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 1.0x10⁶ to 5.1x10⁷ cfu/g, total fungal (TF) count ranged from 1.0x10⁶ to 2.7x10⁷ cfu/g, total coliform (TC) count ranged from 2.0x10⁴ to 8.0x10⁴ cfu/g while hydrocarbon utilizing bacterial (HUB) count ranged from 1.0x10⁵ to 5.0x10⁶ 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, Acenapthylene, 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 because they can degrade hydrocarbon pollutants serving as carbon and energy source for 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 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 community activities and industrial activities.
industrial production and transport of petroleum products and gas flaring by oil companies located within the area.

An understanding of the fate of PAH levels in coastal environments is important, because high levels in coastal ecosystems 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 viewpoint. 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.

II. MATERIALS AND METHODS

A. The Study Area

Iko River estuary is located in Iko town, an oil producing community in Eastern Obolo LGA, Akwa Ibom State in the Niger Delta region, Nigeria. The area lies within latitude 7°30′ N and 7°45′ N, and longitude 7°30′ E and 7°40′ E (Fig. 1). The river has a shallow depth ranging from 1 to 7 m at flood and ebb tide. Iko River takes its rise from Qua Iboe River catchments and drains directly into the Atlantic Ocean at the Bight of Bonny [18]. The adjoining creeks, channels and tributaries form the Iko River estuary which is significant in the provision of suitable breeding sites for the diverse aquatic resources that abound in the area, good fishing ground for artisan fishermen as well as petroleum exploration and production activities. The shoreline of Iko River estuary is fringed with mangrove vegetation, tidal mud flats and pneumatophores of Avicennia exposed during low tide. The macrophytes of the area are predominated by Rhizophora racemosa, R. harrisoni, R. mangle, Avicennia africana and Laguncularia racemosa [19], [6], [20].

B. Collection of Samples

The sampling locations included an abandoned wellhead, an abandoned flow station and a jetty at Iko beach. A core sampler was used to retrieve epipellic sediment with undisturbed sediment water interface. Benthic sediment samples were obtained with the aid of Shipek grab sampler at three different locations within the Iko river estuary into sterile polythene bags, placed in an ice-cooled chest and transported to the microbiology laboratory for analysis. Samples for chemical analysis were collected into Amber glass containers with Teflon-lined cap, stored in the dark at 4°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].

Fig. 1 Map of South-South Nigeria Showing Iko River Estuary

C. Sample Analysis

1) Enumeration of Heterotrophic, Coliform and Hydrocarbon Utilizing Bacteria

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 (0.45µm pore size) and stored in sterile bottles. Inoculated THB and HUB plates were incubated aerobically and anaerobically at room temperature (28 ± 20°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 ± 2°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 was 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 1µl each of the extract was subjected to a GC-MS analysis. Aromatic hydrocarbons were quantified relative to the total volume of 1µl each of the extract was subjected to a GC-MS analysis. Aromatic hydrocarbons were quantified relative to the total volume of 1µl each of the extract was subjected to a GC-MS analysis. Aromatic hydrocarbons were quantified relative to the total volume of 1µl each of the extract was subjected to a GC-MS analysis. Aromatic hydrocarbons were quantified relative to the total volume of 1µl each of the extract was subjected to a GC-MS analysis.

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 1.7x10^7 to 3.3x10^7 cfu/g, 2.3x10^7 to 3.8x10^7 cfu/g and 3.6x10^7 to 4.9x10^7 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 1.2x10^5 to 2.8x10^5 cfu/g, 1.8x10^5 to 2.9x10^5 cfu/g and 3.5x10^5 to 4.5x10^5 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 pacificolis, and Pseudomonas putrefaciens. Among the isolates, Pseudomonas aeruginosa, Micrococcus, Clostridium, Bacillus and Serratia exhibited strong oil degrading capabilities.

### TABLE I

<table>
<thead>
<tr>
<th>Sample Location/No</th>
<th>Benthic Sediment</th>
<th>Epipellic Sediment</th>
<th>Mangrove Rhizosphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>THB</td>
<td>HUB</td>
<td>%HUB</td>
<td>THB</td>
</tr>
<tr>
<td>Well Head 1</td>
<td>2.1(7.32)</td>
<td>1.9(5.28)</td>
<td>72.13</td>
</tr>
<tr>
<td>2</td>
<td>1.7(7.23)</td>
<td>1.2(5.08)</td>
<td>70.26</td>
</tr>
<tr>
<td>3</td>
<td>2.3(7.36)</td>
<td>1.6(5.20)</td>
<td>70.65</td>
</tr>
<tr>
<td>Flow Station 1</td>
<td>3.3(7.52)</td>
<td>2.8(5.45)</td>
<td>72.47</td>
</tr>
<tr>
<td>2</td>
<td>2.5(7.40)</td>
<td>2.0(5.30)</td>
<td>71.62</td>
</tr>
<tr>
<td>3</td>
<td>2.4(7.38)</td>
<td>1.6(5.20)</td>
<td>70.46</td>
</tr>
<tr>
<td>Jetty</td>
<td>1.8(7.26)</td>
<td>1.2(5.08)</td>
<td>69.7</td>
</tr>
<tr>
<td>2</td>
<td>2.9(7.46)</td>
<td>2.2(5.34)</td>
<td>71.58</td>
</tr>
<tr>
<td>3</td>
<td>2.6(7.41)</td>
<td>1.9(5.28)</td>
<td>71.26</td>
</tr>
</tbody>
</table>

Values are means of three determinations. Values in parenthesis are log10. THB = total heterotrophic bacteria, HUB = hydrocarbon utilizing bacteria.
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-Methylanthanthrene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, 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-methylanthanthrene (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 vegetation 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,i)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 fruticans* 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 infaunal 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 rhizophora 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 phyto remediation of other contaminated ecosystem.

REFERENCES

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.

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