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# Biodegradation of Petroleum Hydrocarbons in Contaminated Coastal Environments, Nigeria

SAMSON ONIBIYO

# BIODEGRADATION OF PETROLEUM HYDROCARBONS IN CONTAMINATED COASTAL ENVIRONMENTS, NIGERIA

by

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Under the Direction of Daniel Deocampo, PhD

## ABSTRACT

To compare the degree of biodegradation of petroleum hydrocarbons in sediments from Ikarama and Okwori in the Niger delta, Nigeria, concentrations of n-alkanes and polycyclic aromatic hydrocarbons in the sediments were measured. Analysis was conducted with gas chromatography using mass spectrometry detector. While the decrease in concentrations of n-alkanes and polycyclic aromatic hydrocarbons confirmed the process of biodegradation in the sediments it was not solely fit to substantiate the degree of biodegradation in the sediments. Hence the percentage proportion of n-alkanes and polycyclic aromatic hydrocarbons was used. The degree of biodegradation of n-alkanes in both Okwori and Ikarama was almost similar. However, it was observed polycyclic aromatic hydrocarbons were biodegraded in Okwori sediments than Ikarama sediments and this indicates the degree of biodegradation of petroleum hydrocarbons impacted sediments in Okwori is greater than that of Ikarama.

INDEX WORDS: Biodegradation, Polycyclic Aromatic Hydrocarbons, Hydrocarbon biodegradation, Alkanes, Gas Chromatography, Oil spill

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## DEDICATION

To God, the source of all wisdom, knowledge, understanding, dominion and power. You are my strength.

## ACKNOWLEDGEMENTS

I would like to express my profound gratitude to the graduate admission committee of the Department of Geosciences, headed by Jeremy Diem for giving me the memorable life time opportunity to take my career in Earth Sciences to the next level. Also to my thesis advisor, Daniel Deocampo, I am grateful you accepted me to be your academic advisee to learn from your wealth of geoscientific knowledge. If I have seen farther in geosciences, it is because I sit on the shoulders of giants: Daniel Deocampo, Brian Meyer, Nadine Kabengi, Lawrence Kiage, Crawford Elliot and Lakeisha Tate, you are the giants on whose shoulders I sit. Atieh Tajik, Basirat Lawal and Bone Drake thank you for all the administrative supports you gave me all through my stay in the Department of Geosciences.

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# LIST OF ABBREVIATION

EPA: Environmental Protection Agency

FME: Federal Ministry of Environment

GC-MS: Gas Chromatography-Mass Spectrometry

HMW: High Molecular Weight

HP: Hewlett Packard

IK: Ikarama

LMW: Low Molecular Weight

NOSDRA: National Oil Spill Detection and Response Agency

OK: Okwori

PAHs: Polycyclic Aromatic Hydrocarbons

PCB: Polychlorinated Biphenyl

PTFE: Polytetraflouroethylene

UNEP: United Nation Environmental Program

x

# 1 INTRODUCTION

Accidental oil spills have accounted for 10-15% of all oil that entered the world oceans, and the primary source of anthropogenic marine pollution is the land-based discharges (European Environmental Agency, 2013). Oil spills as a result of maritime accidents or oil and gas platforms and facilities failures, pose enormous environmental and economic threats to local communities, when resulting in release of crude oil into the sea (Palinkas et al., 1993; Arata et al.; Gill et al.,2012; Sammarco et al.,2013). Petroleum based products are the primary sources of energy for industry and daily life activities. In the course of exploration, production, refining, transport and storage of petroleum and petroleum products, the frequent occurrence of leaks and accidental spills is inevitable (Kven volden and Cooper, 2003). Most global petroleum hydrocarbon products can be degraded or otherwise changed by microorganisms: Microorganisms possess the ability to degrade the majority of natural hydrocarbons components, essentially the first saturated and unsaturated alkanes, monoaromatics and low molecular weight polycyclic hydrocarbons (PAHs) (Tippee, 1994; Oolman et al., 1992; Prince, 1993; Singh and Dasai, 1986; Van Hamme and Ward, 2001).The high molecular weight PAHs, resins, and asphaltenes are more resistant to biodegradation. Hydrocarbon-degrading microbes must maintain a constant contact with hydrocarbons using their substrate in order for hydrocarbons uptake to occur (Tippee, 1994; Oolman et al., 1992; Prince, 1993; Singh and Dasai, 1986; Van Hamme and Ward, 2001), and the nature of most insoluble petroleum hydrocarbons inhibit this contact (Bird and Ward, 1996b).

Biodegradation is the dominant and ultimate natural mechanism by which petroleum pollutants are eradicated from an environment (Atlas, 1992; Amund and Nwokoye, 1993; Lal and Khanna, 1996). Jones et al., (1983) reported biodegradation of alkyl aromatics which is a

petroleum-derived aromatic hydrocarbon in marine sediments by *Anthrocebacter*, *Burkholderia, Mycobacterium, Pseudomonas, Sphingomonas and Rhodococcus* and subsequent degradation of n-alkanes profile of the crude oil was detected in the same marine sediments. Adebusoye et al., 2007 also reported microbial degradation of petroleum hydrocarbons in a polluted tropical stream in Lagos, Nigeria, where nine bacteria strains namely, Pseudomonas fluorescents, *P.aeruginosa, Bacillus subtilis, Bacillus sp., Alcaligenes sp., Acinetobacter lwoffi, Flavobacterium sp. Micrococcus roseus, and Corynebacterium sp*. were isolated from the stream.

Essentially, bacteria, yeast, and fungi biodegrade hydrocarbons, and their biodegradation's efficiency varies from 6% (Jones et al., 1970) to 82% (Pinholt et al., 1979) for fungi, 0.13% (Jones et al., 1970) to 50% (Pinholt et al., 1979) for soil bacteria , and 0.003% (Holloway et al., 1980) to 100% ( Mulkinesetal, 1974) for marine bacteria. Bacteria are the most active agents in petroleum hydrocarbon biodegradation and they act as the pioneering degraders of oil spills in polluted environments (Rahman et al., 2003; Brooijmans et al., 2009). Several bacteria are renowned for feeding solely on hydrocarbons as a means of life sustenance (Yakimov et al., 2007). Due to a wide range of molecular structures present in crude oil in soil, freshwater and marine environments, many scientists prescribed the use of mixed microorganism populations, capable of rapidly degrading crude oil in the biotransformation of these complex hazardous environmental contaminants (Bartha and Bossert, 1984; Cooney, 1984 Atlas, 1985; Floodgate, 1984).

### 1.1 Factors Affecting Rate of Biodegradation

Petroleum hydrocarbons have different susceptibilities to microbial attack in the following common accepted decreasing order: n-alkanes > branched alkanes > low molecular

weight aromatics > cyclic alkanes (Perry, 1984). As mentioned earlier the saturates have been recorded to have the highest rate of biodegradation, followed by the light molecular weight aromatics while high molecular weight aromatics and polar compounds have extremely low rates of degradation (Fusey and Oudot, 1984; Jobson et.al, 1972; Walker et al., 1976). However, these patterns are not globally uniform and conventional; greater degradation of naphthalene than hexadecane in water-sediment mixtures from a freshwater lake, alkyl aromatics in preferential order to detectable changes in the n-alkanes profile in marine sediments contaminated with crude oil. Also quicker attack on aromatic hydrocarbons during the biodegradation of crude oil by marine microbial strains from a pristine and a commercial harbor has been recorded (Cooney et al., 1985; Jones et al., 1983; Fedorak and Westlake, 1981). Although, resins and ashphaltenes have been formerly considered to be recalcitrant to biodegradation, they were observed to have been degraded appreciably by using a continuous culture fermentation and a mixed culture of marine bacteria, and co-oxidation process in which non-growth hydrocarbons which are oxidized in the presence of hydrocarbons which can act as growth substrates (Horowitz and Atlas, 1977; Bertrand et al., 1983; Walker et al., 1975; Perry, 1979; Rontani et al., 1985).

The non-uniformity in biodegradation of petroleum hydrocarbons across the globe is due to different favorable factors which are in place at a point in time. In spite of the general trend which biodegradation of petroleum hydrocarbons should follow, no rule-of-thumb can ascertain its rate of occurrence. Many controlling factors have been established to determine biodegradation of petroleum hydrocarbons. According to Brusseau, (1998), the chemical composition and inherent biodegradability of petroleum hydrocarbons are of utmost importance in consideration of biodegradation as a remediation approach.

Nutrients like nitrogen, phosphorus, sometimes iron are the essential precursors in biodegradation of hydrocarbon contaminants (Cooney, 1984). Some of these nutrients could become the controlling factor invariably influencing biodegradation processes. A significant increase of carbon in freshwater and marine water when a large oil spill occurred rendered the available quantities of nitrogen and phosphorus the limiting factors in the oil degradation process (Cooney, 1984). Due to low levels of nitrogen and phosphorus in sea water, their limiting effect is more excruciating in marine environments than freshwater environments. Freshwater wetlands are known for lack of nutrients due to high demands of nutrients by the plants within the environments (Atlas, 1985; Floodgate, 1984; Mitsch and Gosselink, 1993). Hence, nutrients supplements were imperative to improve the biodegradation of oil contaminants (Choi et al., 2002; Kim et al., 2005). Conversely, excessive nutrients availability can prevent the process of biodegradation (Chaillan et al., 2006). Several adverse impacts of high level of Nitrogen-Phosphorus-Potassium (NPK) on the biodegradation of hydrocarbons have been recorded, in which there was no increase in biodegradation rate or an increase only after several months delay (Oudot et al., 1998; Chaineau et al., 2005) and commonly on aromatics (Carmichael and Pfaender, 1997).

In some cases, the effect of salinity on biodegradation of petroleum hydrocarbons has been reported. Shiaris, (1989), showed a commonly positive correlation between salinity and rates of microbial degradation of naphthalene and phenanthrene in estuarine sediments. Kerr and Capone (1988) established a direct proportion between salinity and mineralization of naphthalene in river sediments in which the estuarine sites showed more rapid biodegradation rates over a broad range of salinity than the less saline upstream site. However, Ward and Brock, (1978), recorded the adverse effect of hypersalinity on hydrocarbons mineralization rates. A

decrease in the rate of hydrocarbons metabolism within the increasing salinity range of 3.3 -28.4 psu has been registered as a result of an overall reduction in microbial metabolism in the environment.

The pH of an environment has an effect on the biodegradability of hydrocarbons pollutants in it. Dissimilar to most aquatic environments, pH of the soil is highly variable over a wide range: mine spoil of pH unit 2.5 through 11 in alkaline deserts (Bossert and Bertha, 1984). Most optimal microbial activity is enhanced by a pH close to neutrality, with fungi being more tolerant than bacteria in acidic condition (Atlas, 1988). The rate of biodegradation of gasoline was found to be doubled when its soil pH changed from 4.5 to 7.4. However a significant decrease in the rate was recorded when the pH was further increased to 8.5 (Verstraete et al., 1976).

Adaptation and acclimatization of microorganism can also affect biodegradation of petroleum hydrocarbons. Pre-exposure of a microbial community to hydrocarbons from both anthropogenic and natural sources is vital in biodegradation of hydrocarbon inputs which the microbial community comes in contact with (Bartha and Bossert, 1984; National Academy of Science, 1985). Seeding entails the additive effects of allochthonous microorganisms to the autochthonous microorganism to improve the rate and extent of biodegradation of hydrocarbon pollutants. This phenomenon is highly recommendable when the autochthonous microbial community is incapable or weak to degrade a broad range of complex petroleum hydrocarbon mixtures (Leahy and Colwell, 1990). Atlas, (1977), stated the required criteria for allochthonous seeding organisms to be efficient and not invasive; ability to degrade full range of petroleum components, genetic stability and viability during incubation, fast growth following incubation,

high extent of growth and enzymatic activity in the environment, competitiveness with indigenous microorganism, non-pathogenicity and inability to produce toxic metabolites.

The role of oxidation in biodegradation of hydrocarbons cannot be overemphasized. The priority steps in the catabolism of aliphatic, cyclic and aromatics hydrocarbons by microbial activities require the oxidation of the substrate by oxygenase. Hence, a need for molecular oxygen is required inevitably (Cerniglia, 1984; Perry, 1984; Singer et al., 1985). Oxygen as a limiting condition is not applicable to the upper levels of the water columns in marine and freshwater environments. However, aquatic sediments are considered anoxic except the layers at the sediments surfaces (Cooney, 1984; Floodgate, 1984; Hambrick, 1980). The presence of oxygen in soil depends on rates of microbial oxygen consumption, soil type, soil moisture content and availability of utilizable substrates which can promote oxygen depletion (Bossert and Bartha, 1984). The concentration of oxygen has been observed as the rate-controlling variable in the biodegradation of petroleum hydrocarbons in soil and groundwater (Jamison et al., 1975; Von Wedel et al., 1988). However, several recent studies have shown that monoaromatic hydrocarbons such as benzene, toluene, xylene, and hexadecane and some low molecular weight polycyclic aromatic hydrocarbons can be broken down anaerobically, using nitrate, ferric and sulfate ions as terminal electron acceptors in oxidizing the hydrocarbon pollutants to water and carbon dioxide (Beller et al., 1992; Coates et al., 1996; Lovely et al., 1994; Rabus and Widdel, 1995; Reuter et al., 1994)

Temperature holds a significant role in biodegradation of petroleum hydrocarbons; directly impacting the chemistry of the pollutants, physiology, and diversity of the microbial fauna. Atlas, 1975, observed that at low temperature, toxic low molecular weight hydrocarbons have decreased volatility and increased viscosity which in turn inhibit the commencement of

biodegradation. Temperature also influences the solubility of hydrocarbons (Foght et al., 1996). Although a decrease in temperature reduces the rate of biodegradation, the process takes place over a broad range of temperature. The decreasing order of degradation rates occur in the ranges 30-40 $^0$C, 20-30 $^0$C and 15-20 $^0$C in soil, freshwater and marine environments respectively (Bartha and Bossert, 1984; Cooney, 1984). Venosa and Zhu, 2003, recorded the effects of ambient temperature on both the properties of spilled oil and the microbial activity. Biodegradation of petroleum hydrocarbons has occurred even in psychrophilic environments in temperate areas (Pelletier et al., 2004; Delille et al., 2004)

## 1.2 Background: Oil and Gas Exploration in the Niger Delta, Nigeria

Nigeria is one of the largest oil producing countries in the world. The economy of Nigeria solely depends on the oil and gas industries which carry out most of their exploration and production activities in the Niger Delta (Ijah, 1998). The Niger Delta is a great wetland and marine ecosystems. However, the region has been rendered one of the five world's severely hydrocarbons devastated environments due to unsustainable oil exploration activities (FME, 2006). The oil spill is a regular event in the Nigeria, causes by corrosion of pipelines and tanks, sabotage and vandalization of pipelines, accidents, mishandling of facility and oil production operations (Nwilo and Badejo, 2005). Crude oil spills have devastated both terrestrial and aquatic environments in the Niger Delta for more than 50 years of crude oil exploration and production (Adati, 2012).

In 2011, United Nations Environment Program (UNEP) reported that oil pollution in Ogoni land (in River State, Nigeria) is severe and wild covering land areas, sediments and swampland. The investigation also confirmed high hydrocarbon contaminants in the surface

water throughout the creeks. Bayelsa State is one the most prolific oil and gas states in the Niger Delta. However, it is confronted with the worst environmental despoliation caused by oil and gas exploration and exploitation activities ever recorded in the country. The National Oil Spill Detection and Response Agency (NOSDRA) revealed that Bayelsa State experienced about 40 oil spills in a month. The rate of crude oil spills in Bayelsa State is more destructive than that of the globally reported Ogoni land (NOSDRA, 2014).

The final destinations of the spilled oil vary from one part of the globe to the other, due to many remediations and recovery approaches. In Europe, it has been legislated that as soon as an accidental oil spill occurs, a monitoring program responsible for quantifying the environmental impacts of the spill usually sets up. The assessment of the environmental impact is vital for the decision-making procedures in the choice and execution of an appropriate response and restoration plans (Kirby and Law, 2010). The oil and gas sector in Nigeria by practice and perception has fallen short of its expectation to combat the incessant life threatening environmental pollution in the Niger Delta region. Pollution as a result of activities of oil companies in Nigeria has made life miserable for their host communities; aquatic life is wiped out, agriculture activities are disrupted, and hazardous gasses are released into the atmosphere (Mitchell et al., 2001; Collier and Hoefler, 2005).

The vast difference between Nigeria and the rest of the world, such as the Gulf of Mexico oil spills, is as much in the pattern of response by the United States government, existing environmental regulations and the oil company. The U.S. president personally visited the Deepwater Horizon spill scene on time, and immediate recovery of the polluted areas ensued. Many of Nigeria’s oil-impacted areas are yet to receive adequate attention, even in the few

polluted sites the oil companies claimed to have cleaned up, the nature of remediation and recovery procedures were not adequately documented (UNEP,2011).

The fate of hydrocarbon contaminants in an environment is determined by their chemical nature and the presence of the degradative microbial community. If the polluted area has favorable environmental factors for microbial activities and metabolisms, there is a tendency for the growth of the active and viable community of hydrocarbon utilizing microorganisms (Chikere et al., 2011, 2012b). Considering a massive amount of oil pollution in the Niger Delta region mainly on farmland and rivers, the need to clean up the environment is sacrosanct (Vincent et al., 2011). Conventional methods of physical removal of oil are the first response which is not always virile enough to achieve a complete clean-up process. Likewise, mechanical methods maximally recover 10-15% of crude oil after a major oil spill and the rest of the crude oil is left to worsen the conditions of the contaminated environment (Abu and Dike, 2008)

### *1.2.1 Aerobic Versus Anaerobic Biodegradation of Petroleum Hydrocarbons*

The process of aerobic biodegradation of hydrocarbons involves the breaking down of the contaminants by microorganisms using oxygen as an electron acceptor and mostly the final products are carbon dioxide and water (Habe and Omori, 2003). An introduction of microbial consortia (containing bacteria, fungi and bacteria-fungi mixture) has been recorded to have successfully degraded the 16 priority PAHs to lower degrees of concentrations (Li et al., 2008). Biodegradation of hydrocarbons can take place under both aerobic and anaerobic conditions. However, the rate and degree of hydrocarbons biodegradation are lower, and the range of substrates mineralized is characteristically smaller under anaerobic conditions than aerobic conditions (Coates et al., 1997; Bertrand et al., 1989).There are different categories of

microorganisms that can mineralize hydrocarbons under anaerobic conditions: the facultative anaerobes which use nitrate, iron and manganese as their electron acceptor and the strict anaerobes which use sulfate as their electron acceptor. Many studies have shown the anaerobic oxidation of hydrocarbons by pure bacterial cultures which involve sulfate, nitrate, iron or manganese reducing conditions (Coates et al., 1997; Fries et al., 1994).

## 1.3 Purpose of Study

Numerous studies on the environmental pollution in the Niger Delta have been carried out (Kakulu and Osibanjo, 1992; Daniel and Braide, 2002; Iwegbue et al., 2006; Chikere and Ekwuabu, 2014; Asuquo and Ewa-Oboho, 2004; Nkpaa et al., 2013; Umeh, 2009). Nevertheless, most previous studies have been restricted to small areas and usually done on surface water, groundwater, vegetation, microbial community and fishes. Limited data are available on the occurrence of PAHs and n-alkanes compounds in the soil and sediments of the region. The main objectives of this study are to investigate the level of hydrocarbon contamination in two communities in the Niger Delta and also to compare the biodegradation rates in those communities. The hypothesis is that the rate of biodegradation is faster under aerobic conditions than anaerobic conditions; a marine environment is considered to be more aerobic than a freshwater environment (Bertrand et al., 1989; Coates et al., 1997).

### *1.3.1 Study Areas*

Ikarama is located in Bayelsa state, on latitude $5^{0}08^{'}57.5^{''}$ N and longitude $6^{0}27^{'}10.3^{''}$ E, in the lower delta plain believed to have been formed during the Holocene of the Quaternary period by the accumulation of sedimentary deposits. The main geological setting of Ikarama is sedimentary alluvium. Ikarama is a low land freshwater swamp with elevation 5-7 above mean

sea level. The main soil types in Ikarama are young, shallow, poorly drained (clayey) and acid sulfate soils with texture ranging from medium to fine grains (Wizor and Agbabou,2014)

Okwori is located River state, on latitude $4^{0}22^{'}00^{''}$ N and longitude $6^{0}57^{'}00^{''}$E. It is a typical marine setting in the Niger Delta region; consist of marine sediments which are mainly supplied by the Benue-Niger drainage system and the Cross rivers system which supplied sediments to the eastern delta area of which the south eastern offshore is a prominent portion. The sediments are mainly sand and few pebbles with texture ranging from fine to coarse grains*

*www.offshore-mag.com

The study areas are Ikarama (Bayelsa State) and Okwori (River State), Niger Delta areas in Nigeria. Ikarama is a freshwater environment while Okwori is a marine water environment. The amount of PAHs and n-alkanes in the communities will be measured. The measurement of PAHs and n-alkanes as petroleum hydrocarbon components are chosen in this study due to their biodegradability and bioavailability in these environments. Although it has been established that high molecular weight PAHs are difficult to biodegrade by microbial activities and metabolisms, their significant presence would indicate the level of toxicity, mutagenicity and carcinogenicity they pose to the environment in which they are found beyond the recommended safe limits.

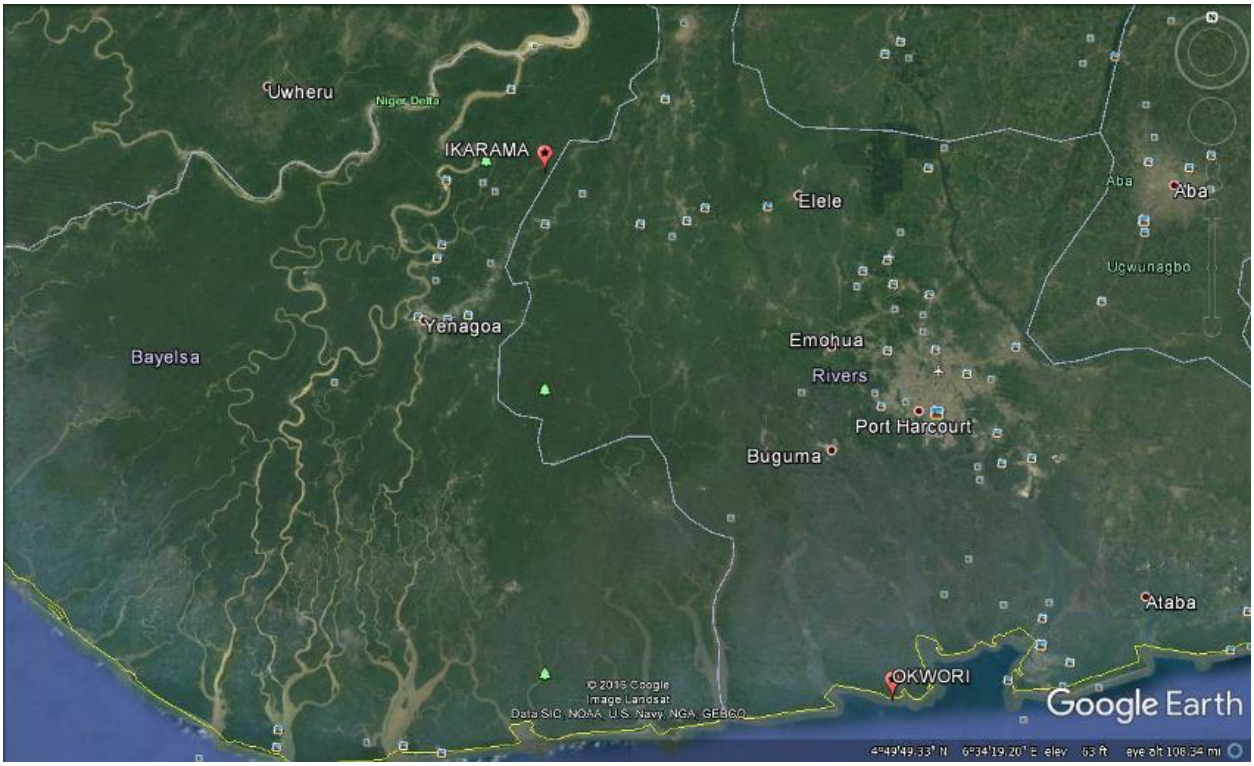
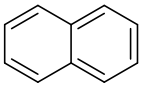
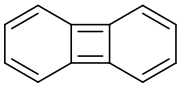


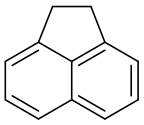

*Figure 1: Study Areas Map*



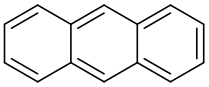
Naphthalene
$C_{10}H_{8,}$
MW: 128.17



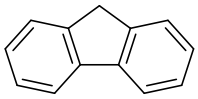
Biphenylene
$C_{12}H_8$
MW: 152.20



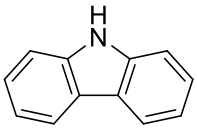
acenaphthene
$C_{12}H_{10}$
MW: 154.21



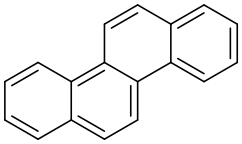
anthracene
$C_{14}H_{10}$
MW: 178.23



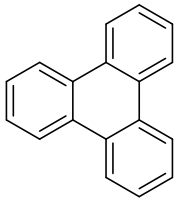
9*H*-fluorene
$C_{13}H_{10}$
MW166.22



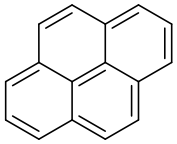

9*H*-carbazole
$C_{12}H_9N$
MW: 167.21



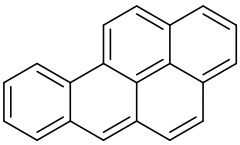
Chrysene
$C_{18}H_{12}$
MW: 228.29



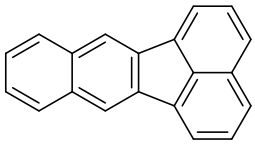
Triphenylene
$C_{18}H_{12}$
MW: 228.29



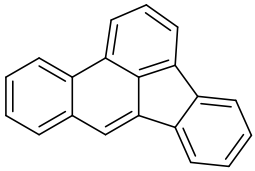
pyrene
$C_{16}H_{10}$
MW: 202.26



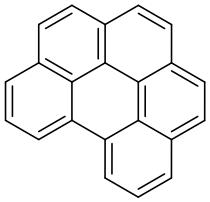
Benzo(a)pyrene
$C_{20}H_{12}$
MW: 252.32



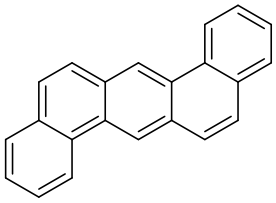
Benzo(K)fluoranthene
$C_{20}H_{12}$
MW: 252.32



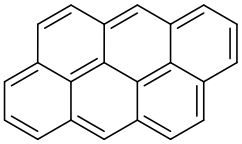
Benzo(e)acephenanthrylene
$C_{20}H_{12}$
MW: 252.32



Benzo(ghi)perylene
$C_{22}H_{12}$
MW: 276.34



Dibenzo(a,h)anthracene
$C_{22}H_{14}$
MW: 278.35



Dibenzo(def,mno)chrysene
$C_{22}H_{12}$
MW: 276.34

MW=Molecular Weight

*Figure 2: Analyzed PAHs' Chemical Formulas, Structures and Molecular Weights*

# 2 MATERIALS AND METHODS

Sediments and water samples were collected at five different points in both Ikarama and Okwori, Niger Delta; 3 sediments samples from Ikarama and 2 sediment samples from Okwori. These sites were selected due to their similarity in the nature of crude oil pollution and difference in their oxidation potentials. Okwori being close to a beach, a marine water environment considered to be more oxidizing than Ikarama, a swampy-freshwater environment. The samples were collected by scooping petroleum hydrocarbons contaminated sediments from the surface landscape randomly from the study areas and stored in colorless borosilicate glass containers with polytetrafluoroethylene (PTFE) lined caps. Ikarama samples were labeled IK1-IK3 and Okwori samples, OK1-OK2. The samples were stored in the refrigerator at 4 $^{0}$C before they were taken to the laboratory for analysis.

## 2.1 Gas Chromatography-Mass Spectrometry (GC-MS) *

The sample is injected into the inlet where it is volatilized and an appreciable portion is taken onto the column aided by the carrier gas ($N_2$ or He). Differential partitioning then separated the sample components into stationary and mobile phases. The separated sample components are removed from the column into the detector where their physicochemical property is detected and a signal is generated. The signal is then amplified and sent to the data system where the chromatograph is electronically constructed.

**Gas inlets:** Gas is fed from cylinders through supply piping into the instrument. It is common to filter gas in order to have highly pure gas and the gas supply pressure may be controlled at the bench appropriately. The gas supply is regulated to the desired flow and then

transferred to the required parts of the instrument. Control is always maintained on the incoming gas and the gas supply to the various parts of the instrument.

**Injector:** Here the sample is volatilized and the resulting gas is moved into the carrier stream entering the GC column.

**Column:** In GC, retention of analyte molecules is as a result of stronger interactions with stationary phase and mobile phase. The interactions could be dispersive, dipole or hydrogen bonding. The sample is separated into its different components in the column. Columns differ in length and internal diameter based on the application type and can either be packed or capillary. Packed columns are packed with a solid support coated with immobilized liquid stationary phase material. Capillary columns are long hollow silica tubes with their inside walls coated with immobilized liquid stationary phase material of various film thickness.

**Column oven:** Temperature in GC is controlled through a heated oven. The oven heats effectively to give excellent thermal control. The oven is cooled using a fan and vent set-up usually at the back of the oven. A hanger or cage is always attached to support the GC column and prevents it from touching the oven walls because this could damage the column. The injector and detector connections are also cased in the GC oven. During isothermal operation, the GC is held at a steady temperature while in temperature programmed GC, the oven temperature is raised based on the analysis temperature program.

Detector: The detector responds to a physicochemical property of the analyte, the response is amplified and an electronic signal is generated for the data system to produce a chromatogram, where the quantitative mass spectrometry of the analytes is taken.

*www.chromacademy.com

To measure the n-alkanes and PAHs hydrocarbons components in the samples, Hewlett Packard 6890 Gas Chromatography oven supplied with an HP 5973 Mass Selective Detector (MSD) and HP 6890 auto-injection unit was used at the University of Georgia, Athens. The analyses were performed by Dr. Sayed M. Hassan in the Laboratory for Environmental Analysis, Center for Applied Isotopes Studies.

Soil samples were extracted with a mixture of equal volumes of acetone and dichloromethane in an ultrasonic bath for three times. The extracts were evaporated to dryness using a stream of nitrogen gas passing over them, and they are then dissolved in toluene for Gas Chromatography-Mass Spectrometer by adapting to Environmental Protection Agency (EPA) method 8275A except thermal extract part of it.

The Gas Chromatography oven program for PAHs and n-alkanes is as follows: Column type – HP 19091J-433.HP-5, 30m X 250μm X 0.25μm film thickness, silicone coated fused silica capillary column kept at the constant pressure of 8.99 psi. The inlet temperature was 250 $^{0}$C, pressure 8.99 psi and split ratio was 50:1.

Oven – initial temperature 50 $^{0}$C, initial time 1 minute, using the ramp rate of 10 $^{0}$C/min to increase to final temperature and time as 260 $^{0}$C and 12 minutes respectively.

*Figure 3: Gas Chromatography- Mass Spectrometry Equipment*

# 3 RESULTS AND DISCUSSION

## 3.1 N-Alkanes Series Hydrocarbons Components

*Table 1: Concentration of aliphatic hydrocarbon components in the samples sediments in the first measurement*

| Number of Carbon Atoms | Ikarama 1 | Ikarama 2 | Ikarama 3 | Okwori 1 | Okwori 2 |
|---|---|---|---|---|---|
| | Concentration, mg/kg | | | | |
| | IK-1 | IK-2 | IK-3 | OK-1 | OK-2 |
| $C_{10}$ | 1.26 | 3.87 | 11.38 | 36.13 | 31.34 |
| $C_{11}$ | 4.83 | 6.50 | 12.01 | 110.23 | 157.57 |
| $C_{12}$ | 7.23 | 9.50 | 18.43 | 326.47 | 423.34 |
| $C_{13}$ | 3.42 | 6.26 | 0.08 | 1076.70 | 1217.69 |
| $C_{14}$ | 7.96 | 17.37 | 30.85 | 525.84 | 452.59 |
| $C_{15}$ | 0.99 | 12.36 | 17.35 | ND | ND |
| $C_{16}$ | 4.16 | 10.70 | 0.00 | 1083.81 | 872.32 |
| $C_{17}$ | 23.51 | 11.12 | 80.10 | 802.65 | 835.37 |
| $C_{18}$ | 9.55 | 5.95 | 34.10 | 303.37 | 576.29 |
| $C_{19}$ | ND | 9.49 | ND | ND | 1300.89 |
| $C_{20}$ | 7.77 | 9.68 | 15.88 | ND | 518.56 |
| $C_{21}$ | 1.59 | 8.45 | 0.09 | ND | 1031.45 |
| $C_{22}$ | 0.31 | 7.25 | 6.88 | 68.02 | 309.18 |
| $C_{23}$ | 0.63 | 7.41 | 6.40 | 460.97 | 772.96 |
| $C_{24}$ | 6.48 | 7.91 | 15.34 | ND | ND |
| $C_{25}$ | 10.34 | 6.43 | 29.03 | 509.33 | 589.78 |
| $C_{26}$ | 6.42 | 3.93 | 18.60 | 310.43 | 477.05 |
| $C_{27}$ | 6.78 | 5.23 | 17.99 | 335.14 | 386.93 |
| $C_{28}$ | 3.89 | 2.97 | 10.07 | 235.37 | 281.17 |
| | 107.11 | 152.39 | 324.59 | 6184.46 | 10234.47 |

**C= number of carbon atoms IK= freshwater environment OK= marine environment**

Table 1 shows the concentrations and the sum of concentrations of aliphatic hydrocarbons contents in the samples sediments from Ikarama (IK) and Okwori (OK) which were measured the first day the samples got to the laboratory. Table 2 shows the concentrations and the sum of concentrations of aliphatic hydrocarbons contents in the same samples measured two days later after they got to the laboratory (not kept in a degradation amenable condition).

Although some of the aliphatic hydrocarbons were not found at detectable level, from Table 1, the minimum value is 0.001 mg/Kg, and maximum value is 1300 mg/Kg; ranging 0.31-23.51 mg/Kg in IK-1 sediments, 2.97-17.37 mg/Kg in IK-2 sediments, 0-80.10 mg/Kg in IK-3 sediments, 36.13-1083.81 mg/Kg in OK-1 sediments and 31.34-1300.89 mg/Kg in OK-2 sediments. Also, Table 2 shows the minimum and maximum values of concentrations of aliphatic hydrocarbons contents in the samples sediments as 0 and 258.729 mg/Kg respectively; ranging 0.062-4.705 mg/Kg in IK-1 sediments, 0.590-3.450 mg/Kg in IK-2 sediments, 0-6.775 mg/Kg in IK-3 sediments, 7.166-214.957 mg/Kg in OK-1 sediments and 6.233-258.729 mg/Kg in OK-2 sediments. The sum of concentrations of aliphatic hydrocarbons contents for IK-1, IK-2, IK-3, OK-1 and OK-2 samples sediments are 107.11 mg/Kg, 152.39 mg/Kg, 324.59 mg/Kg, 6184.46 mg/Kg and 10234.47 mg/Kg respectively as shown in Table 1 while Table 2 gives the sum of the aliphatic hydrocarbons contents in IK-1, IK-2, IK-3, OK-1 and OK-2 samples sediments as 21.435 mg/Kg, 30.266 mg/Kg, 48.578 mg/Kg, 1226.589 mg/Kg and 2035.495 mg/Kg respectively.

*Table 2: Concentration of aliphatic hydrocarbon components in the samples sediments in the second measurement*

| **Number of Carbon Atoms** | IK-1 | IK-2 | IK-3 | OK-1 | OK-2 |
|---|---|---|---|---|---|
| **$C_{10}$** | 0.25 | 0.77 | 2.26 | 7.17 | 6.23 |
| **$C_{11}$** | 0.97 | 1.29 | 2.39 | 21.86 | 31.34 |
| **$C_{12}$** | 1.45 | 1.89 | 3.66 | 64.75 | 84.20 |
| **$C_{13}$** | 0.68 | 1.24 | 0.02 | 213.55 | 242.18 |
| **$C_{14}$** | 1.59 | 3.45 | 6.13 | 104.29 | 90.02 |
| **$C_{15}$** | 0.20 | 2.45 | 3.45 | ND | ND |
| **$C_{16}$** | 0.83 | 2.13 | 0.00 | 214.96 | 173.49 |
| **$C_{17}$** | 4.70 | 2.21 | ND | 159.19 | 166.14 |
| **$C_{18}$** | 1.91 | 1.18 | 6.78 | 60.17 | 114.62 |
| **$C_{19}$** | ND | 1.89 | ND | ND | 258.73 |
| **$C_{20}$** | 1.55 | 1.92 | 3.16 | ND | 103.13 |
| **$C_{21}$** | 0.32 | 1.68 | 0.02 | ND | 205.14 |
| **$C_{22}$** | 0.06 | 1.44 | 1.37 | 13.49 | 61.49 |
| **$C_{23}$** | 0.13 | 1.47 | 1.27 | 91.43 | 153.73 |
| **$C_{24}$** | 1.30 | 1.57 | 3.05 | ND | ND |
| **$C_{25}$** | 2.07 | 1.28 | 5.77 | 101.02 | 117.30 |
| **$C_{26}$** | 1.29 | 0.78 | 3.70 | 61.57 | 94.88 |
| **$C_{27}$** | 1.36 | 1.04 | 3.57 | 66.47 | 76.96 |
| **$C_{28}$** | 0.78 | 0.59 | 2.00 | 46.68 | 55.92 |
| | 21.50 | 30.27 | 48.60 | 1226.60 | 2035.50 |

**C= number of carbon atoms IK= freshwater environment OK= marine environment**

The observed difference in the sum of the concentration of the aliphatic hydrocarbons components in all the samples sediments in Table 1 and Table 2 establish the fact that biodegradation of petroleum hydrocarbons has taken place. The biodegradation of the aliphatic hydrocarbon components in the samples sediments shows an approximate uniform decrease in the sum of the n-alkanes series present in all samples sediments (mathematically; the second measurements are approximately one-fifth of the first measurements). While the rate of biodegradation in the two environments (Ikarama and Okwori) appears to be the same, their degree of biodegradation of n-alkanes is not the same. To establish the extent of biodegradation in the two environments, which is the scope of this study, the percentage proportions of n-

alkanes components in all the samples sediments were calculated from Table 1 and Table 2 to produce Table 3. Using the mathematical relation as below;

$$\boldsymbol{percentage\ proportion} =$$

$$(\boldsymbol{concentration\ of\ a\ component / total\ concentration\ of\ all\ components}) * \mathbf{100}\ \%$$

*Table 3: Percentage proportion of n-alkanes components in all the samples sediments from the first and second measurements*

| Number of Carbon Atoms | % IK-1 | % IK-2 | % IK-3 | % OK-1 | % OK-2 |
|---|---|---|---|---|---|
| $C_{10}$ | 1.17 | 2.54 | 3.51 | 0.58 | 0.31 |
| $C_{11}$ | 4.51 | 4.27 | 3.70 | 1.78 | 1.54 |
| $C_{12}$ | 6.75 | 6.23 | 5.68 | 5.28 | 4.14 |
| $C_{13}$ | 3.19 | 4.11 | 0.03 | 17.41 | 11.90 |
| $C_{14}$ | 7.43 | 11.40 | 9.50 | 8.50 | 4.42 |
| $C_{15}$ | 0.92 | 8.11 | 5.35 | 0 | 0 |
| $C_{16}$ | 3.88 | 7.02 | 0 | 17.52 | 8.52 |
| $C_{17}$ | 21.95 | 7.30 | 24.68 | 12.98 | 8.16 |
| $C_{18}$ | 8.92 | 3.91 | 10.51 | 4.90 | 5.63 |
| $C_{19}$ | 0 | 6.23 | 0 | 0 | 12.71 |
| $C_{20}$ | 7.25 | 6.35 | 4.89 | 0 | 5.07 |
| $C_{21}$ | 1.48 | 5.54 | 0.03 | 0 | 10.08 |
| $C_{22}$ | 0.29 | 4.76 | 2.12 | 1.10 | 3.02 |
| $C_{23}$ | 0.59 | 4.86 | 1.97 | 7.45 | 7.55 |
| $C_{24}$ | 6.05 | 5.19 | 4.73 | 0 | 0 |
| $C_{25}$ | 9.65 | 4.22 | 8.94 | 8.24 | 5.76 |
| $C_{26}$ | 5.99 | 2.58 | 5.73 | 5.02 | 4.66 |
| $C_{27}$ | 6.33 | 3.43 | 5.54 | 5.42 | 3.78 |
| $C_{28}$ | 3.63 | 1.95 | 3.10 | 3.81 | 2.75 |

**C= number of carbon atoms IK= freshwater environment OK= marine environment**

*Table 4: Modified Percentage proportion of n-alkanes components in all the samples sediments to calculate molecular weight index*

| Number of carbon atoms | % IK-1 | % IK-2 | % IK-3 | %OK-1 | %OK-2 | Avg. IK | Avg.OK |
|---|---|---|---|---|---|---|---|
| $C_{10}$ | 1.17 | 2.54 | 4.66 | 0.58 | 0.31 | 2.41 | 0.44 |
| $C_{11}$ | 4.51 | 4.27 | 4.91 | 1.78 | 1.54 | 4.16 | 1.66 |
| $C_{12}$ | 6.75 | 6.23 | 7.54 | 5.28 | 4.14 | 6.22 | 4.71 |
| $C_{13}$ | 3.19 | 4.11 | 0.03 | 17.41 | 11.9 | 2.44 | 14.65 |
| $C_{14}$ | 7.43 | 11.4 | 12.62 | 8.5 | 4.42 | 9.45 | 6.46 |
| $C_{15}$ | 0.92 | 8.11 | 7.1 | 0 | 0 | 4.79 | 0 |
| $C_{16}$ | 3.88 | 7.02 | 0 | 17.52 | 8.52 | 3.64 | 13.02 |
| $C_{17}$ | 21.95 | 7.3 | 0 | 12.98 | 8.16 | 17.98 | 10.57 |
| $C_{18}$ | 8.92 | 3.91 | 13.95 | 4.91 | 5.63 | 7.78 | 5.27 |
| $C_{19}$ | 0 | 6.23 | 0 | 0 | 12.71 | 2.08 | 6.36 |
| $C_{20}$ | 7.25 | 6.35 | 6.5 | 0 | 5.07 | 6.16 | 2.53 |
| $C_{21}$ | 1.48 | 5.54 | 0.038 | 0 | 10.08 | 2.35 | 5.04 |
| $C_{22}$ | 0.29 | 4.76 | 2.82 | 1.1 | 3.02 | 2.39 | 2.06 |
| $C_{23}$ | 0.59 | 4.86 | 2.62 | 7.45 | 7.55 | 2.47 | 7.5 |
| $C_{24}$ | 6.05 | 5.19 | 6.28 | 0 | 0 | 5.32 | 0 |
| $C_{25}$ | 3.7 | 4.22 | 11.87 | 8.24 | 5.76 | 7.61 | 7 |
| $C_{26}$ | 5.99 | 2.58 | 7.61 | 5.02 | 4.66 | 4.77 | 4.84 |
| $C_{27}$ | 6.33 | 3.43 | 7.36 | 5.42 | 3.78 | 5.1 | 4.6 |
| $C_{28}$ | 3.63 | 1.95 | 4.12 | 3.81 | 2.75 | 2.89 | 3.27 |

**C= number of carbon atoms IK= freshwater environment OK= marine environment**

In spite of the differences in the concentrations of aliphatic hydrocarbons contents in all the samples sediments, their percentage proportions remained the same as observed in Table 3. This further confirms the authenticity and reliability in precision of the percentage proportions of n-alkanes components to adjudge the extent to which biodegradation has occurred in the two environments. The lower the percentage proportion value of an aliphatic hydrocarbon, the higher the degree of weathering (biodegradation and dissolution) the sediments have experienced. Decane, Undecane, Dodecane, Tetradecane, Pentadecane, Heptadecane, Octadecane, Eicosane,

Docosane, Tetracosane, Pentacosane, Hexacosane, Heptacosane and Octacosane are more degraded in Okwori sediments due to their lower percentage proportion values than the Ikarama sediments which have higher percentage proportions. Tridecane, Hexadecane, Nonadecane, Heneicosane and Tricosane are more biodegraded in Ikarama than Okwori. Obviously, the degree of biodegradation in Okwori, a marine environment is more than that of Ikarama, a freshwater environment. The extent of biodegradation of n-alkanes in the two environments can be seen in Figure 4 below.

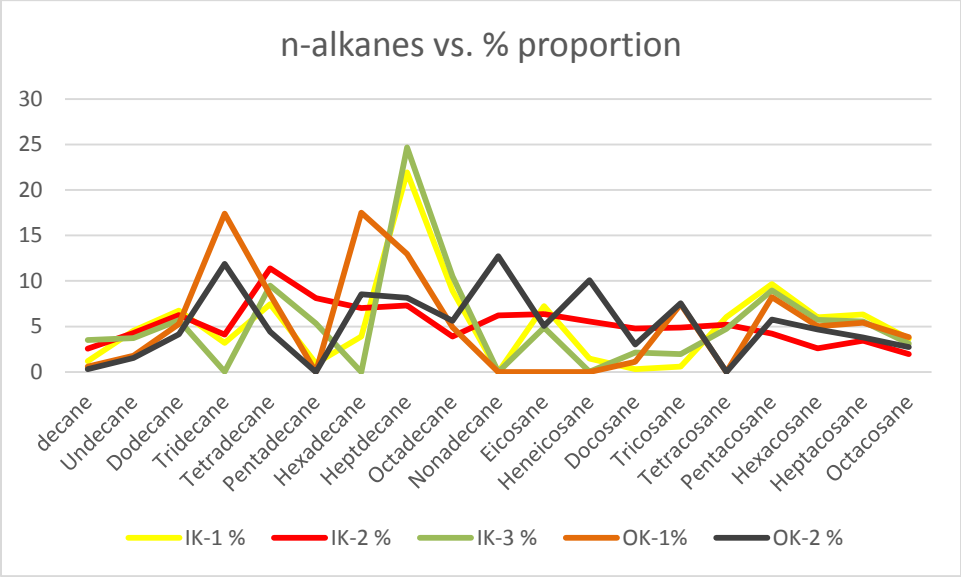



*Figure 4: Plots showing the percentage proportion of n-alkanes present in all the samples sediments*

Also, the molecular weight index of the alkanes is calculated as ratio of sum of lower carbons atoms to sum of higher carbon atoms as follows $MWI_{alkanes} = \Sigma(C_{10} - C_{19}) \Big/ \Sigma(C_{20} - C_{28})$.

From table 4 above, the molecular weight index of Ikarama is approximately 1.6 and that of Okwori is approximately 1.7. This is not a significant difference, so with regard to preferential degradation of short versus long chain alkanes, the two localities are very similar.

## 3.2 Polycyclic Aromatic Hydrocarbons Components

*Table 5: Concentration of polycyclic aromatic hydrocarbon components in the samples sediments in the first measurement*

| | Sample (mg/Kg) | | | | |
|---|---|---|---|---|---|
| **Compound** | IK-1 | IK-2 | IK-3 | OK-1 | OK-2 |
| **Naphthalene** | 0.4264 | 0.6237 | 1.2862 | 13.2302 | 10.9545 |
| **Biphenylene** | 0.0676 | 0.0006 | 0.0115 | 0.0322 | 0.0451 |
| **Acenaphthene** | 0.1 | 0.17 | 1.65 | 9.43 | 11.34 |
| **9H-Fluorene** | 0.0037 | 0.0828 | 0.0258 | 6.9576 | 10.5798 |
| **Anthracene** | 0.0011 | 0.92 | 0.0089 | 40.98 | 54.89 |
| **9H-Carbazole** | 0.0493 | 0 | 0.0032 | 0.011 | 0.0113 |
| **Pyrene** | 0.22 | 0.28 | 0.363 | 0.96 | 1.1528 |
| **Chrysene** | 0.2352 | 0.3666 | 0.8554 | 1.15 | 1.58 |
| **Triphenylene** | 0 | 0 | 0 | 0 | 0 |
| **Benzo [a] pyrene** | 0.0005 | 0.2511 | 0.0012 | 0.0014 | 0.0022 |
| **Benzo [k] fluoranthene** | 0 | 0 | 0 | 0 | 0 |
| **Benz [e] acephenanthrylene** | 0.0014 | 0.0612 | 0.0459 | 0.0025 | 0.0017 |
| **Benzo [ghi] perylene** | 0 | 0 | 0 | 0 | 0.0441 |
| **Dibenz [a,h] anthracene** | 0 | 0 | 0.0014 | 0.0024 | 0.0025 |
| **Dibenzo [def,mno] chrysene** | 0.07 | 0 | 0.2444 | 0.07 | 0.105 |
| | 1.1752 | 2.756 | 4.4969 | 72.8273 | 90.709 |

**IK= freshwater environment OK= marine environment**

Table 5 displays the concentrations and sum of concentrations of PAHs contents in the samples sediments from Ikarama and Okwori, which were measured the first day they got to the laboratory. Table 6 displays the concentrations and sum of concentrations of PAHs contents in the same samples sediments which were measured the fifth day after they got to the laboratory. In some samples sediments few PAHs components were not found at detectable level, but minimum and maximum concentration values in Table 5 are 0 and 54.89 mg/Kg respectively; ranging 0-0.4264 mg/Kg in IK-1 sediments, 0-0.92 mg/Kg in IK-2 sediments, 0-1.65 mg/Kg in IK-3 sediments, 0-40.98 mg/Kg in OK-1 sediments and 0-54.89 mg/Kg in OK-2 sediments. In Table 6, the minimum and maximum values of PAHs components concentrations are 0 and 10.92 mg/Kg respectively; ranging 0-0.08533 mg/Kg in IK-1 sediments, 0-0.18272 mg/Kg in IK-2

sediments, 0-0.32784 mg/Kg in IK-3 sediments, 0-8.12773 mg/Kg in OK-1 sediments, 0-10.92 mg/Kg in OK-2 sediments. The sum of concentrations of PAHs components in all the samples are 1.1752 mg/Kg, 2.756 mg/Kg, 4.4969 mg/Kg, 72.8273 mg/Kg and 90.709 mg/Kg for IK-1, IK-2, IK-3, OK-1 and OK-2 respectively as shown in Table 5 while the sum of concentrations of the PAHs components in all the samples for IK-1, IK-2, IK-3, OK-1 and OK-2 are 0.23518 mg/Kg, 0.54737 mg/Kg, 0.89348 mg/Kg,14.14 mg/Kg and 18.04 mg/Kg respectively as shown in Table 6.

*Table 6: Concentration of polycyclic aromatic hydrocarbon components in the samples sediments in the second measurement*

| | Sample ID | | | | |
|---|---|---|---|---|---|
| **Compound** | IK-1 | IK-2 | IK-3 | OK-1 | OK-2 |
| | Concentration ppm | | | | |
| | IK-1 | IK-2 | IK-3 | OK-1 | OK-2 |
| **Naphthalene** | 0.08533 | 0.12387 | 0.25555 | 2.62400 | 2.17870 |
| **Biphenylene** | 0.01353 | 0.00012 | 0.00228 | 0.00639 | 0.00897 |
| **Acenaphthene** | 0.02001 | 0.03376 | 0.32784 | 1.87029 | 2.25537 |
| **9H-Fluorene** | 0.00074 | 0.01644 | 0.00513 | 1.37993 | 2.10418 |
| **Anthracene** | 0.00022 | 0.18272 | 0.00177 | 8.12773 | 10.92 |
| **9H-Carbazole** | 0.00987 | 0.00000 | 0.00064 | 0.00218 | 0.00225 |
| **Pyrene** | 0.04403 | 0.05561 | 0.07212 | 0.19040 | 0.22928 |
| **Chrysene** | 0.04707 | 0.07281 | 0.16996 | 0.22808 | 0.31424 |
| **Triphenylene** | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00000 |
| **Benzo [a] pyrene** | 0.00010 | 0.04987 | 0.00024 | 0.00028 | 0.00044 |
| **Benzo [k] fluoranthene** | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00000 |
| **Benz [e] acephenanthrylene** | 0.00028 | 0.01215 | 0.00912 | 0.00050 | 0.00034 |
| **Benzo [ghi] perylene** | 0.00000 | 0.00000 | 0.00000 | 0.00000 | 0.00877 |
| **Dibenz [a,h] anthracene** | 0.00000 | 0.00000 | 0.00028 | 0.00048 | 0.00050 |
| **Dibenzo [def,mno] chrysene** | 0.01401 | 0.00000 | 0.04856 | 0.01388 | 0.02088 |
| | 0.23518 | 0.54737 | 0.89348 | 14.44 | 18.04 |

**IK= freshwater environment OK= marine environment**

The decrease in the sum of the concentrations of the PAHs components in the sediments samples in Table 5 and Table 6 indicates that process of biodegradation and other physical processes like

photo-oxidation, air oxidation and volatilization have taken place in the sediments as a means of natural attenuation of the hydrocarbons components.

The higher proportion of low molecular weight-PAHS such as naphthalene, acenaphthene, flourene and anthracene than the high molecular weight-PAHs such as pyrene and chrysene confirms the sources of the hydrocarbons in all the sediments to be petrogenic, according to Rocher et al., (2004). Like in the case of n-alkanes components in all the samples sediments, the rate of decrease in the concentrations of PAHs in the sediments is also the same. Hence, the use of percentage proportions of the PAHs is employed to establish the degree of biodegradation that has occurred in the sample sediment as shown in Table 7 and Table 8.

*Table 7: Percentage proportion of polycyclic aromatic hydrocarbon components in all the samples sediments from the first and second measurements*

| Compounds | % IK-1 | % IK-2 | % IK-3 | % OK-1 | % OK-2 |
|---|---|---|---|---|---|
| **Naphthalene** | 36.28 | 22.63 | 28.60 | 18.17 | 12.10 |
| **Biphenylene** | 5.75 | 0.02 | 0.26 | 0.04 | 0.05 |
| **Acenaphthene** | 8.51 | 6.17 | 36.69 | 12.95 | 12.50 |
| **9H-Flourene** | 0.31 | 3.00 | 0.57 | 9.55 | 11.66 |
| **Anthracene** | 0.09 | 33.38 | 0.20 | 56.27 | 60.51 |
| **9H-Carbazole** | 4.20 | 0 | 0.07 | 0.02 | 0.01 |
| **Pyrene** | 18.72 | 10.16 | 8.07 | 1.32 | 1.27 |
| **Chrysene** | 20.01 | 13.30 | 19.02 | 1.58 | 1.74 |
| **Triphenylene** | 0 | 0 | 0 | 0 | 0 |
| **Benzo [a] pyrene** | 0.04 | 9.11 | 0.027 | 0.002 | 0.002 |
| **Benzo [k] fluoranthene** | 0 | 0 | 0 | 0 | 0 |
| **Benz [e] acephenanthrylene** | 0.20 | 2.22 | 1.02 | 0.003 | 0.002 |
| **Benzo [ghi] perylene** | 0 | 0 | 0 | 0 | 0.05 |
| **Dibenz [a,h] anthracene** | 0 | 0 | 0.03 | 0.003 | 0.003 |
| **Dibenzo [def,mno] chrysene** | 5.96 | 0 | 5.43 | 0.10 | 0.12 |
| | 100 | 100 | 100 | 100 | 100 |

**IK= freshwater environment OK= marine environment**

*Table 8: Modified percentage proportion of polycyclic aromatic hydrocarbon components in selected samples sediments to calculate average molecular weight.*

| Compounds | % IK-1 | % IK-2 | % IK-3 | % OK-1 | % OK-2 | Avg.% IK | Avg. % OK | (Avg. % IK)* MW | (Avg. % OK)* MW |
|---|---|---|---|---|---|---|---|---|---|
| **Naphthalene** | 36.28 | 22.63 | 28.6 | 18.17 | 12.1 | 29.17 | 15.14 | 3738.72 | 1940.49 |
| **Acenaphthene** | 8.51 | 6.17 | 36.69 | 12.95 | 12.5 | 17.12 | 12.73 | 2640.08 | 1963.09 |
| **9H-flourene** | 0.31 | 3 | 0.57 | 9.55 | 11.66 | 1.29 | 10.61 | 214.42 | 1763.59 |
| **Anthracene** | 0.09 | 33.38 | 0.2 | 56.27 | 60.51 | 11.22 | 58.39 | 1999.74 | 10406.85 |
| **Pyrene** | 18.72 | 10.16 | 8.07 | 1.32 | 1.27 | 12.32 | 1.30 | 2491.72 | 262.93 |
| **Chrysene** | 20.01 | 13.3 | 19.02 | 1.58 | 1.74 | 17.44 | 1.66 | 3981.38 | 378.96 |
| | | | | | | | | 15066.06 | 16715.91 |

**IK= freshwater environment OK= marine environment MW= Molecular Weight**

From table 8 above, the sum of the average molecular weight of selected PAHs is calculated using the mathematical relation below $\overline{MW}_{PAH} = \sum Average\ \%PAH * its\ Molecular\ Weight$ . The sum of average molecular weight of Okwori sediment samples is approximately 167 which is higher than that of Ikarama, 151. Based on these values, the Okwori PAHs are somewhat heavier than that of Ikarama - this indicates lighter PAHs have been degraded more at Okwori than Ikarama.

*Table 9: Percentage proportion of the selected few polycyclic aromatic hydrocarbon components in all the samples sediments from the second measurement*

| Compounds | IK-1 % | IK-2 % | IK-3% | OK-1% | OK-2% |
|---|---|---|---|---|---|
| **Naphthalene** | 36.28 | 22.63 | 28.60 | 18.17 | 12.10 |
| **Acenaphthene** | 8.51 | 6.17 | 36.69 | 12.95 | 12.50 |
| **9H-flourene** | 0.31 | 3.00 | 0.57 | 9.55 | 11.66 |
| **Anthracene** | 0.09 | 33.38 | 0.20 | 56.27 | 60.51 |
| **Pyrene** | 18.72 | 10.16 | 8.07 | 1.32 | 1.27 |
| **Chrysene** | 20.01 | 13.30 | 19.02 | 1.58 | 1.74 |
| | | | | | |

**IK= freshwater environment OK= marine environment**

Due to ease in biodegradability and low molecular weight of 2, 3 and 4-benzene rings PAHs through natural attenuation process; Naphthalene, Acenaphthene, Flourene, Anthracene, Pyrene and Chrysene are preferably selected. Table 9 above displays the percentage proportions of the few selected PAHs components due their relative abundant concentrations values which cut across all the samples sediments.

The lower the percentage proportion of a PAH, the greater the extent of biodegradation the sediments have experienced. Naphthalene and acenaphthene are more degraded in Okwori than Ikarama as expected; Okwori being a more oxidizing environment than Ikarama. Flourene and anthracene are less biodegraded in Okwori than Ikarama as against the expectation. This could be due to the oil spill event in Ikarama took place longer ago than the oil spill event in Okwori. Pyrene and chrysene follow suit as in the case of naphthalene and acenaphthene, they are more degraded in Okwori than in Ikarama as expected.

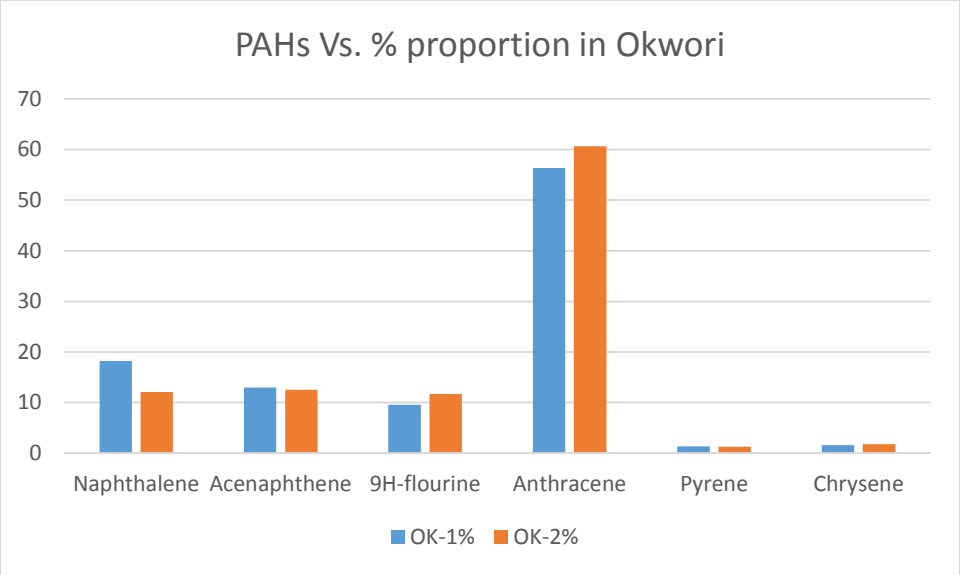



*Figure 5: Bar charts showing the percentage proportions of PAHs in the samples sediments from Okwori*

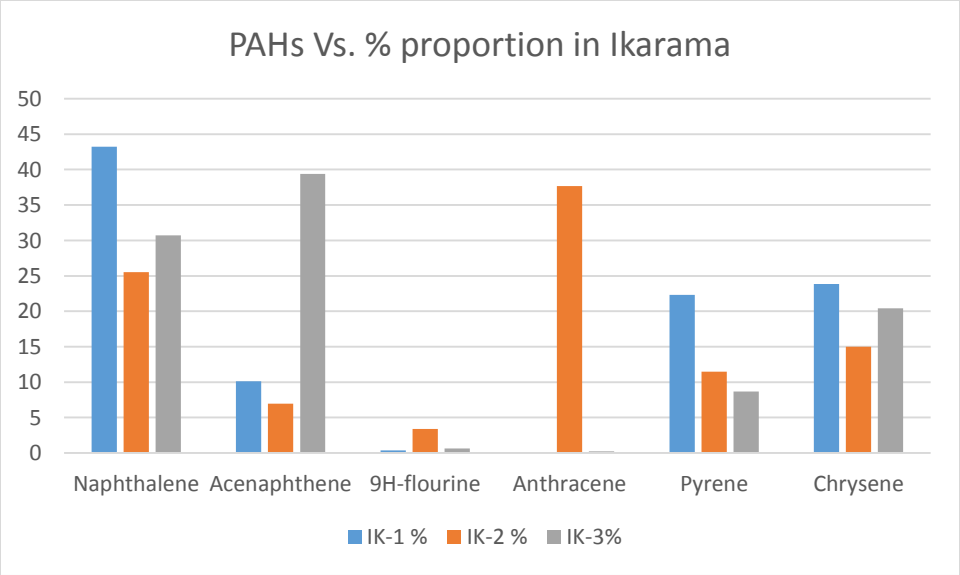



*Figure 6: Bar charts showing the percentage proportions of PAHs in the samples sediments from Ikarama*

The sum of concentrations of PAHs in the study areas are on the high side as compared to other oil spill occurrences. The Sum of concentrations of PAHs in the study areas ranged from 1.175 – 90.709 mg/Kg as compared to the sum of PAHs in the contaminated sediments of study areas conducted by Olajire et al., 2004, in Delta state, Niger Delta region of Nigeria which ranged from 0.0207-0.0721 mg/Kg. Also in a study conducted by Sammarco et al., 2013, the sum of concentrations of PAHs in contaminated sediments associated with BP/Deepwater Horizon oil spill ranged from 0.003- 4.780.960 mg/Kg.

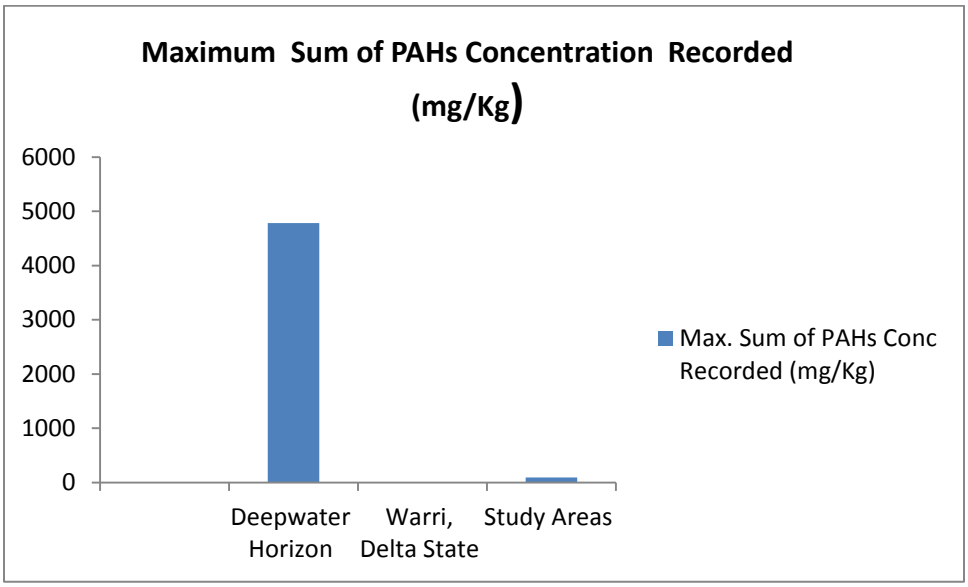



*Figure 7: Maximum Sum of PAHs Recorded in Different Three Oil Spills*

However, the oil spills in the study areas have similarities to that of the Deepwater Horizon oil spill in terms of source and nature of contaminants; emanated from oil well infrastructural failure and typical light crude oil was discharged into the environment. A typical light crude oil like that of DWH oil spill contains 55-90% n-alkanes as saturates, 10-35% PAHs and their alkylated homologues, 0-10% resins and 0-10% asphalthenes, with more than 50% low molecular weight

hydrocarbon constituents (Boehm,2006; Ryerson et al., 2011). Buzzards Bay oil spill in which type 2 fuel oil was discharged into the environment from bunker vessel is different from the study areas spills which released crude oil as the contaminant from failed oil wells.

The concentration levels of some selected PAHs are above the allowable limits according to the EPA standards. These rendered the study areas highly contaminated and appropriate cleanup process should be ensued.

*Table 10: Sediment Quality Criteria versus Recorded Concentrations in Study Areas*

| PAHs | Sediment Concentration Criteria (mg/Kg) | Okwori Sediment Conc. Range (mg/Kg) | Ikarama Sediment Conc. Range (mg/Kg) |
|---|---|---|---|
| Naphthalene | 0.01 | 2.17-10.95 | 0.085-1.29 |
| Flourene | 0.2 | 1.38-10.57 | 0.00074-0.026 |
| Anthracene | 0.06 | 8.12-54.89 | 0.00022-0.92 |
| Pyrene | 0.2 | 0.19-1.15 | 0.044-0.36 |
| Chrysene | 0.2 | 0.23-1.58 | 0.047-0.86 |

# 4 CONCLUSIONS

It is a herculean task to understand the complexity of biodegradation of hydrocarbons process in an environment. The quantitative and qualitative analyzes of the biodegradation process are affected by the nature and amount of hydrocarbons present, surrounding and seasonal environmental conditions and the presence of autochthonous microbial population.

This study shows that microbial mineralization of n-alkanes and PAHs has taken place in the samples sediments from both Ikarama and Okwori. It has been established that the degree of microbial degradation of petroleum hydrocarbons in the aerobic Okwori setting is higher than that of the anaerobic Ikarama setting based on the percentage proportions of the concentrations of hydrocarbons components in all the samples sediments. The observation could be due to the presence of more efficient hydrocarbons degrading microorganisms, photo-oxidation, air oxidation and evaporation of petroleum hydrocarbons in marine environment than in the freshwater environment. As earlier mentioned in the reviewed pieces of literature, bacteria are the most active microorganisms in the biotransformation of hydrocarbons and their biodegradation efficiency is higher in marine environment than in the freshwater environment. Consequently, the degree of biodegradation of petroleum hydrocarbons in Okwori is higher than it is in Ikarama. The presence of efficient petroleum hydrocarbons degrading bacteria community in a marine environment is larger than the one in a freshwater environment due to the encouraging factors as stated earlier in the reviewed literatures.

The porosity and permeability of the sample sediments in the two environments could be a determining factor in the effectiveness of hydrocarbon biodegradation process. The contaminated sediments in Okwori samples are sands which have more effective porosity and permeability than the contaminated sandy clay sediments from Ikarama. This, in turn, increases

the bioavailability and solubility of the petroleum hydrocarbons in Okwori samples sediments to microbial mineralization while that of Ikarama petroleum hydrocarbons tend to sorb to the sandy clay sediments which require greater effort for the microbial community to access the hydrocarbons.

The rate of metabolic activities in the marine environment is higher than the ones in the freshwater environment. Hence, the oxidation processes are higher in Okwori than Ikarama. This makes Okwori more aerobic than Ikarama and more energy is released in Okwori than Ikarama, aiding the greater degree of microbial degradation of petroleum hydrocarbons in Okwori compared to Ikarama.

The Niger Delta has an average annual temperature of approximately 28 $^{0}$C, a range of 24 $^{0}$C- 36 $^{0}$C *. The optimal performance level of biodegradation of petroleum hydrocarbons in the marine environment and freshwater environment is reached in the range of 15 $^{0}$C - 20 $^{0}$C and 20 $^{0}$C - 30 $^{0}$C respectively. The temperature value favors the higher degree of biodegradation of petroleum hydrocarbons in Okwori contaminated sediments which has optimally taken place than in the Ikarama sample sediments. Okwori being an open marine environment is affected by the effect of temperature than Ikarama, a freshwater wetland surrounded by swamp forest to shield the direct impact of sunlight on the contaminated sediments. Consequently, the degree of biodegradation of petroleum hydrocarbons in Ikarama is limited.

*www.eoearth.org/view/article/15483

It has been stated earlier that there is a direct correlation between salinity and biomineralization of low molecular weight petroleum hydrocarbons.

This study also confirms this effect, as a result of Okwori being a marine environment which is more saline than Ikarama, a freshwater environment. Thus, the degree of biodegradation of petroleum hydrocarbons in Okwori is greater than that in Ikarama.

Okwori, a marine environment has a higher energy than the more quiescent Ikarama, a freshwater environment. The constant water wave actions on the contaminated sediments in Okwori enhance the solubility, emulsification, and bioavailability of petroleum hydrocarbons in the sediments and thereby make it easier for the microbial population to access the contaminants.

The biodegradation of petroleum hydrocarbons in aquatic environments is affected mainly by nutrients like nitrogen and phosphorus. Unlike Ikarama, a freshwater environment full of vegetation competing for the absorption of nitrogen and phosphorus as growth nutrients, Okwori environment does not have vegetation to compete with microorganisms in the consumption of the readily available nitrogen and phosphorus.

Therefore, based on this study, it may be concluded that the degree of biodegradation of hydrocarbon contaminants in the marine environment is higher than it is in a freshwater environment. This study also shows high level of contamination in the hydrocarbon impacted areas. Most of the concentrations of PAHs in the contaminated sediment are found to be present in the environments beyond the recommended limits.

Further work to be considered in the study areas is the measurement of pH and Eh of the contaminated sediments and their ambient environments to ascertain the redox potential of the two environments. In addition, data should be collected to determine the concentrations of nitrate, nitrite, ferrous iron, ferric iron, sulfate, sulfite and methane in order to evaluate the dominant anaerobic biodegradation pathways in Ikarama. Also, the measurement of organic carbon contents of the sediments in these two hydrocarbon contaminated environments should be

done to establish the level of contamination to which they are exposed. If the organic carbon contents of the contaminated sediments are known, this will aid the knowledge of the amount of carcinogenic and mutagenic high molecular weight polycyclic aromatic hydrocarbons which are adsorbed to the sediments and have the capacity to render the environments toxic.

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# Appendix

## First Measurement

| Compounds | Conc. In Ikarama Water Sample mg/Kg |
|---|---|
| decane | 0.01 |
| Undecane | 0.02 |
| Dodecane | ND |
| Tridecane | 0.32 |
| Tetradecane | 2.58 |
| Pentadecane | 1.98 |
| Hexadecane | 3.00 |
| Heptdecane | 7.40 |
| Octadecane | 3.80 |
| Nonadecane | ND |
| Eicosane | 1.57 |
| Heneicosane | 1.04 |
| Docosane | 1.14 |
| Tricosane | 1.55 |
| Tetracosane | 2.39 |
| Pentacosane | 2.12 |
| Hexacosane | 1.38 |
| Heptacosane | 1.48 |
| Octacosane | 1.14 |

## Second Measurement

| | |
|---|---|
| Decane | 0.655 |
| Undecane | 1.429 |
| Dodecane | ND |
| Tridecane | 19.024 |
| Tetradecane | 153.649 |
| Pentadecane | 118.071 |
| Hexadecane | 178.601 |
| Heptdecane | 440.607 |
| Octadecane | 225.964 |
| Nonadecane | ND |
| Eicosane | 93.196 |
| Heneicosane | 61.667 |
| Docosane | 68.095 |
| Tricosane | 92.000 |
| Tetracosane | 142.262 |
| Pentacosane | 126.190 |
| Hexacosane | 82.143 |
| Heptacosane | 88.095 |
| Octacosane | 67.905 |