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Evaluation of Sixteen Priority Polycyclic Aromatic Hydrocarbons in Sediment and Impacts on Benthic Marcoinvertebrate Community Structure of the Lagos Lagoon

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Abstract
The Lagos lagoon in Nigeria, receives myriads of organic wastes containing Polycyclic Aromatic Hydrocarbons (PAH). Sixteen (16) PAHs in the sediment and their effects on community structure of the macrobenthic organisms in Apapa, Commandor Channel and Okobabaof the Lagos lagoon were monitored. PAH levels were determined using Hewlett Packard Gas Chromatograph 6890 with flame ionization detector. The levels of PAHs in the sediment samples from Commandor Channel and Okobaba ranged from 0.030- 0.493 mg/kg and 0.010-0.490 mg/kg, respectively. The highest total PAHs was recorded in the Commandor channel station (0.201 mg/kg). The results of the marcobenthic community structure showed that the species richness was 2.509 in Okobaba. 2.950 in Apapa and 2.911 in Commandor Channel. The highest (178) and lowest (82) number of organisms was recorded in the Okobaba and Apapa respectively. Capitella capitata was the main hydrocarbon pollution indicator species identified in the impacted aquatic stations. There was generally high levels of hydrocarbon, low biodiversity which indicated stressed and contaminated study areas. Continuous monitoring of the level of PAHs and its effects on benthic community is necessary for proper management and containment of organic pollution within the Lagos lagoon

Keywords: Apapa, *Capitella capitata*, Commandor Channel, Okobaba, Polycyclic aromatic hydrocarbons (PAHs)

Introduction
Lagoons are ecologically and economically important aquatic ecosystems that provide water and food, primarily in the form of fish to many people worldwide. Lagos lagoon in Nigeria provides a number of important ecosystem services that include fish supply for the indigenous fishing communities of Ilajes and Ijaws [1]. It is a part of the continuous system of lagoons and creeks that are found along the coast of Nigeria from the border with the Republic of Benin to the Niger Delta. The major outlet of freshwater is at Lagos, Nigeria, where it forms an Extensive harbour [2]. However, the Lagos lagoon is a recipient of different forms of waste that contaminates the aquatic system. These contaminants amongst others include the polycyclic aromatic hydrocarbons (PAH) that are series of organic contaminants; ubiquitous in the Marine environment [3]. They are persistent in the environment with long transport potential and can cause adverse environmental effects [4]. Anthropogenic activities in and around the Lagos lagoon have been suggested as the major sources of PAHs [5] contributing to the significant decline of fishery resources thus threatening their long-term sustainability. Lagos lagoon is also a major harbour for ships importing goods into Nigeria via the Tin Can Port and this is another source of contaminants such as oil and antifouling compounds [6]. Sediments are sinks for chemicals present in industrial and domestic effluents that reflect the historic contamination of water bodies [7]. The sediments within the lagoon range between mud, sandy mud, muddy sand and sandy [8]; a reservoir or sink for pollutants especially hydrophobic organic contaminants. These contaminants can be re-suspended in the water column by natural and or anthropogenic phenomena (tides, dredging and flooding) [9]. Hydrophobic organic contaminants in sediments are routinely identified or quantified, but this is often inadequate for assessing the toxic potential of the sediment extracts to living organisms because of the possible additive, synergistic or antagonistic interactions between components of the complex mixture of compounds present [10]. Additionally, biotic communities have been assessed for pathology and bioaccumulation of non-biodegradable compounds [11]. These observations have been linked to diminishing health of its fin and shelf fish communities [12]. These dramatic effects on the health of biota often fail to draw the required attention from the general public and environmental regulators. Therefore, this calls for a need to identify environmental risks posed by pollution in the Lagos lagoon. The objectives of this study are to investigate the occurrence, concentration and distribution

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of PAHs in the sediment and assess the benthic macro-invertebrates community structure of selected areas of the Lagos lagoon with a view to assessing the environmental health in the selected study area.

Materials and Methodology

Study Sites

Lagos lagoon is a tropical, coastal estuary that stretches from Cotonou in the Republic of Benin, and extends to the fringes of the Niger Delta in Nigeria along its 257 km course (located between Latitude 6°26′ 12.48 to 6°31′57″ and Longitude 3°19′ 48″ to 3°30′ 41″). The three (3) sampling areas were selected based on the degree of anthropogenic activities in the areas along Lagos lagoon [10]. The Apapa and Okobaba sites were located adjacent to zones of dense population and municipal waste discharge. Additionally, the Apapa site was near Tin Can Island, which is the site of the largest port in West Africa and houses a high density of petroleum tank farms. The Commandor Channel is located on the Lagos harbour which is also a busy site in which ship's deck and discharge oil (Figure 1).

Figure 1: Geographical Map of the Sampling Areas

Table 1: Coordinates of Sampling Station

Stations	**Latitude**	**Longitude**
Apapa	006° 26' 48.4"	003° 22' 42.8"
Okobaba	006° 29' 26.7"	003°23' 48.5"
Commodore Channel	006° 25'14.1"	003° 24' 26.0"

Sample Collection

Sediment: A total of nine (9) sediment samples were collected from the three (3) selected locations. At each sampling station, the grab was dropped from the edge of an outboard engine boat and then hauled up into the boat. Composite sediment samples was collected from each station and put into labelled aluminum foil for PAHs analysis.

Benthos: Bottom sediments for the analysis of benthos were collected with a Van veen grab sampler (0.1 m^2) in areas of low flow velocity (< 0.3 m/s) in each of the three stations. Thereafter, the sediment was gently stirred and carefully sieved through a 0.5 mm mesh sieve [13].The content of the sieve after washing was transferred into a pre-labelled container and 10% formalin (with Rose Bengal stain) added as preservative [14] for further analysis for benthic macro-invertebrates in the laboratory.

Laboratory Analysis of Benthic Macro-Invertebrates Samples

In the laboratory, the sieved and fixed samples were taken into a white enamel tray and painstakingly sorted with the aid of the magnifying lens for clearer vision. The sorted samples from each sampled station were then put in a transparent glass bottle and preserved with 5% formalin. The organisms were later identified to their lowest taxonomic group and counted. Identification was done with the aid of relevant guides and texts [15, 16].

Extraction of PAHs in Sediment

For the extraction and purification of PAHs the method proposed by [17] was adopted. However, this was done by implementing some slight modifications based on the context of this research. Granular anhydrous sodium sulphate (AR grade, Malinkrodt) was muffled at 400ºC for 4 hrs prior to use. The boiling chips approximately 10/40 mesh were heated to 400ºC for 30 minutes and then extracted with Dichloromethane (DCM) prior to use. All glassware was washed, rinsed with distilled water, dried at 105ºC for 1 hr and then rinsed with DCM prior to use. Exactly 5 g of the sediment was added to 5bg of anhydrous sodium sulphate (Na_2SO_4) and homogenized-using ceramic pestle and mortar, until fully blended and dried. The homogenized sample was then transferred with a spatula into a 250 ml amber bottle. 50 ml of n-hexane was then added to the sample (the first aliquot was used to rinse the sampling bottle so as to include adsorbed material extract) and the solution was sonicated using a mechanical shaker for 30 minutes.This procedure was then repeated thrice to enhance the extraction rate. The sample was then separated using simple separation technique (separation funnel). The filtrate was then collected into a pre-labelled beaker. This sample was then passed through a rotary evaporator until the solvent has fully evaporated.

Silica Gel Clean-Up Procedure

Exactly 5 ml of glass wool was added to GC-packing column glass syringe graduated to 25 ml. 5 g of pre-heated silica gel (drying agent and impurity removal) and 2 g of Na_2SO_4 was then added to the packing column, which was erected on a retort stand. 30 ml of n-hexane was then added into the erected packing column and collected in a fresh beaker. All the n-hexane was fully eluted. 10 ml of Iso-octane was then added to it until the sample was fully dissolved. The dissolved sample was then added to the erected packing column. This was collected in a fresh beaker. 50 ml of Dichloromethane was then added to the column after ensuring that all the samples have been fully eluted. This was then passed through a rotary evaporator until the sample has been fully evaporated. The sample was then finally dissolved using 2 ml of Iso-octane. The samples were vialed using GC vial bottles. The combined extracts were concentrated to 1 ml over a stream of nitrogen before gas chromatography (GC) analysis using Hewlett Packard Gas Chromatograph with flame ionization detector and HP ChemStation Rev. A 09.01 [1206] software.

A total of sixteen (16) PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranathene, benzo[a]pyrene,indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene and benzo[ghi]perylene) congeners were analyzed using modified methods of [18] and [19].

PAH analysis was conducted with the following GC conditions injection temperature at 250°C, flame ionization detector (FID) at 320°C, and separation on a non-polar, general purpose and industry-standard capillary column HP-1 with length 30 m and ID 0.25 μm. The temperature gradient programme started with an initial temperature of 60°C, upon which the first rate increased with 15°C/minutes for 14 minutes maintained for 3 minutes, followed by a second rate of 10°C/minutes for 5 minutes maintained for 4 minutes. The mobile phase (carrier gas) was nitrogen.

Quality Assurance and Quality Control

All the glassware used was cleaned and dried in the oven at 450°C for 4 hrs. The control quality procedure was performed during PAHs analysis consisting of a standard blank for the prepared samples and an internal calibration method for quantification of PAH congeners of each set of samples, and calibration curves were constructed as previously described [20].

Statistical Analyses

Statistical analysis was done using the SPSS software (version 17). To assess changes in PAHs levels at different sampling sites, one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for mean comparisons was applied ($P < 0.05$). Data processing involved the calculation of biological indices such as Margalef's index for species richness, Shannon-Wiener and Simpson's indices for species diversity, and the Equitability index for evenness of the community. All statistical methods used were adapted from [21]. All diversity analyses were carried out using the PAST software.

Results

Analysis of PAHs in Sediments

Table 2, showed the results of sixteen (16) PAHs detected in sediment samples. Naphthalene and acenaphthylene were undetected in all the three (3) stations (0.000±0.000 mg/kg), while in Commandor Channel Acenaphthene (0.000±0.000 mg/kg) was undetected but was found to be the least in Apapa (0.007±0.003 mg/kg) but highest in Okobaba (0.010±0.005 mg/kg).This was however, not significantly ($P > 0.05$) different

from the station in which it was undetected. Fluorene and phenanthrene concentrations in all the stations were not significantly ($P > 0.05$) different from each other. This similar trend was observed in fluoranthene and pyrene concentrations in all the stations. Apapa showed the highest trend for benz(a)anthracene concentration (0.118±0.003 mg/kg), while the least concentration was observed in Commandor Channel (0.108±0.003 mg/kg). However, Commandor channel indicated the highest PAH concentration (0.331±0.010 mg/kg), while Okobaba showed the lowest concentration of this PAH. Benzo(b)fluoranthene concentration was observed to be highest in Okobaba while the lowest concentration was detected in Apapa. This was however, similar to the concentration of the compound observed in Commandor channel. Benzo(k)fluoranthene levels were observed to be highest in Commandor Channel (0.455±0.013 mg/kg), while the lowest concentration was found in Apapa (0.353±0.015 mg/kg). Apapa had the lowest concentration of Benz(a)pyrene(0.302±0.009 mg/kg), while the highest concentration was detected in the Commandor Channel (0.493±0.030 mg/kg). Indeno(1,2,3-cd)pyrene concentration was also found to be highest at the Commandor Channel (0.168±0.020 mg/kg) followed by Okobaba (0.118±0.014 mg/kg), while the least concentration was found in Apapa (0.055±0.012 mg/kg). Benzo(g,h,i)perylene concentration was not detected in Okobaba (0.000±0.000 mg/kg)but was highest in the Commandor channel (0.213±0.022 mg/kg) which was statistically different from the concentration of this compound in Apapa (0.121±0.025 mg/kg).

Table 2: Mean concentration of PAHs (mg/Kg) in the different sampled stations

	Okobaba	**Apapa**	**Commandor Channel**
Naphthalene	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}
Acenaphthylene	0.000±0.000^{a}	0.000±0.000^{a}	0.000±0.000^{a}
Acenaphthene	0.010±0.005^{a}	0.007±0.003^{a,b}	0.000±0.000^{a}
Fluorene	0.034±0.001^{a,b}	0.031±0.001^{a,b}	0.034±0.002^{a,b}
Phenanthrene	0.045±0.002^{a,b}	0.043±0.002^{a,b}	0.052±0.003^{a,b}
Anthracene	0.000±0.000^{a}	0.000±0.000^{a,b}	0.000±0.000^{a}
Fluoranthene	0.054±0.003^{a,b}	0.012±0.004^{a,b}	0.051±0.001^{a,b}
Pyrene	0.042±0.007^{a,b}	0.023±0.002^{a,b}	0.019±0.001^{a,b}
Benz(a)anthracene	0.183±0.022^{c}	0.118±0.003^{c}	0.108±0.003^{b,c}
Chrysene	0.207±0.008^{c}	0.229±0.010^{d}	0.331±0.010^{e}
Benzo(b)fluoranthene	0.490±0.045^{e}	0.350±0.015^{f}	0.416±0.016^{f}
Benzo(k)fluoranthene	0.418±0.027^{d,e}	0.353±0.015^{f}	0.455±0.013^{f}
Benzo(a)pyrene	0.388±0.026^{d}	0.302±0.009^{e}	0.493±0.030^{f}
Dibenz(a,h)anthracene	0.114±0.025^{b,c}	0.077±0.017^{b,c}	0.091±0.020^{f}
Indeno(1,2,3-cd)pyrene	0.118±0.014^{b,c}	0.055±0.012^{a,b,c}	0.168±0.020^{c,d}
Benzo(g,h,i)perylene	0.000±0.000^{a}	0.121±0.025^{c}	0.213±0.022^{d}

*Same superscript in each row showed no-significant difference ($P < 0.01$) using Duncan Multi Range (DMR) test.

Community Structure of Benthic Macroinvertebrates

A summary of the spatial distribution of benthic macro-invertebrates collected during the period of sampling at the study stations Apapa, Commodore Channel and Okobaba (Tables 3 and 4) respectively. A total of 348 individuals comprising two (2) phyla were recorded. The spatial distribution of dominant species during the period of study was highest at Okobaba (158), while lower dominance was recorded at Apapa (64) and Commodore channel (61). Macroinvertebrates abundance steadily increased with reducing particle size. Commandor Channel (27) was dominated by the highest number of annelida, while the least was observed in Okobaba (20) followed by Apapa (17). Okobaba has the highest relative abundance of mollusca species (71).

Table 3: Spatial composition, distribution and abundance of benthic macro-invertebrates of study stations in Lagos lagoon

Taxa	Okobaba	Okobab a	Okobaba	Apap a	Apap a	Apapa	Commodore channel	Commodore channel	Commodore channel	
Phylum: Annelida										***total species per sampling area***
Glycera sp.	1	1	2	-	1	2	3	1	-	11
Nephtys sp.	2	2	2	2	3	1	2	5	2	21
Capitella capitata	3	2	1	1	-	1	1	5	3	17
Nereis sp.	1	2	1	-	4	2	1	-	4	15
Phylum: Mollusca										
Neritina senegalensis	2	2	5	2		1	2	6	1	21
Neritina glabrata	18	4	11	3	1		2	1	7	47
Pachymelania aurita	5	5	5	5	5		5	2	1	33
Tympanotonus fuscatus	23	11	16	2	5	2	1	5	1	66
T.fuscatusVar radula	14	4	8	6	4	1		3	2	42
Dosinia isocarda	4	1	3	4	1	1	2	3	3	22
Iphigenia truncata	4	2	1	1	3	2	2	1	1	17
Tellina nymphalis	-	2	1	4	2	2	2	2	1	16
Aloides trigona	1	-	2		1	4	1	-	-	9
Aloides sulcata		3	1	2	-	1	2	1	1	11
Total Species Per Sampling Period Per Station	**78**	**41**	**59**	**32**	**30**	**20**	**26**	**35**	**27**	**348**

Diversity Indices of Benthic Community Structure

The community structure analysis indicated that Okobaba (0.19) have the highest dominance while similar dominance values were observed in Apapa and commodore channel (0.12). Commandor channel (2.19) and Apapa (2.16) had the highest species richness and evenness for the mollusca group while the least was observed in Okobaba. However, Commandor Channel was dominated by the highest number of annelids, while the least was observed in Apapa which is relative with PAHs level also observed in sediment. Commandor channel also had the highest species richness and the least evenness; this was the reverse in Okobaba (Table 4). Overall, Okobaba (0.19) indicated the highest species dominance, which was the least in Apapa for the macrobenthos while Apapa had the highest species richness, Brillouin, Menhinick, and evenness. The Shannon diversity test indicated that there was no significant difference ($P > 0.001$) for the diversity of the macrobenthic community between Okobaba and Apapa. This was a similar result obtained (but at $P > 0.05$) between Apapa and Commandor channel. Okobaba and Commandor Channel also showed a similar diversity in the marcobenthic community ($P > 0.001$).

Table 4: Community structure analysis of the study areas in Lagos lagoon

Phylum Mollusca	**Commodore_channel_**	**Okobaba**	**Apapa**
Taxa_S	10	10	10
Individuals	61	158	65
Dominance_D	0.12	0.19	0.12
Simpson_1-D	0.88	0.81	0.88
Shannon_H	2.20	1.91	2.21
Evenness_e^H/S	0.90	0.67	0.91
Brillouin	1.95	1.80	1.98
Menhinick	1.28	0.80	1.24
Margalef	2.19	1.78	2.16
Equitability_J	0.95	0.83	0.96
Fisher_alpha	3.40	2.37	3.30
PhylumAnnelida			
Taxa_S	4	4	4
Individuals	27	20	17
Dominance_D	0.28	0.26	0.29
Simpson_1-D	0.72	0.74	0.71
Shannon_H	1.33	1.37	1.29
Evenness_e^H/S	0.94	0.98	0.91
Brillouin	1.15	1.14	1.05
Menhinick	0.77	0.89	0.97
Margalef	0.91	1.00	1.06
Equitability_J	0.96	0.99	0.93
Fisher_alpha	1.30	1.50	1.649

Discussion

The study established sixteen (16) priority PAHs congeners in sediment within the selected areas of the Lagos lagoon and their effects on the benthic macro invertebrates' community structure. The range of values of polycyclic aromatic hydrocarbons studied, demonstrated large fluctuations within the period of study possibly influenced by anthropogenic activities. This observation corroborates with reports of earlier workers [22, 23 & 10] in Southwest Nigeria. PAHs are environmental contaminants found to be ubiquitous in aquatic ecosystem through several anthropogenic activities that could be generated during incomplete combustion petrol, wood, coal and different organic materials. Among the other sources of anthropogenic PAHs generation are industrial waste burning, open domestic waste burning, open landfill burning, seepage, indiscriminate disposal of petrol waste from roadside, mechanic and motor shops, oil refineries as well as vehicular emission. The partitioning in

the atmosphere between the gaseous and particulate phase influences the transportation of PAHs from the atmospheric phase to the aquatic environment affecting the entire community of the aquatic ecosystem and invariably human. Several Polycyclic aromatic hydrocarbons have been found to be carcinogenic, mutagenic and very toxic. PAHs are highly lipophilic thus readily absorbed from the gastrointestinal tract of many aquatic biota that are easily distributed through the organs due to the affinity for fatty tissues.

The analysis of the community structure of benthic macro-invertebrates of the selected sampled area of the Lagos lagoon revealed two (2) major phyla; Mollusca and Annelida . This is in concordance with the findings of [24] who observed mollusca dominance in the southern -western lagoon. Spatial composition, distribution and abundance of benthic macro-invertebrates of the study stations indicated that Okobaba was dominated by *Tympanotonus fuscatus* and *Neritina glabrata* while Apapa station was dominated by *T.fuscatus Var radula* and *Pachymelania aurita* whereas the Commandor Channel was dominated by *Dosinia isocarda* and *Pachymelania aurita* were similar results obtained by [25]. It was observed that *T. fuscatus* had the highest relative abundance in all the locations. This trend of abundance was also observed by [26]. The abundance of Polychaetes and a reduction in diversity in the sampling sites indicated regions with high molecular weight PAHs, which are persistent [27]. Mirza and Gray [28], also noted decreased species diversity along a gradient from relatively unpolluted areas to areas highly impacted by organic pollution. Mirza and Gray [28] reported that C. *capitata, Polydora spp*. and *Nereimyra punctata* were highly abundant at the most polluted sites and were therefore, designated as indicators of polluted conditions. One important characteristic of this decrease in diversity was the increase in species dominance most notably by Polychaetes. Among the three (3) species, both *C. capitata* and *Polydora spp*. have been characterized by [29] as indicators of organic enrichment. The assumption underlying the environmental studies is that PAHs may have adverse effects on organisms in the receiving water. Many invertebrates, such as molluscs and crustaceans, tend to accumulate PAHs without obvious detrimental effects [30, 31]. However, various studies have reported toxic effects, even at moderate PAH concentrations [32]. [33] Apparently, the ability to metabolize PAHs differs among species and classes of organisms, which may account for some of the reasons potential hazard, tend to differ among species. In Polychaetes, the metabolic ability varies considerably among species and may not be predicted on the basis of standard taxonomic classifications [34].

In molluscs, it was generally recognized that the overall ecological effects of contaminants will be exposed through the natural communities of species, since a community represents a system of species interactions and, accordingly, integrates and reflects the responses of all affected species. The study of natural species communities is therefore, a key element in many monitoring programmes addressing the spatial and temporal effects of contaminants. However, the effects may be minimal compared to the influences of natural abiotic factors and may also be overshadowed by large biological variation. The inherent difficulty may lie in correctly linking biotic responses and environmental influences, in particular when it comes to detecting and interpreting subtle changes [35]. Despite the complexity of the systems, the importance of community or ecosystem studies cannot be ignored [36]. Consequently, there is an increased abuse of the lagoon as it is being used for sewage, refuse disposal and other anthropogenic activities. The general degradation of the environment arising from human activities is high in the lagoon and if it continues unchecked, could lead to modification in biotic community structure and dynamics in response to changes in spatial complexity of the vegetative structure, shading effects of dense canopies, amount and location of plant biomass, densities of vegetation patches, plant detritus deposition rate, growth rates, dissolved oxygen levels and rates of evapotranspiration [37]. Therefore, there is need for proper, continuous monitoring and enforcement of laws in order to curtail human activities in the area thus protecting the environment.

Conclusion

Apapa, Okobaba and commodore Channel of Lagos Lagoon can become grossly contaminated with these increasing trends of PAHs level in sediments within the sampling areas. Also, the observed effects of PAHs level in the sediments probably indicated effects of these compounds on the distribution of benthic community structure. However, the presence of tolerant species (*C. capitata* and *Nereis sp.*) in Commandor channel and Okobaba may serve to confirm that the stations were organically polluted. Additionally, low density of Polychaetes (pollution indicator species) and more abundance of Mollusca (pollution sensitive species) in Okobaba indicated a relatively healthy community.

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