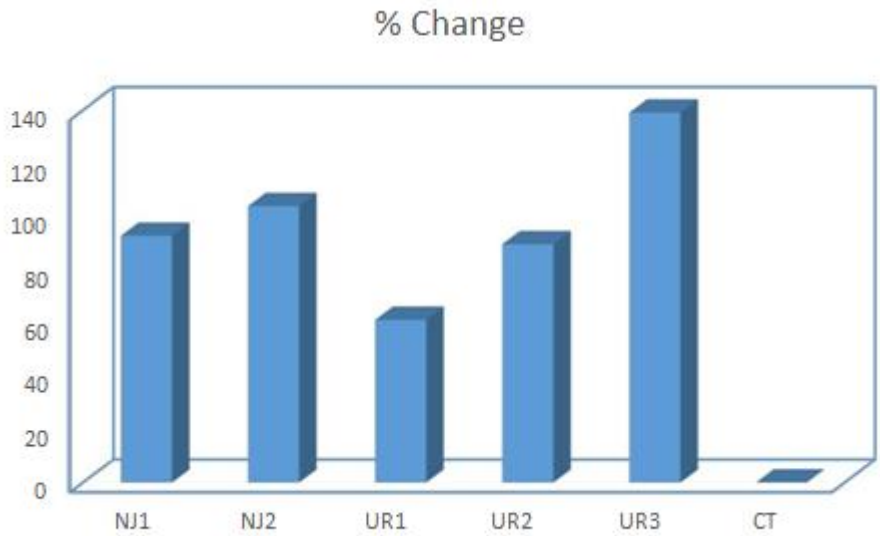

Fig. 4: Percentage (%) change in soil dehydrogenase activity.

Fig.4 show the percentage (%) change in DHA of sampling locations compared to the control sample. Results show that UR3 had a percentage increase in DHA of 139.10% which was the highest. NJ1, NJ2, UR1 and UR2 have values of 92.94%, 104.04%, 61.42% and 89.76% respectively.

Discussion

Petroleum products, when present in soil, creates an unsatisfactory condition for soil organisms, mainly due to poor aeration, immobilization of soil nutrients and lowering of soil pH which results in petroleum mediated reduction in the number of hydrocarbon degrading microorganisms (Visser and Parkinson, 1992; Brzezińska*et al.*,2001; Osuji and Nwoye, 2007; Brzezinska*et al*., 2001; Pan and Yu, 2011). Results obtained shows a significant increase in dehydrogenase activity in the polluted soil samples than in the control samples (Fig 4.2 andFig 4.3). Spent engine oil caused a significant ($p<0.05$) change in soil dehydrogenase activity. This could be as a result of increase in total microbial respiratory rate. Dehydrogenase activity in soil is a measure of microbial activity and respiration rate (Schinner*et al*., 1996; Ihem*eet al*., 2017). The increase in dehydrogenase activity can be due to the activities of specific microorganisms in the metabolism of polyaromatic hydrocarbons(Burns, 1982). Oxido-reductases 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 (Dick, 1997; Wolińska, and Bennicelli, 2010; Wolinska and Stepniewska, 2012). Similar observations were made in earlier reports by Odjegba and Sadiq (2002) who observed that that soil respiration increased initially when spent engine oil was added to soil and initiated by soil dehydrogenases(Burns, 1982; Sonia *et al*., 2014).

Conclusion

The outcome of this research has clearly shown that spent engine oil alters enzymatic activities of dehydrogenase that is present in the soil. The enzymatic activity provides information on the microbial properties of the soil when exposed to oil pollution. However, the soil dehydrogenase activity increased as the metabolic rate of the microorganisms present in the soil samples increased.

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