

Density of Hydrocarbonoclastic Bacteria and Polycyclic Aromatic Hydrocarbon Accumulation in Iko River Mangrove Ecosystem, Nigeria

Ime R. Udotong, Samuel I. Eduok, Joseph P. Essien, and Basil N. Ita

Abstract—Sediment and mangrove root samples from Iko River Estuary, Nigeria were analyzed for microbial and polycyclic aromatic hydrocarbon (PAH) content. The total heterotrophic bacterial (THB) count ranged from 1.1×10^7 to 5.1×10^7 cfu/g, total fungal (TF) count ranged from 1.0×10^6 to 2.7×10^6 cfu/g, total coliform (TC) count ranged from 2.0×10^4 to 8.0×10^4 cfu/g while hydrocarbon utilizing bacterial (HUB) count ranged from 1.0×10^5 to 5.0×10^5 cfu/g. There was a range of positive correlation ($r = 0.72$ to 0.93) between THB count and total HUB count, respectively. The organisms were *Staphylococcus aureus*, *Bacillus cereus*, *Flavobacterium breve*, *Pseudomonas aeruginosa*, *Erwinia amylovora*, *Escherichia coli*, *Enterobacter* sp, *Desulfovibrio* sp, *Acinetobacter iwoffii*, *Chromobacterium violaceum*, *Micrococcus sedentarius*, *Corynebacterium* sp, and *Pseudomonas putrefaciens*. The PAH were Naphthalene, 2-Methylnaphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenzo(a,h)anthracene, Benzo(g,h,i)perylene, Indeno(1,2,3-d)pyrene with individual PAH concentrations that ranged from 0.20mg/kg to 1.02mg/kg, 0.20mg/kg to 1.07mg/kg and 0.2mg/kg to 4.43mg/kg in the benthic sediment, epipellic sediment and mangrove roots, respectively. Total PAH ranged from 6.30 to 9.93mg/kg, 6.30 to 9.13mg/kg and 9.66 to 16.68mg/kg in the benthic sediment, epipellic sediment and mangrove roots, respectively. The high concentrations in the mangrove roots are indicative of bioaccumulation of the pollutant in the plant tissue. The microorganisms are of ecological significance and the detectable quantities of polycyclic aromatic hydrocarbon could be partitioned and accumulated in tissues of infaunal and epifaunal organisms in the study area.

Keywords—Hydrocarbonoclastic bacteria, Iko River estuary, Mangrove, Polycyclic aromatic hydrocarbon.

I. INTRODUCTION

THE natural environment contains a wide variety of hydrocarbons of biogenic, petrogenic and pyrogenic origin [1]. Hydrocarbons are ubiquitous organic pollutants that contaminate the environment [2]. Most of the hydrocarbons, especially polycyclic aromatic hydrocarbon (PAH) belong to a class of environmentally-persistent compounds which are widespread in aquatic and terrestrial ecosystems. Owing to their hydrophobic properties, most PAH dissolve only sparingly in water and are taken up readily by suspended particles which are coated in a complex matrix of organic matter in aquatic environment [3]. As a result of lipophilicity and particle settlement, sediment tends to be the major sink for PAH in lakes, estuaries and oceans [4], [5]. Sediment also serve as a source of these pollutants due to the constant water agitation associated with tidal flushing, wave and increased navigation due to petroleum exploitation in the area [6].

Although hydrocarbon may undergo photolysis, chemical oxidation, volatilization and other physical and chemical processes, microbial degradation is a major process affecting their fate in the environment [7], [2]. Therefore, some hydrocarbon pollutants serve as carbon and energy source for microorganisms while others may destroy microbial populations depending on the pollutant and the concentration present in the sediment [8], [9]. The pollutants in the sediment have both beneficial and destructive function on microbial population [10], and the presence of hydrocarbon degrading microorganism may affect the amount of hydrocarbon present in the sediment [11].

The Iko river estuary is a mesotidal estuary and like other estuaries located in the Niger Delta, Nigeria, it supports productive processes as it provides an active habitat for biotic components of the aqua-terrestrial ecosystem. It also provides a good fishing ground for the inhabitant of the area and serve as a source of water for industrial as well as domestic use. Iko river estuary is also prone to pollution because it serves as route for crude oil exploitation receiving point- and non-point sources of anthropogenic perturbations from local and

I. R. Udotong is with the Department of Microbiology, University of Uyo, P.M.B.1017, Uyo, Akwa Ibom State, Nigeria (phone:234-8023008640) on sabbatical leave at Applied Ecology Dept., Environmental Systems Division, Snampromgetti S.P.A., Via Toniolo, 1-61032 Fano (PU), Italy (phone: +39 0721 1682 227, e-mail: ime.udotong@usiicld.com).

S. I. Eduok is with the Department of Microbiology, University of Uyo, PMB 1017, Uyo, Nigeria (e-mail: seduok@yahoo.com).

J. P. Essien is presently with the Health Safety and Environment Department, Shell Petroleum Development Company of Nigeria, Port Harcourt, Rivers State, Nigeria on leave from the Department of Microbiology, University of Uyo, PMB 1017, Uyo, Nigeria (e-mail: jomato652003@yahoo.com).

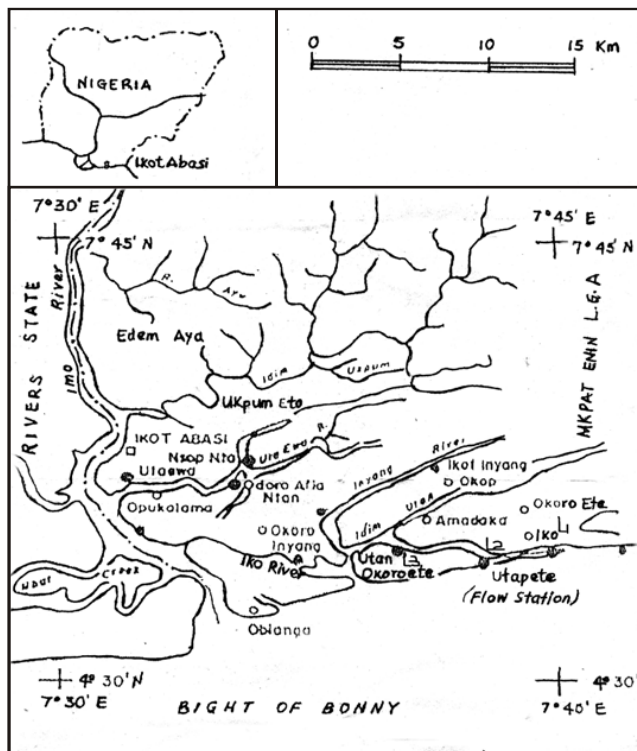
B. N. Ita is with the Department of Chemistry, University of Uyo, PMB 1017, Uyo, Nigeria (e-mail: basil-ita@yahoo.com).

An understanding of the fate of PAH levels in coastal environments is important, because high levels in coastal ecosystem may pose a threat to human public health and the well-being of aquatic biota [5], [8]. Due to their lipophilic nature, PAHs have a high potential for biomagnification through trophic transfers [12], [13], [14]. PAH are also known to exert acutely toxic effects and/or possess mutagenic, teratogenic or carcinogenic properties [15], [16], [17]. This study was designed to evaluate the density of hydrocarbon degraders and PAHs level in Iko River mangrove ecosystem close to the abandoned oil facilities of Shell Petroleum Development Company (SPDC), Nigeria in order to provide a baseline reference on the impact of petroleum exploration and gas flaring on the mangrove ecosystem. This information is very significant especially from a bioaccumulation and ecotoxicological view point. The relationship between the microbial density and distribution and type of polycyclic aromatic hydrocarbon and concentration in sediment would give a general overview on the changes in the build-up of the chemical and biological activities within the Iko River mangrove ecosystem.

A. The Study Area

B. Collection of Samples

40°C with a maximum holding time of 6 h before extraction. Prop roots of the mangrove (*Rhizophora racemosa*) were obtained with the aid of machete into polythene bags. Sampling was done during the rainy season (month of August). Equal amount of sediment samples and mangrove rhizosphere were collected from each location and formed into a composite sample to reduce the total number of samples and associated cost of the analysis. The composite sample was thoroughly homogenized. Precisely 10g subsample of the homogenized sample was serially diluted for microbiological analysis [21].



C. Sample Analysis

The counts of total heterotrophic bacteria (THB) in the sediment and mangrove rhizosphere was enumerated by pour plate technique [21] using diluents prepared with 25% Ringer's solution and cultured on nutrient agar (Difco) while total coliform (TC) counts were determined using the membrane filter techniques [22]. The hydrocarbon utilizing bacterial counts (HUB) were enumerated by the spread plate technique using oil-mineral salt medium (MSM). The media were supplemented with cycloheximide (100µg/ml and benomyl (50µg/ml) to prevent fungal growth [23]. The crude oil used was sterilized by filtering through Millipore filter (0.45µ pore size) and stored in sterile bottles. Inoculated THB and HUB plates were incubated aerobically and anaerobically at room temperature ($28 \pm 20^\circ\text{C}$) for 24 hours and 5 to 14 days respectively and thereafter enumerated [21], [24].

Representative bacterial colonies were purified by repeated subculturing and maintained as stock on nutrient agar slants. The identification of the isolates was done by comparing the cultural, morphological and biochemical characteristics of the cultures with the characteristics of known taxa using the Bergey's manual of determinative bacteriology [25], and Cowan and Steel's manual for the identification of medical bacteria [26].

2) Enumeration of Total Fungal (TF) Counts

The total fungal (TF) count in the sediment and mangrove rhizosphere was enumerated by pour plate technique [21] using diluents prepared with 25% Ringer's solution and cultured on sabouraud dextrose agar (Difco). Inoculated TF plates were incubated aerobically at room temperature ($28 \pm 2^\circ\text{C}$) for 48 to 72 hours and thereafter enumerated [21], [24]. Representative fungal colonies were purified by repeated subculturing and maintained as stock on sabouraud dextrose agar slants.

3) Chemical Analysis

The analysis of PAH concentration followed a standard procedure [22], [27]. Each of dried and ground sample spiked with squalene and 32-alkane were serially extracted with 100 mL methyl isobutyl ketone (Analar grade). Each extract was allowed to settle, centrifuged for 5 min and decanted. The extracts were concentrated on a rotatory evaporator maintained at 20°C to a volume of about 5mL. A sample volume of 1 μL each of the extract was subjected to a GC-MS (Hewlett Packard model 5890). Concentrations of polycyclic aromatic hydrocarbons were quantified relative to the total peaks as these were converted to weights using hydrocarbon standard calibration [28]. Duplicates and method blanks were similarly treated using the same reagents to test for the precision, accuracy and reagent purity used in the analytical procedure. This method has been previously adopted by [29].

4) Statistical Analysis

Correlation analysis of data were performed using Analyse-It General 1.73 statistical software® on Log – transformed estimates of densities of heterotrophic and hydrocarbon utilizing bacteria (Log cfu/g) with levels of significance maintained at 95% for each test. Interpretation was done based

on [30] rule of thumb for interpreting the size of a correlation coefficient.

III. RESULTS AND DISCUSSION

The microbial count of sediment samples from three locations in Iko River estuary are presented in Table I. The results obtained revealed that the total heterotrophic bacterial (THB) count ranged from 1.7×10^7 to 3.3×10^7 cfu/g, 2.3×10^7 to 3.8×10^7 cfu/g and 3.6×10^7 to 4.9×10^7 cfu/g in the benthic sediment, epipellic sediment and mangrove rhizosphere, respectively. Total hydrocarbon utilizing bacterial (HUB) counts in the benthic sediment, epipellic sediment and mangrove rhizosphere ranged from 1.2×10^5 to 2.8×10^5 cfu/g, 1.8×10^5 to 2.9×10^5 cfu/g and 3.5×10^5 to 4.5×10^5 cfu/g, respectively. There was a very high positive linear correlation ($r = 0.9313$) between the total heterotrophic bacteria in mangrove rhizosphere (THBmr) and hydrocarbon utilizing bacteria in mangrove rhizosphere (HUBmr), indicating that increase in the rhizosphere heterotrophic densities resulted in a corresponding increase in hydrocarbon utilizing bacteria.

A range of high positive linear correlation ($r = 0.7167$ to $r = 0.8353$) was obtained in the relationship between THB and HUB in the benthic sediment, epipellic sediment and mangrove rhizosphere (Table II).

This is an indication that the densities of HUB in the sediment and rhizosphere are influenced by the THB microbial densities.

The results obtained from the bacteriological analysis of sediments and mangrove rhizosphere revealed the presence of the following microorganisms: *Staphylococcus aureus*, *Bacillus cereus*, *Flavobacterium breve*, *Pseudomonas aeruginosa*, *Erwinia amylovora*, *Escherichia coli*, *Enterobacter sp.*, *Acinetobacter iwoffii*, *Chromobacterium violaceum*, *Micrococcus sedentarius*, *Desulfovibrio sp.*, *Serratia marcescens*, *Clostridium sp.*, *Corynebacterium sp.*, *Pseudomonas paucimobilis*, and *Pseudomonas putrefaciens*. Among the isolates, *Pseudomonas aeruginosa*, *Micrococcus*, *Clostridium*, *Bacillus* and *Serratia* exhibited strong oil degrading capabilities.

TABLE I
MICROBIAL LOAD IN SEDIMENTS FROM IKO RIVER ESTUARY

Sample Location/No		Benthic Sediment			Epipellic Sediment			Mangrove Rhizosphere		
		THB	HUB	%HUB	THB	HUB	%HUB	THB	HUB	%HUB
Well Head	1	2.1(7.32)	1.9(5.28)	72.13	2.6(7.41)	2.0(5.30)	71.52	3.9(7.59)	3.6(5.56)	73.25
	2	1.7(7.23)	1.2(5.08)	70.26	2.3(7.36)	1.8(5.26)	71.46	3.6(7.56)	3.5(5.54)	73.28
	3	2.3(7.36)	1.6(5.20)	70.65	3.1(7.49)	2.9(5.46)	72.9	4.7(7.67)	4.3(5.63)	73.4
Flow Station	1	3.3(7.52)	2.8(5.45)	72.47	3.4(7.53)	2.8(5.45)	72.38	4.4(7.64)	4.1(5.61)	73.43
	2	2.5(7.40)	2.0(5.30)	71.62	3.0(7.48)	2.4(5.38)	71.93	4.8(7.68)	4.5(5.65)	73.57
	3	2.4(7.38)	1.6(5.20)	70.46	3.2(7.51)	2.3(5.36)	71.37	4.6(7.66)	4.3(5.63)	73.5
Jetty	1	1.8(7.26)	1.2(5.08)	69.7	3.1(7.49)	2.4(5.38)	71.83	4.9(7.69)	4.3(5.63)	73.21
	2	2.9(7.46)	2.2(5.34)	71.58	3.3(7.52)	2.6(5.41)	71.94	4.5(7.65)	4.5(5.65)	73.86
	3	2.6(7.41)	1.9(5.28)	71.26	3.8(7.58)	2.5(5.40)	71.24	4.7(7.67)	4.4(5.64)	73.53

Values are means of three determinations. Values in parenthesis are \log_{10} , THB = total heterotrophic bacteria, HUB = hydrocarbon utilizing bacteria

TABLE II
CORRELATION BETWEEN MICROBIAL GROUPS IN THE BENTHIC SEDIMENT, EPIPELLEIC SEDIMENT, AND MANGROVE RHIZOSPHERE

	%HUBb	%HUBe	%HUBmr	THBb	THBe	THBmr	HUBb	HUBe	HUBmr
%HUBb									
%HUBe	-0.1375								
%HUBmr	-0.1890	0.0558							
HETb	0.0683	0.3166	0.6927						
HETe	-0.2903	0.1271	0.5394	0.7167					
HETmr	-0.4468	0.2976	0.3243	0.3563	0.7772				
HUBb	0.9564	-0.0325	0.0174	0.3554	-0.056	-0.3032			
HUBe	-0.2919	0.7030	0.4215	0.7086	0.7947	0.7377	-0.0596		
HUBmr	-0.4315	0.2610	0.6465	0.5541	0.8353	0.9313	-0.2363	0.7575	

e = Epipellic Sediment, b = Benthic Sediment, mr = Mangrove Rhizosphere

0.00 to 0.30 (-0.00 to -0.30)

Little if any correlation

0.30 to 0.50 (-0.30 to -0.50)

Low positive (negative) correlation

0.50 to 0.70 (-0.50 to -0.70)

Moderate positive (negative) correlation

0.70 to 0.90 (-0.70 to -0.90)

High positive (negative) correlation

0.90 to 1.00 (-0.90 to -1.00)

Very high positive (negative) correlation

TABLE III
POLYCYCLIC AROMATIC HYDROCARBON LEVEL IN SEDIMENT AND MANGROVE ROOT

Parameter	Concentration (mg/kg)								
	Benthic sediment			Epipellic sediment			Mangrove root		
	L1B	L2B	L3B	L1E	L2E	L3E	L1M	L2M	L3M
Naphthalene	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2-Methylnaphthalene	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Acenaphthylene	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Acenaphthene	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Fluorene	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.70
Phenanthrene	0.20	0.20	0.20	0.20	0.20	0.20	0.26	0.26	0.26
Anthracene	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Fluoranthene	0.20	0.20	0.20	0.20	0.30	0.20	0.20	0.20	0.20
Pyrene	0.20	0.35	0.20	0.20	0.23	0.20	0.20	0.20	0.20
Benzo(a)anthracene	0.20	0.93	0.20	0.20	0.32	0.20	1.56	1.70	1.34
Chrysene	0.30	0.59	0.30	0.30	0.36	0.30	0.67	0.75	0.56
Benzo(b)fluoranthene	0.20	0.37	0.20	0.20	0.30	0.20	0.38	0.38	0.38
Benzo(k)fluoranthene	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Benzo(a)pyrene	0.20	0.20	0.20	0.20	0.20	0.20	0.61	0.65	0.61
Dibenzo(a,h)anthracene	0.20	1.67	0.20	0.20	1.70	0.20	1.24	3.71	1.08
Benzo(g,h,l)perylene	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Indeno(1,2,3-d)pyrene	0.20	1.02	0.20	0.20	1.12	0.20	0.98	4.43	0.63
Total PAH	6.30	9.93	6.30	6.30	9.13	6.30	10.5	16.68	9.66

L = location, B = benthic sediment, E = epipellic sediment, M = mangrove root

The isolates include members of the autochthonous and allochthonous microbial community transported to the estuarine sediment by point and non-point sources. The point sources include effluent discharge and direct defecation through aqua privy constructed at the near shore; the non-point sources include surface runoffs. The point source addition of pollutant can produce distinct and predictable changes in microbial community [31].

Polycyclic aromatic hydrocarbon detected in the Iko River estuarine epipellic and benthic sediments and mangrove root in varying concentrations are presented in Table III. These were Naphthalene, 2- Methylnaphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenzo(a,h)anthracene,

Benzo(g,h,l)perylene, Indeno(1,2,3-d)pyrene. These PAHs are introduced into the estuary by oil spills, sabotage to well heads, disposal of industrial waste and other human activities in addition to persistent gas flaring from oil facilities with horizontal nozzle pointed at the sediment and vegetation. The concentration of some PAH in the epipellic and benthic sediment were the same as those in the mangrove root for naphthalene (1.00 mg/kg), 2- methylnaphthalene (0.20mg/kg), acenaphthylene (1.00mg/kg), acenaphthene (1.00mg/kg), fluorene (0.70mg/kg), anthracene (0.40mg/kg), Benzo(k)fluoranthene (0.20mg/kg) and benzo(g,h,l)perylene (0.20mg/kg). The microorganisms isolated from the Iko River estuarine sediment are capable of overcoming the deleterious effects of petrogenic waste and polycyclic aromatic hydrocarbon present in the sediment. Though direct degradation of PAH by the estuarine organisms was not

carried out in this study, most of the microorganisms found in the sediment and mangrove rhizosphere have the potential to degrade PAH, use same as carbon and energy source. This was evident in the high %HUB with a range of 69.7% to 73.86% in the samples. The pollution status of Iko River Mangrove Ecosystem through petroleum E & P activities should be higher compared to the result of this study. This clearly showed that the microorganisms present in the sediment are able to degrade the various components of the oil. *Pseudomonas paucimobilis* is implicated with the degradation of fluoranthene as a sole carbon and energy source [9]. Also *P. aeruginosa* and *Flavobacterium sp* have been reported to metabolize fluoranthene, pyrene, chrysene and benzo(a)anthracene [32] *Chromobacterium violaceum* is involved in nutrient recycling and *E. coli* is indicative of recent water pollution with fecal matter.

There are at least three possible reasons for the low concentration of PAH contaminant in the epipellic and benthic sediments. This could be as a result of uptake by the plants, phytodegradation or phytovolatilization due to the tropical climate, in addition to rhizodegradation. Several field studies have demonstrated the disappearance of PAH in vegetated ecosystem which is in agreement with the result of this study. Chrysene, benzo(a)anthracene, benzo(a)pyrene and dibenzo(a)anthracene had greater disappearance in vegetated soils than in nonvegetated soils [33]. Anthracene and pyrene had greater disappearance in vegetated soils than in unvegetated soils [34] and pyrene was mineralized at a greater rate in a planted system than in an unplanted system [35].

The concentration of fluoranthene (0.30mg/kg) was higher in the epipellic sediment at location 2. Also, pyrene had a slightly raised concentration of 0.23mg/kg and 0.35mg/kg in epipellic and benthic sediments respectively at location 2, indicative of fresh input of the pollutant into the ecosystem from the flow station. Among the high molecular weight PAH, benzo(k)fluoranthene and benzo(g,h,l)perylene had uniform concentration of 0.20mg/kg in the samples. Others such as benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene and indeno(1,2,3-d)pyrene were detected at elevated concentrations in the mangrove root than the background concentration in the epipellic and benthic sediments indicating bioaccumulation of these PAHs. Among the low molecular weight PAH only phenanthrene was detected in the mangrove root at a concentration of 0.26 mg/kg above the background concentration of 0.20mg/kg in the sediments.

The exposure and accumulation of these PAHs could negatively impact the estuarine diversities and density of the biota feeding on the mangrove trees. The various probabilities of PAHs exposure, accumulation, biomagnifications and their toxicological implications have been elucidated in several studies. Oil pollution from oil or gas exploration, petroleum and accidental spills severely damages mangrove ecosystems [36]. Oiling of mangrove has a number of significant consequences. One of the most immediate and obvious is defoliation of the trees. The toxicity of the oil may depend on environmental condition and oil had the greatest effect on survival and growth of *Rhizophora mangle* when the trees are in hot, bright outdoor conditions [37]. Also, toxicity has been

shown to differ among mangrove species [38]. The Iko River mangrove ecosystem is steadily undergoing long-term changes in the community structure with differential mortality of the mangrove trees with a steady surge in the population of *Nypa palm*. Few stands of brackish water palm *Nypa fruticosa* and *Phoenix reclinata* that occurred in some places [19] has surreptitiously encroached and colonize a vast area of the ecosystem being more resistant or adaptable to the environmental pollution by petroleum related activities. The most obvious impact of the rapid mangrove mortality is coastal erosion and degradation of the shoreline with some coastal fishing settlements, a school, health center, and about two-thirds of Edonwik village in Eastern Obolo L.G.A. of Akwa Ibom State completely washed off while other areas are persistently threatened by the powerful ocean surge. The menace of erosion is exacerbated by the high mortality rates of the mangrove species which were natural barriers against the ocean waves and the shallow continental shelf where these settlements are located. This increase in coastal erosion and submerging of settlement may be connected to the rise in global water level as a result of climate change.

Iko River Estuary is a site that poses acute risks for humans and other ecologically sensitive biota due to exposure to PAH and other persistent organic pollutants. Though humans are not eating any part of the mangrove plant, there is the possibility that the natural vegetation in the area is contributing to the food chain exposures. The potential avenues of ecological exposure could occur in certain plant fruit, seeds and leaves as the roots and stems are not often edible. Also, the estuarine oyster that firmly adhere to the prop roots of these plants, plant eating animals, crab, arthropods etc. that constitute the lower level food chain for the in-faunal and epi-faunal organisms could be direct avenues of PAH transmission along the food chain. For instance, [39], in their study found out that some herbivorous crabs do not simply graze on fallen leaves; some actually forage in the canopy of the tree. Also, the biota constituting the lower level food chain could have elevated tissue burden of the pollutant due to their filter-feeding and burrowing habits and ultimately be the route for transmission of these pollutants to humans. Several of these PAHs are known for their carcinogenic, mutagenic and teratogenic properties and also implicated in causing reproductive problems [40] for the aquatic biota and ultimately man.

There were high percentages of hydrocarbon utilizing microorganisms in the benthic sediment (72.13%), epipellic sediments (72.93%) and mangrove rhizosphere (73.86%), an indication that Iko River mangrove ecosystem is highly polluted with petrogenic waste with the various hydrocarbon degraders having a high cell recovery and adaptability to the pollutant. This could be due to pre-exposure of these organisms to oily waste in ecosystem which enhanced their degradation potential. Several studies [41], [42], [43], [44] have shown the potential of the estuarine microbial isolates in crude oil degradation. The low and almost uniformly distributed concentration of the PAH pollutants in the sediments could largely be due to the degrading potential of the microorganisms that are ubiquitous in the ecosystem. Thus after entering the mangrove ecosystem either from

airborne deposition and petroleum exploration and production activities, the PAH could be rapidly degraded before significant plant uptake occurs. In addition, the higher molecular weight PAH could adsorb firmly to sediment particles, colloids and organic matter and become unavailable for uptake by plants and subsequently transported due to the hydrodynamic conditions in the estuary and deposited at other sites.

IV. CONCLUSION

The Iko River Estuarine epipellic sediment, benthic sediment and mangrove rhizosphere harbors distinct microbial population of ecological and biogeochemical importance with the polycyclic aromatic hydrocarbon concentration that could be partitioning and accumulated in tissues of aquatic biota and biomagnify along the food chain. The estuarine ecosystem is polluted by indiscriminate disposal of industrial effluent, oil spillage, gas flaring, disposal of domestic waste and fecal matter by inhabitants of the area. The concentration of PAH in the epipellic and benthic sediments and mangrove plant could be regarded as baseline environmental concentration and bioaccumulation in the plant tissues respectively. The result of this study is a clear indication that the plant can bioaccumulate a number of PAH since the background concentration of PAH in the sediment is lower than that in the plant tissue. The mangrove plant (*Rhizophora racemosa*) could be used as a biomarker of PAH exposure in the Iko River mangrove ecosystem and possibly as a means of phytoremediation of other contaminated ecosystem.

REFERENCES

- [1] P. D. Boehm, "Petroleum in the marine environment: Physical and chemical methods," US National Res Council, 1981.
- [2] B. T. Ashok, S. Saxena, K. P. Singh, and J. Mistral, "Biodegradation of polycyclic aromatic hydrocarbons in soils around Mathura oil refinery, India," *World J. Microbiol. Biotechnol.*, vol.11, pp.691 – 692, 1995.
- [3] L. Deschenes, P. Lafrance, J. P. Villeneuve, and R. Samson, "Adding sodium dodecyl sulfate and *Pseudomonas aeruginosa* UG2 biosurfactants inhibits polycyclic aromatic hydrocarbon biodegradation in a weathered creosote-contaminated soil," *Appl. Microbiol. Biotechnol.*, vol. 46, pp. 638-656, 1996.
- [4] M. I. Lee, M. V. Novotny, and K. D. Bartle, "Analytical Chemistry of Polycyclic Aromatic Hydrocarbon," Academic Press New York, 1981, pp 472- 491.
- [5] M. P. Shiaris, "Seasonal biotransformation of naphthalene, phenanthrene, and benzo (a) pyrene in surficial estuarine sediments," *Applied and Environmental Microbiology*, vol. 55, no. 6, pp. 1391 – 1399, 1989.
- [6] J. Ekwere, E. B. Akpan, and E. E. U. Ntekim, "Geochemical studies of sediments in Qua Iboe estuary and associated creek South Eastern Nigeria," *Tropical Journal of Applied Sciences*, vol.2, pp. 91 - 95, 1992.
- [7] B. T. Ashok and S. Sexana, "Biodegradation of polycyclic aromatic hydrocarbons – a review," *Journal of Science and Industrial Research*, vol. 54, pp. 443 – 451, 1995.
- [8] I. R. Udotong, "Environmental monitoring and effect of petroleum production effluent on some biota of the lower Qua Iboe River estuary," PhD Thesis, River State University of Science and Technology, Nkpolu, Port Harcourt, Nigeria, 2000.
- [9] R. A. Kanaly, and S. Harayama, "Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria, a minireview," *Marine Biotechnology*, vol.18, no.8, pp.2059 - 2067, 2000.
- [10] Y. I. Sorokin, "Microheterotrophic organisms in marine ecosystems," in A. R. Longhort (ed) *Analysis of Marine Ecosystems*, Academic Press, London, 1981, pp. 293 - 342.
- [11] E. A. S. Linley, and R. C. Newell, (1981) "Micro heterotrophic communities associated with the degradation of kelp debris," *Kieler Meerestorsch*, vol.5, pp. 345 - 355, 1981.
- [12] P. Y. Lu, R. L. Metcalf, N. Plummer, and D. Mandel, "The environmental fate of three carcinogens: benzo-(a)-pyrene, benzidine, and vinyl chloride evaluated in laboratory model ecosystems," *Arch. Environ. Contam. Toxicol.*, vol.6, pp.129 - 142, 1977.
- [13] W. H. Clement, J. T. Oris, and T. E. Wissing, "Accumulation and food chain transfer of flouranthene and benzo[a]pyrene in *Chironomus riparius* and *Lepomis macrochirus*," *Arch. Environ. Contam. Toxicol.*, vol. 26, pp. 261-266, 1994.
- [14] M. R. Twiss, L. Granier, P. Lafrance, and P. G. C. Campbell, "Bioaccumulation of 2,2',5,5'-tetrachlorobiphenyl and pyrene by picoplanktonic acid concentrations and pH," *Environ. Toxicol. Chem.*, Vol.18, pp.2063 - 2069, 1999.
- [15] D. H. Philips, "Fifty years of benzo(a)pyrene," *Nature*, vol. 303, pp. 468 – 472, 1983.
- [16] C. E. Cerniglia and M. A. Heitkamp, "Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment," pp. 41-68, in U. Varanasi (ed.), *Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment*. CRC Press, Inc., Boca Raton, Fla., 1989.
- [17] International Agency for Research on Cancer, "Monographs on the evaluation of carcinogenic risks to human," vol. 1 - 49, 1972 – 1990.
- [18] U. J. Ekpe, U. Ekanem, and E. R. Akpan, "Temporal changes in some water quality parameters in Iko and Uta Ewa River, South Eastern Nigeria," *Global Journal of Pure and Applied Sciences*, vol.1, pp. 63 - 68, 1995.
- [19] R. P. King, (1991) "Some aspect of the reproductive strategy of *Illisha africana* in Qua Iboe estuary, Nigeria," *Cybius*, vol. 15, no.3, pp. 239 - 257.
- [20] I. E. Ukpong, "An ordination study of Mangrove Swamp Communities in West Africa," *Vegetatio*, vol.116, pp. 147-159, 1995.
- [21] W. F. Harrigan, and M. E. McCance, "Laboratory methods in food and dairy microbiology," Academic Press, London, 1990.
- [22] APHA, "Standard methods for the examination of water and waste water," 20th ed. American Public Health Association, 1998.
- [23] L. Kinkle, M. Wilson, and S. E. Lindow, "Effects of scale on the assessment of epiphytic bacteria population. *Microbial Ecology*, vol. 29, pp.283-297, 1995.
- [24] E.N. Amadi and S.A. Braide, "Distribution of Petroleum hydrocarbon degraders around petroleum- related facilities in a mangrove swamp of the Niger Delta," *Journal of Nigerian Environmental Society*, vol. 1, no 2, pp. 187 – 192, 2003.
- [25] J. G. Holt, N. R. Krieg, P. H. A. Sneath, J. J. Staley, and S. T. William, "Bergey's manual of determinative bacteriology," 9th ed. William and Wilkins, Baltimore, 1994.
- [26] G. J. Barrow and R. L. A. Feltham, "Cowan and Steel's Manual for the Identification of Medical Bacteria," 5th edition, Cambridge University Press, Leicester, pp. 330, 1992.
- [27] M. Radojevic and V. N. Bashkin, "Practical environmental analysis," Royal Society of Chemistry, 1999, pp. 465.
- [28] FEPA, "National Guidelines for Spilled Oil Fingerprinting," Federal Environmental Protection Agency, Government Press, Abuja, 2001.
- [29] J. P. Essien, S. P. Antai, and N. U. Benson, "Microalgae biodiversity and biomass status in Qua Iboe estuary mangrove swamp, Nigeria," *Aquatic Ecology*, vol. 42, pp. 71 – 81, 2008.
- [30] D. E. Hinkle, W. Wiersma, and S. G. Jars, "Applied statistics for the behavioural sciences" 3rd ed. Houghton Mifflin Company, Boston 1994, pp 507 - 510.
- [31] J. C. Fetzer, "The chemistry and analysis of the large polycyclic aromatic hydrocarbon," John Wiley, New York, 2000, pp 231-237.
- [32] D. Trzesieka – Mlynarz and O. P. Ward, "Degradation of polycyclic aromatic hydrocarbons (PAHs) by a mixed culture and its component pure culture obtained from PAH – contaminated soil," *Can. Journal of Microbiology*, vol. 41, pp. 470 – 476, 1995.
- [33] W. Aprill and R. C. Sims, "Evaluation of the use of prairie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil," *Chemosphere*, vol. 20, pp. 253 - 265, 1990.

- [34] K. A. Reilley, M. K. Banks, and A. P. Schwab, "Dissipation of polycyclic aromatic hydrocarbon in the rhizosphere," *J. Environ. Qual.*, vol. 25, pp. 212 – 219, 1996.
- [35] A. M. Ferro, R. C. Sims, and B. Bugbee, "Hycrest crested wheatgrass accelerates the degradation of pentachlorophenol in soil," *J. Environ. Qual.*, vol. 23, pp. 272 - 279, 1994a.
- [36] M. Mastaller, "Destruction of mangrove wetlands-causes and consequences" *Natural Resources and Development*, vol.43-44, pp. 37 - 57, 1996.
- [37] C. E. Proffitt, D. J. Delvin, and M. Lindsey, "Effects of oil on mangrove seedlings grown under different environmental conditions," *Marine Pollution Bulletin*, vol.30, no.12, pp. 788 -793, 1995.
- [38] C. C. Lamparelli, F. O. Rodrigues, and D. O. de Moura, "A long-term assessment of an oil spill in a mangrove forest in Sao Paulo, Brazil," in *Mangrove Ecosystem Studies in Latin America and Africa*. B. Kjerfve, L.D. Lacerda and S. Diop, eds, pp 191-203. UNESCO, Paris, 1997.
- [39] S. Cannicci, S. Ritossa, R. K. Ruwa, and M. Vannini, "Tree fidelity and hole fidelity in the tree crab *Sesarma leptosoma* (Decapoda: Grapsidae)," *Journal of Experimental Marine Biology and Ecology*, vol.196, no.1-2, pp. 299 - 311, 1996a.
- [40] A. Luch, "The carcinogenic effects of polycyclic aromatic hydrocarbons, Imperial College Press, London, 2005.
- [41] U. J. J. Ijah, and L. I. Ukpe, "Biodegradation of crude oil by *Bacillus* strains 28A and 61B isolated from oil spilled soil," *Waste Management*, vol.12, pp. 55 - 60, 1992.
- [42] A. Y. Itah, "Biodegradation of Qua Iboe light crude oil by coastal marine yeast strains isolated from oil spilled site at Iko, Akwa Ibom State, Nigeria," *Journal of Applied Sciences*, vol. 2, pp. 412 – 427, 1999.
- [43] A. Y. Itah and J. P. Essien, "Petroleum hydrocarbon degrading capabilities and growth profile of bacteria from crude oil polluted ultisol and brackish water," *Global Journal of Pure and Applied Science*, vol.1, pp. 507 - 512, 2001.
- [44] J. P. Essien, A. Y. Itah, and S. I. Eduok, "Influence of electrical conductivity on microorganisms and rate of crude oil mineralization in Niger Delta ultisol," *Global Journal of Pure and Applied Science*, vol.9, pp.199 - 203, 2003.

Ime R. Udotong was born in Ikpe Annang, Etim Ekpo LGA, Akwa Ibom State, Nigeria on 23 July 1960. He holds a Higher National Diploma (HND) in Applied Biology from Rivers State University of Science & Technology, Port Harcourt, Nigeria; Master of Philosophy (M.Phil) in Applied Microbiology (Waste Management option) and a Doctor of Philosophy (Ph.D) in Applied Microbiology (Environmental Monitoring & Impact Assessment option) from same University.

He was promoted an Associate Professor of Environmental Microbiology on 1st October 2002 and seconded from the Department of Microbiology, University of Uyo, Nigeria to work as the MANAGING DIRECTOR of University of Uyo Consultancy Ltd from 23rd August 2003 to 31st January 2008. Besides research and training at the undergraduate and post graduate levels, he has worked as Environmental Consultant / Expert to multi-national Oil & Gas companies in Nigeria and Europe. He is presently on Sabbatical leave at Applied Ecology Dept., Environmental Systems Division, Snamprogetti S.p.A., Via Toniolo, 1-61032 Fano (PU), Italy as ENVIRONMENTAL CONSULTANT / EXPERT with effect from 1st September 2008. He has published 22 articles in local and international Journals, 16 Technical Reports, 6 book chapters and 4 International and several Conference papers to his credit. His current research interest is in the Microbial ecology of and impacts of Oil & Gas E&P activities on wetland soils.

Dr. Udotong is a member of Nigerian Society for Microbiology (NSM), Nigerian Institute of Food Science & Technology (NiFST) and Nigerian Environmental Society (NES). As Environmental Consultant / Expert, he has consulted severally for Shell Petroleum Development Company of Nigeria Ltd (SPDC), Nigerian Agip Oil Company Ltd (NAOC), Mobil Producing Nigeria Ltd – a subsidiary of Exxon Mobil and Elf Petroleum Nigeria Ltd (EPNL).