EFFECTS OF HYDROCARBON ON THE ACTIVITIES OF HETEROTROPHIC AND BIOLUMINISCENT BACTERIA AND FERTILITY OF A TROPICAL ULTISOL

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ABSTRACT

The effects of hydrocarbon on the activities of heterotrophic and bioluminescent bacteria as well as the fertility status of an ultisol in Uyo, Akwa Ibom State Nigeria were determine using standard bacteriological and analytical techniques. The research findings have shown that hydrocarbon affects the activities of heterotrophic and biolumincent bacteria as well as the fertility of soil. The effects are dose dependent and varied with the bacterial community, nature or type of soil enzymes as well as the indices of fertility determined. The densities of heterotrophic bacteria were retarded while the density of hydrocarbon degraders increased. The later was remarkable in soil contaminated with 5% of crude oil. The increase in the activities of the oil degraders in the hydrocarbon contaminated soil would definitely enhance the growth of the non-oil degraders. Therefore, the low percentages of hydrocarbon utilizing bacteria/heterotrophic bacteria (HUB:HET) ratios obtained may be due to proliferation of heterotrophic bacteria. A reduction (3.2-2.01 log_{10}CFU/g) in the densities of bioluminescent bacteria accompanied by the loss of the ability to luminesce was observed after hydrocarbon contamination. The densities of diazotrophs (2.39-1.2 log_{10}CFU/g) and phosphate solubilizers (1.9-1.2 log_{10}CFU/g) were also significantly reduced after hydrocarbon contamination. The results of the soil enzymes assay have shown that hydrocarbon contamination significantly ($p \leq 0.05$) increased the activities of all the four soil enzymes activities tested. Values were within the range of 0.79 in the garden (control) soil to 2.09 ($cm^3H_2Kg^{-1}$d.w.soild^{-1}) in the test soil. However, depending on the level of contamination, simulation of soil with crude oil affected both the physical and chemical attributes especially the nutritional status of the test soil. Crude oil contamination of soil is common in the Niger Delta of Nigeria. It retards soil fertility. Timely remediation is hereby recommended for the clean-up of hydrocarbon contaminated arable lands to improve agricultural productivity in the area.

INTRODUCTION

Land degradation and declining soil fertility are critical problems affecting agricultural productivity and human welfare in Sub-Saharan Africa. The problem of land degradation is rife in the Niger Delta region of Nigeria where crude oil exploration has a significant environmental impact on arable land through oil spillage. Onshore oil spills destroy crops and aquaculture through contamination of the soil, surface- and even ground-water sources in swampy environments. Soil quality is one of the significant agro-ecosystem components for which management efforts must intensify in order to achieve sustainability. Soil quality has been estimated with soil physical, chemical and biological factors (Alrumman, Standing and Paton, 2015). Various biological indicators are known to be related to soil quality, for example microbial biomass, bioluminescent bacteria, soil respiration, soil enzyme activities, earthworms and nematodes number (Alrumman, Standing and Paton, 2015).

Biological indicators are very important in hazard assessment. Other than that, they also play an important role in determining the efficiency of remediation of hydrocarbon-polluted soils. Bioluminescence has been reported among hazard indicator microorganisms and has been used as an index of various characteristics, including ability to utilize organic compounds (Breitung *et al.,* 1996; Thomulka and Lange, 1995).Toxicant bioassay that uses bioluminescent bacteria in its analysis has numerous advantages. These include rapid, economic, small test volume, and high sensitivity to a broad spectrum of toxic compounds. This study was conducted to investigate the effect of hydrocarbon contamination on bioluminescent bacteria, soil enzymes and other fertility indices of an ultisol in Uyo, Akwa Ibom State Nigeria.

MATERIALS AND METHODS

The soil used for the experiment was the sandy-loamy soil of the Experimental Farm of the Department of Botany& Ecological Studies, University of Uyo. Treatments were completely randomized. Three 2m x 2m plots prepared as crop beds were contaminated with 0.5%, 1.0% and 1.5% levels ($^{V}/_{W}$)) of hydrocarbon source and allowed to conditioned for two weeks. An uncontaminated bed served as control. Soil samples were collected from the top (0-10 cm) of the beds, air-dried and sieved to <5 mm. Samples meant for physicochemical analysis were kept in polyethylene bags, while samples for microbiological analysis were collected with the aid of a sterile hand trowel, stored in sterile glass bottles and transported to the laboratory for immediate analysis.

The total culture-able heterotrophic bacterial, Hydrocarbon degrading microorganisms, phosphate solubilizing bacteria, nitrifying bacterial, bioluminescent bacterial and fungal load was determined using the pour and streak plate techniques respectively as described by Cappuccino and Sherman, (2002). Samples used for enzyme analysis were sieved in a mesh diameter of 2 mm. The enzyme analysis includethe determination of soil dehydrogenases activity (DHA) with 2,3,5-triphenyltetrazolium Chloride (TTC) substrate (Öhlinger, 1996), urease (Ure) Activity according to Alef and Nannpieri (1998), as well as acid phosphatase (Pac) and alkaline phosphatase (Pal) activity according to the method described by Alef *et al.* (1998). The grain size analysis (particle size distributions) of soil samples was determined by the hydrometer method (AOAC, 1999).Using the standard analytical protocol (APHA, 1992) soil samples were analysed for pH, moisture content, organic matter, total organic carbon (TOC), available phosphorus, total nitrogen (TON), nutritive salts ($-NO_2$, $-NO_3$, $-SO_4^{2-}$, $-NH_4^+$, Cl), particle sizes and Electrical conductivity.

RESULTS AND DISCUSSION

The result in Figure 1 represents the microbiological properties of the garden (control) soil and the hydrocarbon treated soil. The results revealed heterotrophic bacterial densities ranged from 4.96$\log_{10}$CFU/g in soil treated with 1.5% (highest contamination level) hydrocarbon to 5.21 $\log_{10}$CFU/gin control soil, while the densities of hydrocarbon degraders ranged from 2.58 $\log_{10}$CFU/gin the uncontaminated soil (control) to 5.3$\log_{10}$CFU/g in the soil treated with 1.5% hydrocarbon. The HUB/HET ratio ranged from 0.49in the uncontaminated soil (control) to 1.09in the soil treated with 1.5% hydrocarbon. A reduction (3.2-2.01$\log_{10}$CFU/g) in the densities of bioluminescent bacteria accompanied by the loss of the ability to luminesce was observed after hydrocarbon contamination. The densities of diazotrophs (2.39-1.2$\log_{10}$CFU/g) and phosphate solubilizers (1.9-1.2$\log_{10}$CFU/g) were also significantly reduced after hydrocarbon contamination.

This study has shown that hydrocarbon contamination on had a profound effect on soil microbial communities. A 2-log reduction was observed for heterotrophic bacteria. Similarly, the densities of diazotrophs, phosphate solubilizers and bioluminescent bacteria were also adversely affected. The effect increased with increase in level of hydrocarbon contamination. The negative effect of hydrocarbon on microorganisms cannot be overemphasized. Hydrocarbon toxicity is a well-known phenomenon that has been reported by previous studies (Essien *et al.,* (2005). The increase in the number of culturable hydrocarbon degrading bacteria

(HDB) however, demonstrates how rapidly indigenous soil organisms adapt to the influx of new substrates. The high carbon content of hydrocarbon presents a suitable substrate for microorganisms. Studies have reported that increase in the number of HDB shares a positive correlation with increase in hydrocarbon concentration in soil (Margesin *et al.,* 2000; Dindar *et al.*, 2017). In this study the HDB/THB ratio of 0.49-1.09 revealed a soil environment that is remarkably stressed by hydrocarbons. Low percentages of hydrocarbon utilizing bacteria/heterotrophic bacteria (HUB:HET) ratios were obtained from the contaminated soil and may be due to proliferation of heterotrophic bacteria. The increase in the activities of the oil degraders in the hydrocarbon contaminated soil would definitely enhance the growth of the non-oil degraders. This implies that the low percent HUB:HET ratio observed in soils contaminated with 0.5% and 1.0% hydrocarbon may result from high heterotrophic microbial activities and plausibly lead to "a hastened" natural remediation after crude oil pollution. On the other hand, the high HUB:HET percentages recorded for soil contaminated with 1.5% crude oil may be associated with the slow oil-degrading activities of the oil degraders.

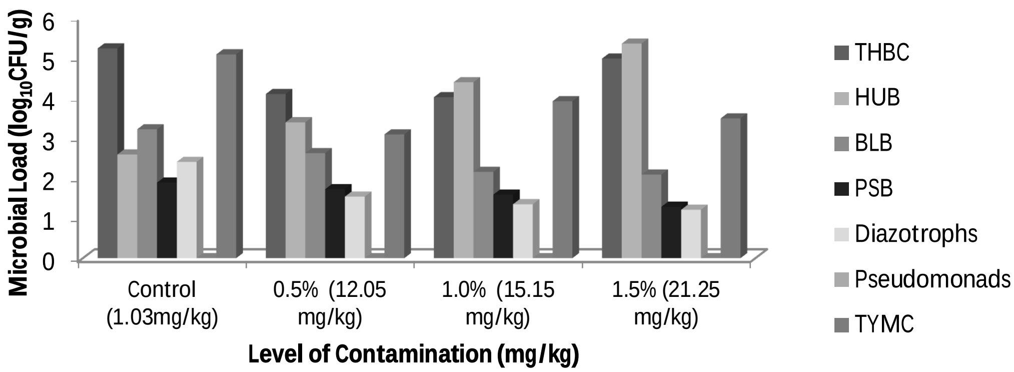

Figure 1: Microbial load (log_{10}CFU/g) of the Treated and Untreated Soil Samples

The results presented in Figures 2 - 5 have revealed that hydrocarbon contamination was observed to significantly ($p \leq 0.05$) increased the activities of all the four soil enzymes tested. Values were within the range of 0.79 in the garden (control) soil to 2.09 ($cm^3H_2Kg^{-1}$d.w.soild^{-1}) in the test soil. Values for urease, alkaline and acid phosphatases were within the range of (1.4-2.19mgN-NH_4Kg^{-1}d.w.soilh^{-1}), (2.2-2.33mmPNpKg^{-1}d.w.soilh^{-1}) and (1.81-1.94mmPNpKg^{-1}d.w.soilh^{-1}) respectively in the garden (control) soil and soil exposed to 1.5% hydrocarbon contamination.

Enzyme activities are commonly used as indicators of soil health because they play an important role in nutrient cycling and can be sensitive indicators of pollution with hydrocarbons (Dindar *et al.,* 2017). In this study, hydrocarbon treatment altered the biochemical properties of soil. It was observed that the treatments stimulated the activities of dehydrogenases, urease, alkaline phosphatase and acid phosphatase in soil. Dehydrogenase activity (DHA) is a measure of the oxidation–reduction capability of the active soil microbial biomass (Shaw and Burns, 2006). Soil characteristics such as organic matter and soil texture are known to affect DHA. Generally, an increase in soil organic matter will be accompanied by a corresponding increase in soil DHA. Unlike DHA, phosphatase activity (PA) phosphatases are not purely intracellular and are not dependent on cell activity (Shaw and Burns, 2006). They are also not ubiquitous and may be absent from parts of the microbial community (Shaw and Burns, 2006). Phosphatase activity is influenced by soil phosphorus status and pH. Urease enzyme plays an important role in the microbial mineralization of nitrogen compounds in the soil. The increase in urease activity observed in this study could therefore be attributed to the influx of nitrogen compounds from the dead microbiota and fauna affected by hydrocarbon contamination.

The results of the soil physical and chemical characteristics (Table 1) have shown that the garden soil is predominantly sandy. The particle size arrangement of soil determines the texture of the soil, while soil texture determines the water absorption capacity, water storage, ease of tilling as well as soil aeration and fertility (Olalekan and Ezugwu, 2017). This study has revealed an acidic soil (pH of 6.39) characterized with low level of total organic carbon content (1.59%). The total nitrogen content of 0.38%, available phosphorus level of 0.14% and nutritive salts levels of 0.03 mg/kg, 0.26 mg/kg, 0.13 mg/kg and 0.10 mg/kg recorded for nitrate, sulphate, phosphate and ammonium respectively revealed a relatively fertile soil with a sandy loam texture and bulk density of 1.19kg/cm^3. However, depending on the level of contamination, simulation of soil with crude oil affected both the physical and chemical attributes especially the nutritional status of the test soil. It increases the acidity, total organic carbon bulk density and the nutritive salts content of the soil although the chloride content was slightly reduced.

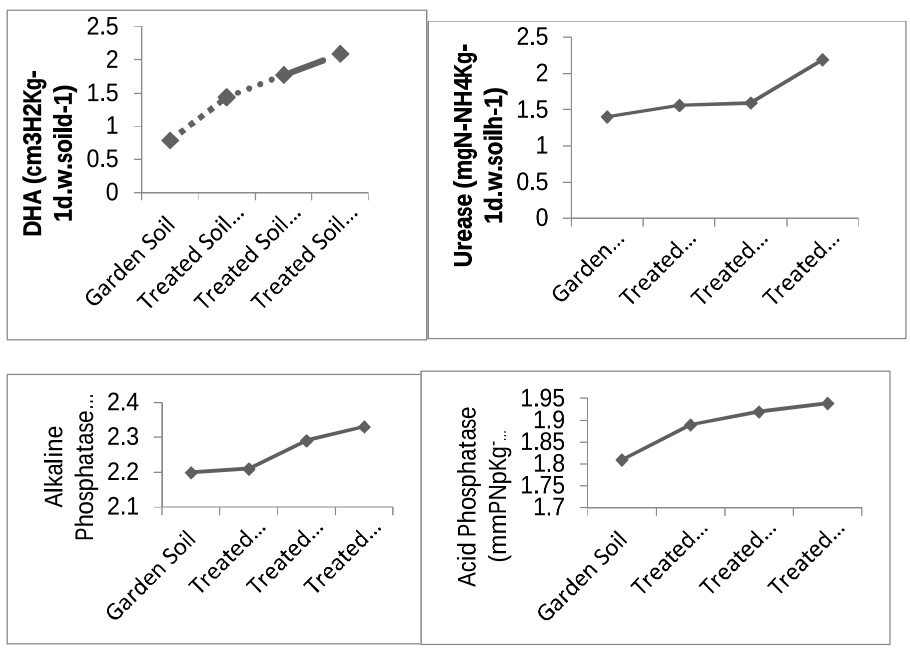

Figure 2-5: Enzyme activity of the garden soil and hydrocarbon treated samples (a) Dehydrogenase (b) Urease (c) Alkaline phosphatase (d) Acid phosphatase

The pH range of 6.39 revealed an acid soil and acid soils are known to exhibit intensive leaching, low exchangeable basic cations content and slow microbial activity (Olalekan and Ezugwu, 2017).Hydrocarbon contamination resulted in a slight increase in the silt content as compared to the control due to compaction of soil particles. A decrease in soil pH and electrical conductivity was observed with increase in contamination levels. Moses and Uwah (2016) reported that decrease in soil pH can lead to an increase in trace metal contents of the soil while a marked decrease in electrical conductivity can result in low permeability and slow water infiltration into the soil.

Table 1: Properties of untreated and hydrocarbon treated soil

Parameter	Garden soil (Control)	Treated Soil (0.5%)	Treated Soil (1.0%)	Treated Soil (1.5%)
Sand (%)	52.0	51.0	50	50
Clay (%)	27.0	29.0	28	28
Silt (%)	20.0	21.0	22	22
Texture Class	SCL	SCL	SCL	SCL
Bulk Density (kg/cm^3)	1.19	1.20	1.20	1.20
Electrical Conductivity(µs/cm)	47.66	17.22	17.21	17.21
Organic Matter (%)	2.12	2.136	2.139	2.180
Crude Nitrogen (%)	0.308	0.317	0.319	0.384
Available P	0.14	0.026	0.022	0.019
Moisture Content (%)	61.03	61.34	61.59	61.82
TOC (%)	1.59	2.603	3.308	4.203
C/N Ratio	5.16	8.21	10.37	10.95
pH	6.39	5.7	5.8	5.8
Total Alkalinity (mg/kg)	4.006	5.308	5.329	5.411
Nitrite (mg/kg)	0.0102	0.0102	0.0102	0.0104
Nitrate (mg/kg)	0.0262	0.194	0.209	0.222
Sulphate (mg/kg)	0.261	0.301	0.307	0.309
Phosphate (mg/kg)	0.135	0.228	0.233	0.239
Ammonium (mg/kg)	0.120	0.532	0.586	0.611
Chloride (mg/kg)	3.060	3.012	3.015	3.019

The total organic carbon (TOC) and phosphorus content of the soil used for the study are low with the percentage availability of 1.59%, and 0.14% respectively. The TOC level of test soil was less than 12% reported for soils derived from mineral sources (Olalekan and Ezugwu, 2017) and is consistent with findings in previous reports (Essien and Udotong, 1999: Moses and Uwah, 2016; Olalekan and Ezugwu, 2017; Dindar *et al*., 2017). The low level of carbon, nitrogen and phosphorus (C, N and P) in the garden soil samples might have been caused by leaching/ erosion on the study area. Hydrocarbon contamination resulted in a slight increase in the silt content as compared to the control due to compaction of soil particles. A decrease in soil pH and electrical conductivity was observed with increase in contamination levels. Moses and Uwah (2016) reported that decrease in soil pH can lead to an increase in trace metal contents of the soil while a marked decrease in electrical conductivity can result in low permeability and slow water infiltration into the soil. Nitrogen and organic carbon increased with an increase in treatment concentration for all the crops. The observed values of organic carbon were above the 2.0% critical level required for normal plant growth. This may be attributed to slow decomposition by heterotrophs stressed by hydrocarbon contaminates. However, the carbon level may reduce as microorganisms adapt to soil conditions and are able to utilize the carbon content of the soil for their metabolism (Uquetan *et al*., 2017).

CONCLUSION AND RECOMMENDATION

The research findings have shown that soil contamination with hydrocarbon affects the activities of heterotrophic and biolumincent bacteria as well as the fertility of soil. The effects are dose dependent and varied with the bacterial community, nature or type of soil enzymes as well as the physicochemical indices of soil fertility. It had a stimulatory effect on the activities of dehydrogenases, urease, alkaline phosphatase and acid phosphatase and showed a significantly negative effect on soil beneficial microorganisms. Crude oil contamination of soil is common in the Niger Delta of Nigeria. It retards soil fertility. Timely remediation is hereby recommended for the clean-up of hydrocarbon contaminated arable lands to improve agricultural productivity in the region.

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