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Molecular Evaluation of Soil Respiration in Crude Oil Pollution

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A B S T R A C T

Oxygen diffusion into the soil ecosystem is imperative for the sustainability of life in the soil. This study evaluated selected microbial exudates as indices of soil respiration in a crude oil polluted soil ecosystem ex-situ using biochemical and physicochemical tools to determine the following parameters; total petroleum hydrocarbon (TPH), dehydrogenases, catalase, ATP, and pH. The result of the study at $1.5 - 3.5\%$ contamination across days-zero to -28 following crude oil impact presented an augmented increase in TPH from 0.02 ± 0.00 to 0.10 ± 0.00 with an increase in the activity of soil dehydrogenases from 4.90 ± 0.01 to 8.80 ± 0.04 katal which was significant ($p<0.05$). Conversely, the activity of soil catalase suffered inhibition from 0.20 ± 0.00 to 0.11 ± 0.00 katal. ATP synthesis decreased from control to 0.24 ± 0.00mg/100g on week one and increased to 0.60 ± 0.10mg/100mg significantly ($p<0.05$) on week four with contamination as the pH was reduced from 5.8 ± 0.00 to 4.1 ± 0.00 significantly, thus creating hypoxia and a reducing environment, a demonstration of soil respiration.

A R T I C L E   I N F O

1. INTRODUCTION

Crude oil has been the main source of energy and hence a large global environmental pollutant. Its environmental pollution could arise from equipment failure during drilling, processing, or transportation. Petroleum hydrocarbon being a complex mixture of non-aqueous and hydrophobic components such as n-alkane, aromatics, resins, and asphaltenes are toxic, mutagenic, and carcinogenic to living beings (Mandal *et al.*, 2012). Therefore, the release to the environment is strictly controlled as they are classified as priority environmental pollutants by US Environmental Protection Agency, due to their adverse impaction on human health and the environment.

Soil pH is a physical property and an important parameter to monitor especially during the emergence of xenobiotics such as hydrocarbon (Ebulue, 2020). It refers to acidity or alkalinity, which is a measure of hydrogen ion concentration [H+] in the soil; and it is defined by the equation; pH = – Log [H+].

Soil enzymatic activities which are molecular indices of soil respiration have a central role in the soil environment. They are used as attractive bio-indicators for monitoring various impacts on the soil (Ebulue *et al.*, 2017; Ebulue, 2021). By their activities, they bio-transform toxic petroleum products, hydrocarbons, heavy metals, and pesticides into harmless compounds (Ebulue *et al.*, 2017).

Dehydrogenases (EC 1.1.1.1) which by their oxidative activities Ebulue *et al.* (2017) catalyze the removal of hydrogen atoms from substrates which culminates in the degradation of soil organic matter (Margesin *et al.*, 2000). The activity of soil dehydrogenases is an index of soil respiration Ebulue *et al.* (2017) in response to organic matter input in the soil environment.

Catalase (EC 1.11.1.6), an antioxidant enzyme generates oxygen from hydrogen peroxide. The enzyme is widely present in nature, which accounts for its diverse activities in the soil. The activities of catalase and dehydrogenases are used as biomarkers of hydrocarbon-polluted soil ecosystem due to their sensitivity Ebulue *et al.* (2017) as they give information on the microbial activities in the soil. Their values have been suggested to be used as a simple toxicity test Rogers and Li, (1985) and respiratory index in hydrocarbon pollution.

Adenosine triphosphate (ATP) is the primary energy source /storage molecule in living cells, and directly reflects the amount of microbial carbon and thus the biomass and activity of the microbial community. The ATP content of the soil has been used for several years as an indicator of biological activity Ausmus, (1973) and more recently, as a means of estimating the microbial biomass Jenkinson *et al.*, (1979) and respiratory index. The soil biomass maintains an ATP level of the same order as that in eukaryotic and prokaryotic organisms undergoing active growth in vitro, as listed by Knowles, (1977). ATP, therefore, provides a useful indicator of life in soil and respiratory index.

2. METHODS
2.1. Methods: Research Design

This research was designed for an a-thirty-five-day investigation in consideration of the volatility and biodegradability of hydrocarbons: Day- zero, Day- 14, Day- 28, and Day- 35; within which the aforementioned parameters were evaluated.

2.2. Materials: Sample Collections

The soil sample was obtained from the premises of Federal University Technology, Owerri, Imo State, Nigeria with an auger inserted about fifteen centimetres into the soil; while the crude oil was obtained from Port Harcourt Refining Company, Rivers State, Nigeria.

2.3. Soil Preparation

Ten grams (10g) of soil sample was contaminated with crude oil at different concentrations (1.5, 2.5, and 3.5% w/w) for the determination of the aforementioned parameters.

2.4. Determination of pH

Advanced Bench pH Meters 3510 suitable for easy readout of pH with a resolution of three decimal places and automatic or manual buffer selection was used.

2.5. Determination of Total Petroleum Hydrocarbon (TPH)

The principle is based on the estimation of the total petroleum hydrocarbon (TPH) in the soil concerning the standard curve derived from fresh crude oil diluted with toluene using the equation $y = 1.094x$; where y = absorbance and x = concentration.

For the procedure, total petroleum hydrocarbon content was determined gravimetrically, to provide an estimate of the available total hydrocarbon with time and the liquid phase of the extract were measured spectrophotometrically at 420nm. The total petroleum hydrocarbon (TPH) in the soil was estimated concerning the standard curve using the equation $y = 1.094x$; where y = absorbance and x = concentration.

2.6. Determination of Dehydrogenases Activity

For the principle, dehydrogenases convert 2,3,5-triphenyl tetrazolium chloride (TTC) to formazan. The absorbance of formazan was read spectrophotometrically at 485nm.

For the protocol, the protocol of Ebulue, (2021) was used where the absorbance of formazan was read spectrophotometrically at 485 nm after contaminating the soil with different concentrations of crude oil. The concentration of formazan was evaluated using the molar extinction coefficient of dehydrogenase at $15433 \text{Mol}^{-1} \text{ cm}^{-1}$ Dushoff *et al.,* (1965); and the activity was determined thereafter from Beer-Lambert's law, A = ECL as follows: A = ECL were C = A/EL, and activity in katal.

2.7. Determination of Soil Catalase Activity

For the principle, catalase generates oxygen in the presence of H_2O_2. The activity was determined by the method of Cohen *et al*. (1970), where decomposed hydrogen peroxide was measured by reacting it with excess potassium tetraoxomanganate (VII), $(KMnO_4)$ and residual $KMnO_4$ was measured spectrophotometrically at 480 nm.

For the protocol, one-tenth ml of a buffered sieved soil sample (supernatant) was introduced into differently labeled test tubes containing 0.5 mL of 2 mMol hydrogen peroxide and a blank containing 0.5 ml of distilled water. Enzymatic reactions were initiated by adding sequentially, at the same fixed interval, 1 mL of 6N tetraoxosulphate (VI) acid, (H_2SO_4) to each of the labeled test tubes containing soil contaminated with crude oil at: 1.5, 2.5, and 3.5 %w/w (oil-soil mixture); to the blank, 7 ml of 0.1N $KMnO_4$ was added within 30 seconds and thoroughly mixed.

The spectrophotometric standard was prepared by adding 7 mL of 0.1 N $KMnO_4$ to a mixture of 5.5 mL of 0.05N phosphate buffer, pH 7, and 1 mL of 6 N H_2SO_4. The spectrophotometer was then zeroed with distilled water before taking absorbance readings at 480 nm.

The concentration of catalase was determined using the Beer-Lambert's law, A = ECL with the molar extinction coefficient of catalase at $4.02 \text{ mol}^{-1} \text{ cm}^{-1}$; and the activity was determined thereafter as follows: A = ECL, where C = A/EL (activity in katal).

2.8. Adenosine Triphosphate (ATP) Concentrations of Soil Microbial Biomass

Microbial biomass as determined by the fumigation/ incubation technique subjects a fresh soil sample to chloroform fumigation which causes cell walls to lyse and denature and the cellular contents to become extractable in 0.5M K_2SO_4. Adenosine triphosphate (ATP) was calculated from the biomass carbon using the relationship Jenkinson, (1979).

Biomass C in soil = 120 (ATP content of the soil).

Where; y = Biomass C, x = ATP.

From the regression equation, Y = (120) x.

2.9. Statistical analysis

The results were expressed as mean ± standard deviation (SD). All results were compared concerning the control. Comparisons between the concentrations and control were made by using Statistical Package for Social Sciences (SPSS) version 20 and One-way Analysis of Variance (ANOVA). Differences at $p < 0.05$ were considered significant.

3. RESULTS
3.1. Determination of pH

The pH of crude oil-contaminated soil is shown in **Figure 1**. Relative to the control, there was a progressive reduction in pH values which was statistically significant (p<0.05) between treatment groups.
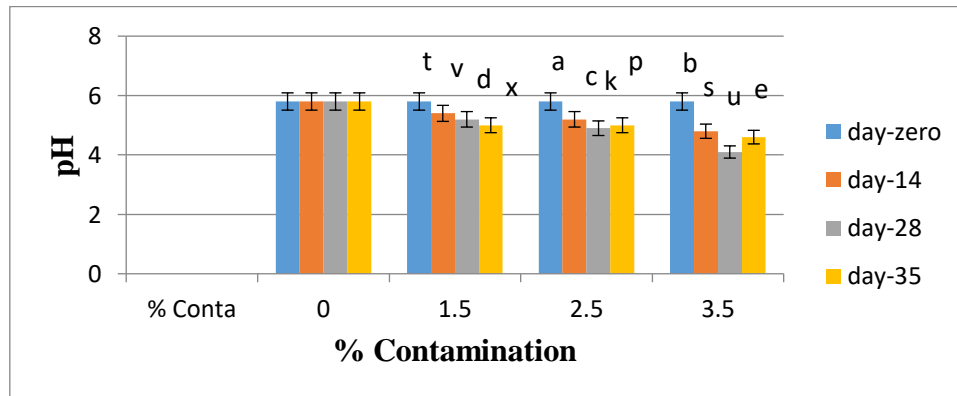

Figure 1. pH of the soil polluted with crude oil. Comparison between groups: Bars with different letters differs significantly (p<0.05).

3.2. Determination of TPH

The total petroleum hydrocarbon (TPH) of crude oil-contaminated soil is shown in **Figure 2**. Following contamination, there was a synergistic increase in total petroleum hydrocarbon (TPH) which decreased over time significantly (p<0.05) when compared between groups.
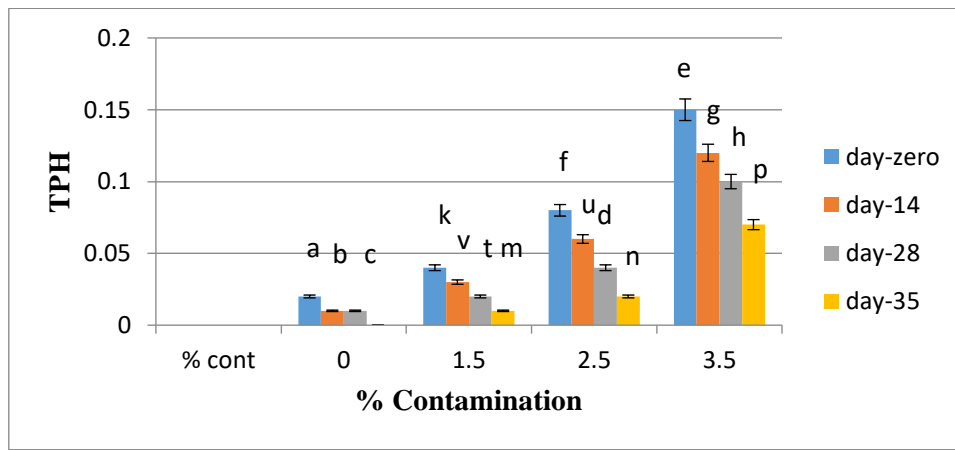
Figure 2. TPH of the soil polluted with crude oil. Comparison between groups: Bars with different letters differs significantly (p<0.05).

3.3. Determination of Dehydrogenases Activity

Relative to the control, the crude oil stimulated the activity of soil dehydrogenases in a concentration and time-dependent manner up to day-28 but declined on day-35 as presented in **Figure 3**, which was significant (p<0.05) between groups.
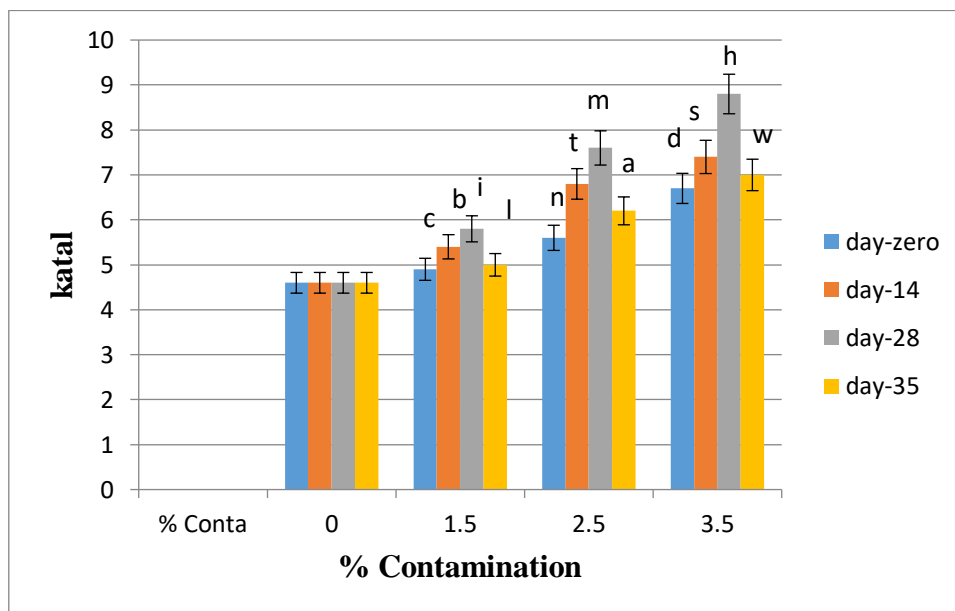

Figure 3. Activity of soil dehydrogenases in the soil polluted with crude oil. Comparison between groups: Bars with different letters differs significantly (p<0.05).

3.4. Determination of The Activity of Catalase

The activity of soil catalase was inhibited relative to the control as shown in **Figure 4**. The inhibition which was in a concentration and time-dependent manner except on day-35 was significantly different (p<0.05) between groups.
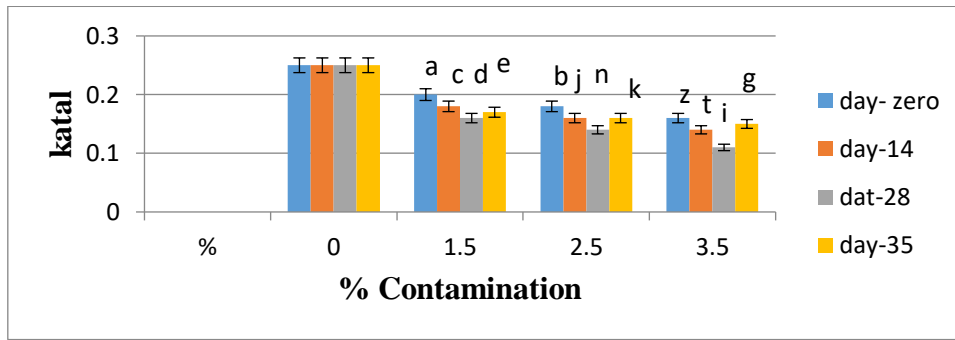
Figure 4. Activity of soil catalase in the soil polluted with crude oil. Comparison between groups: Bars with different letters differs significantly (p<0.05).

3.5. Determination of ATP

There was a sequential decrease in ATP synthesis relative to control on week one across all the significant treatments (p<0.05). Thereafter, was the increase in ATP synthesis which was significant (p<0.05) as presented in **Figure 5**.

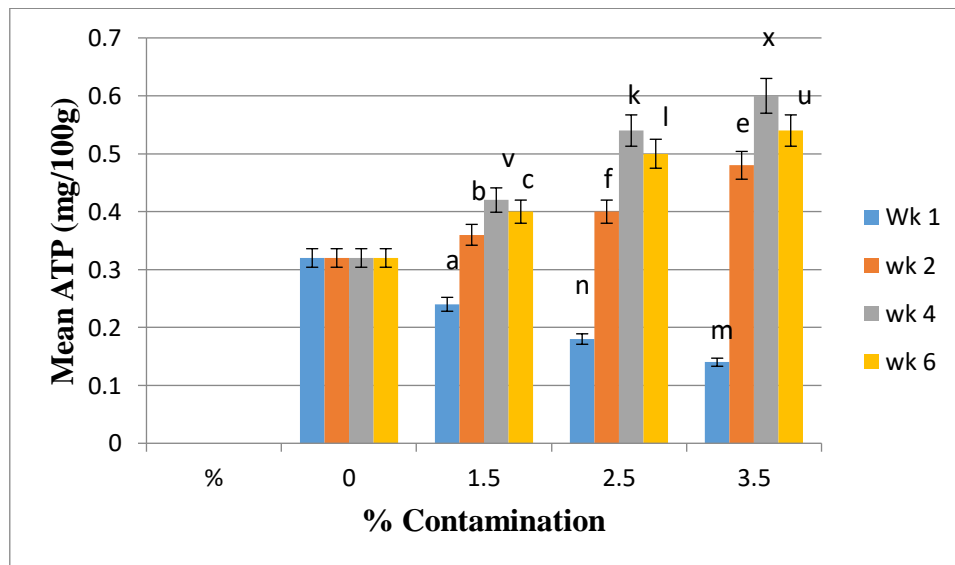**Figure 5**. ATP content of crude oil-polluted soil. Comparison between groups: Bars with different letters differs significantly (p<0.05).

4. DISCUSSION

The intense infusion of degradable hydrocarbon from crude oil impaction would have stimulated aerobic and anaerobic microbial metabolism; and so, as oxygen became limiting, utilization of alternate electron acceptors produced an increased reducing environment. The consequence is a lowered pH. In this study, there was a significant (p<0.05) reduction in pH which paralleled hydrocarbon input. The lowered pH reflected accelerated metabolism and accelerated demand for electron acceptors, thus creating a reducing environment. This increase in acidity would likely affect plant growth, microbial mineralization, succession and metabolism, and leaching of metals. This is replete with a report of Osuji and Nwoye (2007), Osam *et al*. (2013), Ebulue *et al*. (2017), and Ebulue (2020).

Hydrocarbon from crude oil impact inhibited the activity of soil catalase significantly (p<0.05) in a concentration and time-dependent manner. This inhibition could arise from hypoxic and reducing environment occasioned from the pollution. Thus, any condition that

creates oxygen tension is irritable to catalase and this corroborated with Ebulue *et al.* (2017) implying that the genes for catalase were not transcribed.

On the other hand, the result revealed a significant ($p < 0.05$) increase in the activity of soil dehydrogenases in a concentration and time-dependent manner. This stimulatory effect in the activity of this enzyme could be as a result of induction of hydrocarbonclastic organisms Ebulue *et al.* (2017) which increased the respiratory rate, Wyszkowska *et al.* (2002), and Wyszkowska and Kucharski (2005) in the environment. Dehydrogenases being oxidoreductase are involved in soil respiration which was also reported by Dominguez-Rosado and Pitchel (2004) that an increase in soil respiration was initiated by soil dehydrogenases following the impact of hydrocarbon, implying that the genes were transcribed in a hydrocarbon polluted soil ecosystem.

The result revealed that soil adenosine triphosphate (ATP) concentration suffered a lag phase on week one at $1.0 – 3.5\%$ contaminations. This shortfall in ATP synthesis was not unconnected with the toxic effect of crude oil on the microbial community. ATP provides a useful indicator of life and respiration in the soil. This study has demonstrated that soil ATP and biomass carbon are closely related, thus soil ATP is a measure of soil microbial biomass. The consequent reduction in microbial biomass was reflected in a decline in enzyme induction and ATP synthesis on week one. However, over time, the induction of hydrocarbon-degrading microorganisms (the hydrocarbonclastics) culminated in the increase in enzyme induction, ATP synthesis, and respiration.

5. CONCLUSION

Hydrocarbons from crude oil impact altered the pH, enzymatic activities of dehydrogenases and catalase as well as ATP content of the soil ecosystem. Oxidoreductases catalyzed oxidation-reduction processes which culminated in soil respiration. There is a strong correlation among the respiratory indices to the soil pH; thus, enzymatic catabolic oxidation of hydrocarbons led to the production of organic acid which is assimilated into the microbial TCA cycle for the synthesis of ATP.

6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

7. REFERENCES

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