EVALUATION OF SOIL ENZYMATIC ACTIVITIES AFTER TREATMENT WITH VARIOUS HYDROCARBONS

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Abstract: Pollution of farm land is ubiquitous in oil producing regions. The intent of this study was to evaluate the influence of various hydrocarbons on soil catalase (CAT) as well as dehydrogenase(DH) activity. The experiment was consisted of known amount of soil treated with varying amounts of various hydrocarbons and left to stand for twelve days. At four days interval, the activities of CAT and DH were assayed using standard methods. The results showed a significant ($p<0.05$) disparity in enzyme activity. CAT activity was lesser in petrol infused soil comparative to the other hydrocarbons as number of days progressed, with engine oil tainted soil exhibiting more sustained activity. Also, a significant increase ($p<0.05$) was noted in DH activity as number of days progressed comparative to control values. Similarly, the various hydrocarbons triggered a similar tendency in the modification of soil DH activity, as they affected CAT activities. Generally, the toxic effect of the hydrocarbons is in the manner of kerosene > diesel > petrol > engine oil. The results in overall showed that hydrocarbons perturb soil enzyme reactions.

Keywords: Catalase, dehydrogenase hydrocarbons, pollution, soil

Introduction

Chemicals derived from petroleum are employed in many ways such as household solvents and specialty chemicals, all are important routes hydrocarbons pollute man's environment (Eneh, 2011; Achuba, 2018). Moreso, mechanized farming that employs heavy duty machinescontributes its quota to farm lands pollution during collection or storing petroleum oils as well as unguided disposal of spent petroleum oil into the natural environment (Odjegba and Sadiq, 2002). The pollution of soil alters the nutrient status thereby decreasing its productive capacity (Achuba and Iserhienrhien, 2018).

Enzymes are important component of soil which is responsible for soil biochemical reactions because they participate in the conversion of organic substances into plant nutrients (Zahir *et al.*, 2005; Kumari *et al.*, 2016; Chikezie *et al.*, 2017). Previous report hinted on soil enzyme perturbation by petroleum oil [8] (Kaczynska *et al.*, 2015). The study involves determination of two major soil enzymes, CAT and DH activities. The two enzymes are vital in soil biotransformation reactions (Li *et al.*, 2005; Kaczynska *et al.*, 2015). These enzymes are sensitive to pollutionand their values are used as toxicity testing instrument (Li *et al.*, 2005). Therefore, the study aimed at determiningthe consequence ofhydrocarbons marinating onCAT and DH activities in soil.

Materials and Methods

Materials

Warri Refining and Petrochemical Company supplied the petroleum hydrocarbons. The collection of soil and physical property was reported earlier (Achuba, 2006). Reagents used were analytical grade.

Extract preparation and determination of soil catalase activity

The extract for the determination of catalase was prepared following the protocol described previously (Achuba and Peretiemo-Clarke, 2008). To prepare this extract, 10 g and 100 ml of phosphate buffer of pH 7.4 was homogenized in pre-cold mortar (4.0° C). The mixture was filtered and the filtrate subjected to centrifugation for ten minutes at 7000 g to produce the supernatant used for enzyme assay. Catalase activity was assayed for as reported by Rani *et al.* (2004). In a test tube, I ml of phosphate buffer, pH 7.5, hydrogen peroxide (0.5 ml of 0.2M) and 0.5 ml of enzyme extract were added and incubated at various times (1, 2 and 3 min). The reaction was stopped by adding 5% dichromate/acetic acid mixture. The activity of catalase was measured by monitoring the consumption of hydrogen peroxide/min.

Determination of soil dehydrogenase activity

Kaczyńska *et al.* (2015) protocol was adopted with extinction coefficient proposed byDushoff *et al.* (1965) in order to estimate soil dehydrogenase activity.The soil (6 g), 0.06 g CaCO3, and 1 cm^3 3% aqueous solution of 1, 3, 5-phenyl-tetrazolium chloride and 2.5 cm^3 distilled water were added into a beaker. The mixture was incubated for 24 h at 37°C with water bath. This was followed by addition of 25 cm^3 methyl alcohol. The content was mixed thoroughly and subjected to filtration. The filtrate was made up to mark with methyl alcohol and mixed thoroughly. The absorbance of the filtrate was read 485 nm.

Results and Discussion

Previous investigation has indicated the variation of soil enzyme activities by spent engine oil (Kaczynska *et al.*, 2015). This is in unison with this research in whichsoil CAT activity was varied by the various hydrocarbons in relation to thenumber of days of treatment of soil and with the type of hydrocarbon applied. After four days of post treatment, all the hydrocarbons tested decreased soil CAT activity comparable to the control. And this was concentration dependent but the activity in kerosene treated soil was lower comparable to the other hydrocarbons (Table 1). However, on the eighth day, the activity of the enzyme increased comparable to the control (Table 2). These findings are predicated onearlier studies which reported that petroleum contamination lead to poor physical and biological properties of soil culminating in decrease in soil enzyme activities (Alkorta and Garbisu, 2001; Maila and Cloete, 2005; Kaczynska *et al.*, 2015; Wang, *et al.*, 2013). Similarly, increase in soil catalase activity has been noted after biodegradation of soil pollutant (Achuba and Peretiemo-Clarke, 2008). This may account for the decrease in CAT activity at initial days after treatment and subsequent increase as number of days progress (Tables 1 and 2).

Table 1: Effect of four days treatment with different hydrocarbons on soil catalase activity

Level of hydrocarbons in soil (%)	Catalase activities (nMol min^{-1} g^{-1})			
	Kerosene	Diesel	Engine oil	Petrol
0.25	7.07 ± 0.54^{a}	8.20 ± 0.48^{a}	7.73 ± 0.55^{a}	6.28 ± 0.95^{a}
0.50	7.33 ± 0.42^{a}	8.75 ± 0.62^{a}	6.01 ± 0.17^{a}	6.38 ± 0.49^{a}
1.00	5.35 ± 0.61^{b}	6.26 ± 0.71^{b}	4.63 ± 0.49^{c}	4.11 ± 0.45^{b}
1.50	4.5 ± 0.63^{c}	4.76 ± 0.83^{c}	3.04 ± 0.21^{c}	2.71 ± 0.61^{d}
2.00	3.06 ± 0.67^{c}	3.68 ± 0.59^{c}	2.29 ± 0.41^{d}	1.95 ± 0.24^{d}
0.00	8.76 ± 1.60^{a}	8.76 ± 1.60^{a}	8.76 ± 1.60^{a}	8.76 ± 1.60^{a}

Superscripts with different letters indicate values significantly different from control value at $P < 0.01$

Table 2: Effect of eight days treatment with different hydrocarbons on soil catalase activity

Level of hydrocarbons in soil (%)	Catalase activities (nMol min^{-1} g^{-1})			
	Kerosene	Diesel	Engine oil	Petrol
0.25	9.99 ± 0.16^{a}	9.06 ± 0.27^{a}	6.41 ± 0.63^{b}	11.69 ± 0.56^{f}
0.50	8.53 ± 0.80^{a}	8.15 ± 0.67^{a}	5.78 ± 0.31^{b}	11.71 ± 0.56^{f}
1.00	6.94 ± 0.72^{b}	6.72 ± 0.55^{b}	4.32 ± 0.51^{c}	7.42 ± 0.81^{b}
1.50	6.98 ± 0.50^{b}	6.77 ± 0.32^{b}	6.21 ± 0.60^{b}	9.38 ± 0.98^{a}
2.00	6.46 ± 0.52^{b}	6.15 ± 0.49^{b}	4.63 ± 0.72^{c}	10.40 ± 0.92^{f}
0.00	8.76 ± 1.60^{a}	8.76 ± 1.60^{a}	8.76 ± 1.60^{a}	8.76 ± 1.60^{a}

Superscripts with different letters indicate values significantly different from control value at P < 0.

Table 3: Effect of twelve days treatment with different hydrocarbons on soil catalase activity

Level of hydrocarbons in soil (%)	Catalase activities (nMol min^{-1} g^{-1})			
	Kerosene	Diesel	Engine oil	Petrol
0.25	5.31 ± 0.90^{c}	7.51 ± 0.95^{a}	8.68 ± 0.50^{a}	5.96 ± 0.94^{b}
0.50	7.32 ± 0.37^{a}	8.25 ± 0.29^{a}	9.65 ± 0.44^{a}	6.27 ± 0.43^{b}
1.00	6.00 ± 0.11^{b}	8.38 ± 0.92^{a}	10.71 ± 0.53^{f}	4.69 ± 0.93^{c}
1.50	7.21 ± 0.43^{a}	8.42 ± 0.42^{a}	11.52 ± 0.49^{f}	5.47 ± 0.63^{b}
2.00	7.53 ± 0.65^{a}	8.02 ± 0.74^{a}	6.12 ± 0.47^{b}	6.64 ± 0.54^{b}
0.00	8.76 ± 1.60^{a}	8.76 ± 1.60^{a}	8.76 ± 1.60^{a}	8.76 ± 1.60^{a}

Superscripts with different letters indicate values significantly different from control value at P < 0.01

The subsequent increase in enzyme activity has been impinged onstimulation of microbial consortium that produces enzymes required for the biodegradation of available hydrocarbon (Kaczynska *et al.,* 2015). The enhanced biodegradation culminates in reduced available soil carbon content. This might be the rationale for the decrease in the activity of the enzyme after twelve days of post treatment of soilwith petrol and kerosene (Table 3). This alteration in soil catalase activity is inimical to soil health since this enzyme has been reported to play active part in oxidoreduction reaction, a vital process in soil nutrient mobilization (Zahir *et al.*, 2001).

Earlier reports indicated a proportionate reductionin CAT activity as biodegradation decreases (Klamerus-Iwan *et al.,* 2015; Wolińska *et al.,* 2016). Hence, the CAT activity varied between the hydrocarbons treated soil. These observations indicate that rate of biodegradation is highest in petrol treated soil and least in engine oil. This standpoint is hinged on lower levels of the enzyme in petrol-treated soil comparable to the other hydrocarbons.

The result of the current investigation showed that CAT and DH activities portrayed similar trend. The activity of the enzymes oscillated between four (Table 4), eight days (Table 5) and twelve days (Table 6) of post treatment with the four types of hydrocarbons.

Table 4: Effect of four days treatment with different hydrocarbons on soil dehydrogenase activity

Level of hydrocarbons in soil (%)	Dehydrogenase activities (µMolg^{-1})			
	Kerosene	Diesel	Engine oil	Petrol
0.25	15.91±1.53^{a}	16.97±0.39^{a}	19.29±0.84^{b}	17.97±0.64^{a}
0.50	18.00 ± 0.58^{b}	18.93 ±0.60^{b}	23.08 ± 0.53^{c}	19.92±0.50^{b}
1.00	21.45±0.94^{c}	24.56±0.93^{c}	27.94±1.62^{d}	28.52±1.38^{c}
1.50	25.56 ± 0.96^{c}	27.85 ±1.31^{d}	35.44 ± 2.49^{f}	31.09 ± 0.50^{f}
2.00	26.86 ± 0.65^{c}	31.85 ±1.47^{f}	40.20 ± 2.19^{f}	32.71 ± 0.63^{f}
0.00	14.01±1.05^{a}	14.01± 1.05^{a}	14.01 ± 1.05^{a}	14.01 ± 1.05^{a}

Superscripts with different letters indicate values significantly different from control value at P < 0.01

Table 5: Effect of eight days treatment with different hydrocarbons on soil dehydrogenase activity

Level of hydrocarbons in soil (%)	Dehydrogenase activities (µMolg^{-1})			
	Kerosene	Diesel	Engine oil	Petrol
0.25	17.76±0.49^{a}	20.50±1.57^{b}	22.41±0.66^{b}	20.50±1.5^{b}
0.50	21.42±1.02^{b}	22.21±1.56^{b}	26.16±0.71^{b}	21.49±1.30^{b}
1.00	25.20±0.90^{b}	27.86±1.80^{c}	33.15±1.76^{c}	31.78±0.92^{c}
1.50	30.06 ± 2.43^{c}	30.69±0.86^{c}	42.11±2.46^{d}	36.67±3.10^{d}
2.00	31.98 ± 1.79^{c}	38.06 ±2.94^{d}	46.34±4.46^{d}	41.00±2.57^{f}
0.00	14.01±1.05^{a}	14.01±1.05^{a}	14.01±1.05^{a}	14.01±1.05^{a}

Superscripts with different letter indicates values significantly different from control value at P< 0.0

Table 6: Effect of twelve days treatment with different hydrocarbons on soil dehydrogenase activity

Level of hydrocarbons in soil (%)	Dehydrogenase activities (µMolg^{-1})			
	Kerosene	Diesel	Engine oil	Petrol
0.25	14.09±1.15^{a}	14.37±0.97^{a}	16.43 ± 0.74^{a}	15.63±3.25^{a}
0.50	15.89±1.45^{a}	16.62±0.41^{a}	19.33 ± 2.08^{b}	17.12±1.17^{a}
1.00	21.76±1.16^{b}	20.54±0.81^{b}	22.73 ± 1.99^{c}	21.72±1.24^{b}
1.50	25.93±0.89^{b}	25.63±0.79^{b}	27.43 ± 1.31^{c}	25.93±0.89^{b}
2.00	23.62±10.08^{b}	29.08±2.20^{c}	31.56 ± 2.21^{d}	29.02±1.09^{c}
0.00	14.01±1.05^{a}	14.01±1.05^{a}	14.01 ± 1.05^{a}	14.01±1.05^{a}

Superscripts with different letters indicate values significantly different from control value at P < 0.01

This observation agrees with previous study (Kaczynska *et al.,* 2015. Enhancement of soil enzyme activity is a consequence of natural mobilization of soil microbial consortium related to the metabolism of available organic carbon; however, the decrease in the enzyme activity could be due to exhaustion of carbon content of the soil as the number days after treatment progress.Previous study reported decrease in soil DH activity after biodegradation of petroleum (Jain *et al.,* 2009; Kaczynska *et al.,* 2015). In addition, the overall results indicated that the toxicity of the hydrocarbon showed increasing trend of kerosene > diesel > petrol > engine oil. The high toxicity of kerosene and diesel was previously reported (Wemedo *et al.,* 2002).

In conclusion, this study established that the four types of hydrocarbon altered the activities of the studied enzymes. This could impact on soil nutrient status. Moreover, kerosene had more effect than the other three hydrocarbons in short term.

Conflict of Interest

Authors declare that there is no conflict of interest reported on this work.

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