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Assessment of the Heterotrophic and Crude Oil Utilizing Microorganisms of Imo River Estuary of the Niger Delta Mangrove Ecosystem

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Abstract

Sediment and water samples from Imo river estuary were analyzed to assess the influence of seasonal variation on the microbial populations. The results obtained shows that the sediment samples had a significantly ($P < 0.05$) higher counts of total heterotrophic bacteria (THB) than the surface and sub-surface water samples. The counts obtained in the dry season were significantly higher ($P<0.05$) than the wet season. There were no significant difference ($P > 0.05$) in the population of THB in the surface water during the wet and dry season ($2.23 \times 10^6 \pm 2.23$ cfuml^{-1} and $2.39 \times 10^6 \pm 1.63$ cfuml^{-1}) respectively, while there were significant difference ($P < 0.05$) in the population of total heterotrophic bacteria (THB) in the sub-surface water during the dry and wet season ($2.27 \times 10^6 \pm 2.00$ cfuml^{-1} and $2.13 \times 10^6 \pm 1.84$ cfuml^{-1}) respectively. The total heterotrophic fungi (THF) densities in the surface water were $1.17 \times 10^5 \pm 0.93$ cfuml^{-1} and $1.38 \times 10^5 \pm 0.63$ cfuml^{-1} during the wet and dry season respectively. The mean THF densities of $1.15 \times 10^5 \pm 0.63$ cfuml^{-1} and $1.30 \times 10^5 \pm 0.48$ cfuml^{-1} were observed in the sub-surface water during the wet and dry season respectively. The mean densities for sediments were $1.42 \times 10^6 \pm 1.19$ cfug^{-1} and $1.60 \times 10^6 \pm 1.05$ cfug^{-1} during the wet and dry season respectively. Significant difference ($P < 0.05$) in the population of total heterotrophic and crude oil utilizing microorganisms for crude oil polluted and pristine samples were also observed. It can be concluded that the microbial population of Imo river estuary is higher during the dry season than the rainy season.

Keywords: Heterotrophic, Crude oil, Sediment, Surface, Sub-surface

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Introduction

Aquatic microorganisms play a significant ecological and biogeochemical role in marine ecosystems due to their high abundance relative to the overlying water column they play a key role in the decomposition of organic matter, nutrient cycling and carbon flux (Edlund and Jansson, 2006; Head *et al*., 2006, Ekpo *et al*., 2012). It is known that dense aggregations of microorganisms may rapidly colonize clean surfaces of both hard and soft substrates in the sea, following adsorbed organic molecules which may serve as a stimulus for the initial immigrants: bacteria, fungi, and unicellular algae. Microbial communities are structured by temporal and spatial variability of physicochemical and biotic parameters (Hewson *et al*., 2007). Microbial communities readily respond at faster rates (compared to other benthic organisms) to environmental and pollution changes. This reflects their micro environmental conditions and "communicates" this information to other biota in their vicinity and therefore plays key roles in benthic-pelagic coupling. The estimation of microbial abundances as well as their genetic diversity under in situ conditions is therefore the most fundamental objective of aquatic microbial ecology (Hewson *et al*., 2007).

The detection of microbial diversity and their variation in water and surface sediments is of great practical and scientific relevance, especially in coastal ecosystems. Recently, the analysis of changes in surface sediment microbial community has been used for detecting and monitoring the biological effects of human activities in the marine environment (Zhang *et al*., 2008). If the microbial community structure in soft-benthic habitat is determined by their environment, then pollution loading or organic enrichment is expected to shift their composition, and a counter shift toward the original community should be evident after the abatement of pollution discharge. Microorganisms in water and sediments attract other biota by providing resources (e.g. food, habitat, shelter), or, by signaling settlement sites with increased fitness expectations (i.e. high survival probabilities until reproduction) and, conversely to avoid others with low fitness potential.

In Nigeria, the Niger Delta region produces more than 80% of the country's crude oil. There is presently an unprecedented increase in the upstream and downstream activities of the oil and allied companies in this area (Chikere *et al*., 2009). Over the years, these oil companies have generated myriad of pollutants in the form of gaseous emissions, oil spills, effluents and solid waste (Chikere *et al*., 2009) that have polluted the marine environment beyond sustainability.

Heightened navigational activities in inland and coastal waters of the Niger Delta region is another anthropogenic source of petroleum pollution of the aquatic environment. The aim of this study is to estimate the population of heterotrophic microorganisms including aerobic heterotrophic bacteria, and fungi (moulds) and oil degrading microorganisms in Imo river estuary of the Niger Delta mangrove ecosystem.

Materials and Methods

Study Site

The study site for this research work was Imo river estuary in the Niger Delta region of Nigeria. Pristine water and sediment samples were collected from Great Qua river Calabar. Imo River estuary lies between latitude 04^0 $34^0$52N and longitude 007^0 $32^0$59E, with an elevation of 11 m above sea level. Five sampling points were used.

Sample Collection

Using a decontaminated shovel and a scoop, the desired thickness and volume of sediment from the sampling area was collected and transferred into an appropriate sample or homogenization container. Surface water was decanted from the sample prior to sealing and transfer, care was taken to retain the fine sediment fraction during this process. Collection of surface and sub-surface water samples was done aseptically into clean one (1) liter capacity plastic bottles. The bottles were open to fill and closed below the water surface. All containers were rinsed at least three times with water that was to be analyzed (APHA, 1998). After collection, the samples were refrigerated at about 4°C during the transport to the laboratory (Radojevic and Bashkin, 1999; APHA, 1985, 1998).

Microbiological Analysis

Sediment and water samples for microbial analysis were collected aseptically, labeled and stored in ice packed plastic coolers and transported to the laboratory where analysis within 24 hours of collection was done. The sediment samples were homogenized before analysis.

Ten (10 g) of each sample was weighed out, added to 90 ml of sterile deionized water and vigorously shaken for 1 minute using a vortex shaker to dislodge the microbiota. This method disrupted the flocculent material and randomly disrupts the protists. Treated samples were allowed to settle for 10 minutes prior to withdrawal of supernatant for serial dilution. Ten-fold serial dilution of the sediment and water samples was carried out for enumeration of densities of the different microbial groups.

Estimation of Microbial Densities of Sediment and Water Samples

Several methods and media were used for the enumeration of the various microbial groups. The densities of the following microbial groups were determined: Total heterotrophic bacteria (THB), total heterotrophic fungi (THF), crude oil utilizing bacteria (CUB), crude oil utilizing fungi (CUF)

Culture Media

The analytical media employed in the course of this research included: nutrient agar (NA), Sabouroud dextrose agar (SDA), mineral salts medium (MSM), thiosulfate citrate-bile salts-sucrose agar (TCBS), Salmonella-Shigella agar (SSA) and agar - agar. The media were prepared according to recommendations by the manufacturers (DIFCO, 1984).

Estimation of Densities of Heterotrophic Microorganisms

The counts of total heterotrophic bacteria in sediment and water samples were determined by the pour plate techniques (Chikere *et al.,* 2009,) using nutrient agar (NA). The NA medium was amended with nystatin ($50\mu gml^{-1}$) in order to prevent the growth of fungal contaminants. The total heterotrophic fungi count was determined by pour plate technique using Sabouroud dextrose agar (SDA) supplemented with streptomycin ($50\mu gml^{-1}$) to inhibit the growth of bacterial contaminants (Martini *et al.,* 1980; Barnett and Hunter, 1972). Inoculated NA plates were incubated at 28^0C for 24 hours, while the SDA plates were incubated at room temperature for 3 days before enumeration of microbial colonies.

Isolation, Purification and Maintenance of Pure Microbial Isolates

Distinct or representative colonies from the culture plates were selected for characterization. The test results for bacteria were evaluated using characteristics presented in *Bergy's manual of determinative bacteriology* (Holt *et al.,* 1994). Representative colonies of fungi isolates were characterized and identified based on their cultural and morphological features as described by Barnett and Hunter (1987). Bacterial colonies were repeatedly transferred to freshly prepared nutrient agar plates by the streak-plate method and allowed to grow for 24 hours before stocking. Similarly, distinct fungal colonies were subcultured repeatedly on freshly prepared Sabouroud dextrose agar plates for 72 hours before stocking.

Enumeration of Crude Oil Utilizing Microorganisms

The counts of crude oil utilizing bacteria and fungi were enumerated by pour plate techniques (Mills *et al*., 1978; Obire *et al*., 2008) using vapour phase transfer technique on mineral salts medium (MSM). For the enumeration of oil degrading bacteria, the medium was supplemented with $50\mu gml^{-1}$ fungizol miconazole nitrate to prevent the growth of fungal contaminants. On the other hand, mineral salts medium supplemented with $50\mu gml^{-1}$ streptomycin to inhibit the growth of bacterial contaminants was used to ensure the enumeration of oil degrading fungi. In both cases the crude oil used was sterilized by millipore filtration (0.45 μm pore size) and stored in sterile bottles. The plates were incubated at room temperature for 5 days before enumeration.

Statistical Analysis

Data collected were subjected to 2x3x6 factorial experiment in a Completely Randomized Design (CRD), significant means were separated using Least Significant Difference (LSD) test at 5% probability level.

Results

Total Heterotrophic Bacteria (THB)

In this study, the results show that the season of study influenced microbial proliferation as significantly ($P < 0.05$) higher microbial population levels were observed in sediments during the dry season. It was observed that the sediment samples produce significantly ($P < 0.05$) higher total heterotrophic bacteria (THB) count than the surface and sub-surface water samples. However, the result shows that sediment samples during the dry season produced significantly higher ($P < 0.05$) THB with a mean density of $2.55 \times 10^7 \pm 2.34$ than the wet season with a mean density of $2.46 \times 10^7 \pm 2.20$. There were no significant difference ($P > 0.05$) in THB between the mean values of the surface water during the wet and dry season ($2.23 \times 10^6 \pm 2.23$ and $2.39 \times 10^6 \pm 1.63$) respectively, while there were significant difference ($P < 0.05$) between the mean values of total heterotrophic bacteria (THB) in sub-surface water during the dry and wet season ($2.27 \times 10^6 \pm 2.00$ and $2.13 \times 10^6 \pm 1.84$) respectively. The sub-surface water samples during the wet season had the lowest microbial count with a mean density of $2.13 \times 10^6 \pm 1.84$. The result revealed that in Imo river estuary, there were no significant difference ($P > 0.05$) in total heterotrophic bacteria (THB) in all the sampling points in a given microhabitat (Table 1).

Table 1: Influence of source of sample collection and sampling points on microbial populations

	Surface water (cfuml^{-1})						Sub-surface water (cfuml^{-1})						Sediment (cfug^{-1})					
	P	**SP1**	**SP2**	**SP3**	**SP4**	**SP5**	**P**	**SP1**	**SP2**	**SP3**	**SP4**	**SP5**	**P**	**SP1**	**SP2**	**SP3**	**SP4**	**SP5**
THB	$1.95^{c} \times 10^{5} \pm 2.30$	$2.39^{b} \times 10^{6} \pm 1.98$	$2.33^{b} \times 10^{6} \pm 0.84$	$2.46^{b} \times 10^{6} \pm 0.16$	$2.39^{b} \times 10^{6} \pm 0.59$	$2.33^{b} \times 10^{6} \pm 0.23$	$1.16^{c} \times 10^{5} \pm 0.15$	$2.36^{b} \times 10^{5} \pm 1.53$	$2.37^{b} \times 10^{6} \pm 2.32$	$2.28^{b} \times 10^{b} \pm 0.98$	$2.36^{b} \times 10^{6} \pm 0.56$	$2.30^{b} \times 10^{6} \pm 0.18$	$2.36^{b} \times 10^{6} \pm 0.17$	$2.63^{a} \times 10^{7} \pm 0.10$	$2.43^{a} \times 10^{7} \pm 0.94$	$2.58^{a} \times 10^{7} \pm 1.32$	$2.50 \times 10^{7} \pm 1.04$	$2.52^{a} \times 10^{7} \pm 0.95$
CUB	$1.21^{c} \times 10^{4} \pm 0.18$	$1.43^{b} \times 10^{5} \pm 0.94$	$1.22^{b} \times 10^{5} \pm 0.23$	$1.33^{b} \times 10^{5} \pm 0.58$	$1.24^{b} \times 10^{5} \pm 0.19$	$1.18^{b} \times 10^{5} \pm 0.26$	$1.04^{c} \times 10^{4} \pm 1.54$	$1.31^{b} \times 10^{5} \pm 0.91$	$1.21 \times 10^{5} \pm 0.83$	$1.14^{b} \times 10^{5} \pm 0.43$	$1.41 \times 10^{5} \pm 1.90$	$1.38^{b} \times 10^{5} \pm 0.0.24$	$1.49^{b} \times 10^{5} \pm 1.90$	$1.51^{b} \times 10^{6} \pm 0.18$	$1.62 \times 10^{6} \pm 0.17$	$1.73^{a} \times 10^{6} \pm 0.23$	$1.53 \times 10^{6} \pm 0.95$	$1.58^{a} \times 10^{6} \pm 1.60$
THF	$9.6^{c} \times 10^{3} \pm 0.98$	$1.38^{b} \times 10^{5} \pm 0.23$	$1.36^{b} \times 10^{5} \pm 0.20$	$1.27^{b} \times 10^{5} \pm 0.28$	$1.07^{b} \times 10^{5} \pm 0.34$	$1.61^{b} \times 10^{5} \pm 0.56$	$7.5^{c} \times 10^{3} \pm 0.19$	$1.18^{b} \times 10^{5} \pm 0.51$	$1.16^{b} \times 10^{5} \pm 1.06$	$1.17^{b} \times 10^{5} \pm 0.82$	$1.48 \times 10^{5} \pm 0.19$	$1.60^{b} \times 10^{5} \pm 1.52$	$1.34^{b} \times 10^{5} \pm 1.63$	$1.44^{a} \times 10^{5} \pm 0.54$	$1.52 \times 10^{6} \pm 0.23$	$1.55^{a} \times 10^{6} \pm 0.91$	$1.46 \times 20^{6} \pm 0.60$	$1.74^{a} \times 10^{6} \pm 1.08$
CUF	$7.1^{d} \times 10^{2} \pm 0.30$	$8.0^{c} \times 10^{3} \pm 0.18$	$7.9^{c} \times 10^{3} \pm 0.10$	$9.3^{c} \times 10^{3} \pm 0.63$	$8.0^{c} \times 100 \pm 0.19$	$8.6^{c} \times 10^{3} \pm 0.20$	$5.0^{d} \times 10^{2} \pm 0.15$	$8.9^{c} \times 10^{3} \pm 0.22$	$9.7^{c} \times 10^{3} \pm 0.24$	$9.7^{c} \times 10^{3} \pm 0.12$	$8.0^{c} \times 10^{3} \pm 0.18$	$8.6^{c} \times 10^{3} \pm 0.21$	$1.11^{b} \times 10^{4} \pm 0.43$	$1.08^{b} \times 10^{4} \pm 0.50$	$9.1^{b} \times 10^{4} \pm 0.26$	$1.08^{a} \times 10^{5} \pm 0.32$	$1.21^{a} \times 10^{5} \pm 0.12$	$1.04^{a} \times 10^{5} \pm 0.20$

Means with the same superscript along the horizontal array represent no significant difference (P>0.05).

KEY: THB = total heterotrophic bacteria, THF = total heterotrophic fungi, CUB = crude oil utilizing bacteria, CUF = crude oil utilizing fungi, cfu = colony forming unit, P = pristine, SP_n = sampling point number. LSD: THB =2.53, CUB =1.48, THF =2.64, CUF = 1.28

Crude Oil Utilizing Bacteria (CUB)

The densities of crude oil utilizing microorganisms were significantly ($P < 0.05$) low compared to total heterotrophic counts. The results show that there were significant difference ($P < 0.05$) in population of crude oil utilizers with respect to season (Table 3). The mean densities of crude oil utilizing bacteria observed in Imo river estuary were $1.22 \times 10^5 \pm 1.20$ cfuml^{-1} and $1.32 \times 10^5 \pm 1.05$ cfuml^{-1} in surface water during the wet and dry season respectively, in the sub-surface water, the mean densities observed were $1.18 \times 10^5 \pm 1.06$ cfuml^{-1} and $1.32 \times 10^5 \pm 0.98$ cfuml^{-1} during the wet and dry season respectively, while for the sediments, the mean densities observed were $1.52 \times 10^6 \pm 2.03$ cfug^{-1} and $1.63 \times 10^6 \pm 1.34$ cfug^{-1} during the wet and dry season respectively. The results show that there were no significant difference ($P > 0.05$) in crude oil utilizing bacteria (CUB) in sediments and sub-surface water during the wet and dry season, while surface water samples produced significantly ($P < 0.05$) higher density of CUB during the wet season than dry season.

Total Heterotrophic Fungi (THF)

The total heterotrophic fungi (THF) density (Table 3) revealed that in the surface water, the mean densities observed were $1.17 \times 10^5 \pm 0.93$ cfuml^{-1} and $1.38 \times 10^5 \pm 0.63$ cfuml^{-1} during the wet and dry season respectively, the mean densities of $1.15 \times 10^5 \pm 0.63$ cfuml^{-1} and $1.30 \times 10^5 \pm 0.48$ cfuml^{-1} were observed in sub-surface water during the wet and dry season respectively, while in the sediments, the mean densities observed were $1.42 \times 10^6 \pm 1.19$ cfug^{-1} and $1.60 \times 10^6 \pm 1.05$ cfug^{-1} during the wet and dry season respectively. The result shows that the sediment samples produce a significantly ($P < 0.05$) higher counts of total heterotrophic fungi during the dry season than the wet season and was significantly ($P < 0.05$) higher than the densities observed in both surface and sub-surface water. There were no significant differences ($P > 0.05$) in total heterotrophic fungi counts (THF) in surface and sub-surface water throughout the study. The influence of season and sampling point on total heterotrophic fungi (THF) shows that there were no significant differences ($P > 0.05$) in the sampling points throughout the study (Table 2).

Table 2: Influence of season and sampling points on the microbial population of the samples

	Wet season (August)						Dry season (November)						
	P	SP1	SP2	SP3	SP4	SP5	P	SP1	SP2	SP3	SP4	SP5	LSD
THB (10^6)	1.87^b ±2.3	2.38^a± 1.56	2.31^a ±1.20	2.67^a± 0.98	2.35^a± 1.02	2.33^a± 1.16	2.05^b± 2.4	2.54^a± 0.95	2.45± 1.52^a	2.51± 1.06	2.48^a± 0.91	2.44^a± 2.10	2.06
CUB (10^5)	1.17^a ±0.5	1.40^a± 0.98	1.29^a ±1.02	1.34^a± 0.68	1.32^a± 0.89	1.30^a± 1.12	1.32^a± 0.73	1.43^a± 0.63	1.41± 0.55^a	1.46± 0.80	1.49^a± 1.01	1.46^a± 0.62	1.21
THF (10^5)	9.3^a ±0.21	1.26± 0.90	1.31^a ±1.13	1.24^a± 0.56	1.23^a± 0.24	1.51^a± 0.83	1.11^a± 0.51	1.40^a± 0.98	1.38± 0.63^a	1.41± 0.19	1.45^a± 1.04	1.78^a± 0.97	2.16
CUF (10^3)	7.0^b ±0.23	8.1^b± 0.81	8.1^b± 0.53	9.0^a±0.63	8.4^a±0.53	8.6^a±0.18	8.5^a±0.24	9.9^a±0.91	9.2±0.16^a	1.04± 0.20	1.03^a± 0.18	1.01^a± 0.28	1.06

Means with the same superscript along the horizontal array represent no significant difference (P>0.05).
KEY: THB = total heterotrophic bacteria, THF = total heterotrophic fungi, CUB = crude oil utilizing bacteria, CUF = crude oil utilizing fungi, cfu = colony forming unit, P = pristine, SP_n = sampling point number. LSD: THB = 2.06, CUB = 1.21, THF = 2.16, CUF =1.05.

Crude Oil Utilizing Fungi (Cuf)

The mean density of crude oil utilizing fungi (CUF) in Imo river estuary also shows similar patterns as observed for total heterotrophic fungi (THF). The mean densities observed for the surface water were 7.2 x 10^3 ± 0.23 cfuml^{-1} and 8.9 x 10^3 ± 0.63 cfuml^{-1} during the wet and dry season respectively. In the sub-surface water, the mean densities were 7.4 x 10^3 ± 0.78 cfuml$^-$ and 8.8 x 10^3 ± 0.84 cfuml^{-1} during the wet and dry season respectively, while for the sediments, the mean densities observed were 9.9 x 10^4 ± 0.94 cfug^{-1} and 1.15 x 10^5 ±0.98 cfug^{-1} during the wet and dry season respectively (Table 3). The result shows that the sediments in Imo river estuary had a significantly (P < 0.05) higher density of crude oil utilizing fungi (CUF) during the dry season than the wet season, while there were no significant difference (P > 0.05) in density of CUF in surface and sub-surface water. Furthermore, the result shows that the density of CUF in sediments was significantly (P < 0.05) higher than the density observed in the overlaying water and sub-surface water samples (Table 3).

Table 3: Influence of season and source of sample collection on the microbial population of the samples

	Wet season (August)			Dry season (November)			
	SW (cfuml^{-1})	SSW (cfuml^{-1})	SED (cfug^{-1})	SW (cfuml^{-1})	SSW (cfuml^{-1})	SED (cfug^{-1})	LSD
THB	$2.23^{c}x10^{6}\pm2.23$	$2.13^{d}x10^{6}\pm1.84$	$2.46^{b}x10^{7}\pm2.20$	$2.39^{c}x10^{6}\pm1.63$	$2.27^{c}x10^{6}\pm2.00$	$2.55^{a}x10^{7}\pm2.34$	1.46
CUB	$1.22^{a}x10^{5}\pm1.20$	$1.18^{b}x10^{5}\pm1.06$	$1.52^{a}x10^{6}$ ±2.03	$1.32^{b}x10^{6}\pm1.05$	$1.32^{b}x10^{5}\pm0.98$	$1.63^{a}x10^{6}\pm1.34$	8.5
THF	$1.17^{c}x10^{5}\pm0.93$	$1.15^{c}x10^{5}\pm0.63$	$1.42^{b}x10^{4}\pm1.19$	$1.38^{c}x10^{3}\pm0.63$	$1.30^{c}x10^{3}\pm0.48$	$1.60^{a}x10^{5}\pm1.05$	1.52
CUF	$7.2^{c}\times10^{3}\pm0.23$	$7.4^{c}\times10^{3}\pm0.78$	$9.9^{b}\times10^{4}\pm0.94$	$8.9^{c}\times10^{3}\pm0.63$	$8.8^{c}\times10^{3}\pm0.84$	$1.15^{a}\times10^{5}\pm0.98$	1.05

Means with the same superscript along the horizontal array represent no significant difference (P>0.05).
KEY: SW = surface water, SSW = sub-surface water, SED = sediment, THB = total heterotrophic bacteria, THF = total heterotrophic fungi, CUB = crude oil utilizing bacteria, CUF = crude oil utilizing fungi, cfu = colony forming unit.

Discussion

Water and sediment microbial community are a major component of microbial food webs, biogeochemical cycles and energy flow. Bacteria and fungi are the predominant organisms in these microhabitats (Youssef *et al.,* 2010). Their biodiversity is structured and determined by the temporal and spatial variability of physicochemical and biotic parameters and thus, can reflect local environmental conditions (Urakawa *et al*., 1999; Zhang *et al.,* 2008). In this study, the results obtained showed that the season of study influences microbial proliferation as significantly ($P < 0.05$) higher population levels were observed especially in sediments during the dry season. It was observed that the sediments sample produced significantly ($P < 0.05$) higher total heterotrophic bacteria (THB) count than the surface and sub-surface water samples. The increase in microbial levels of heterotrophic microorganisms especially in sediments and sub-surface water during the dry season can be linked with the slight increase in the temperature of the estuarine ecosystem during the study period. The slightly lower population of heterotrophic microorganisms during the wet season may be due to changes in biological oxygen demand, dissolved oxygen levels, temperature and salinity.

The low microbial populations observed in Great Qua river may be due to low levels of nutrients and productivity level in the river environment. The main reason for high abundance of microbial populations in estuaries is the high productivity rate because estuaries provide habitats for a large number of organisms and the presence of phytoplankton (mainly the diatoms and dinoflagellates) which are the primary producers in estuaries (Branch, 1999). The results show that sediment samples had a significantly ($P < 0.05$) higher counts of total heterotrophic fungi than surface and sub-surface water. The strong link between bacteria and phytoplankton earlier reported by Wolta (1982) may be the main reason for higher bacteria abundance than any other microorganism in the estuarine ecosystem.

The densities of crude oil utilizing microorganisms were low compared to total heterotrophic counts. The results show that there was a significant difference ($P < 0.05$) in crude oil utilizers with respect to season and microhabitats. This may have resulted from the high heterotrophic activities in the environment. In Great Qua river (pristine site) lower densities of crude oil utilizing microorganisms were observed. From the results, it is observed that, the percentage of petroleum degrading microorganisms in the total viable count increases with increase amount of petroleum hydrocarbons at the sites under study. So we can rely on the level of hydrocarbon utilizing microorganisms as a sensitive index of environmental exposure to hydrocarbons. This is in agreement with a number of reports (Atlas and Bartha, 1992; Atlas, 1981; Walker and Colwell, 1976; Agbor *et al*., 2013) who reported that the number of hydrocarbon utilizing microorganisms and their proportion in the heterotrophic community increases upon exposure to petroleum or other hydrocarbon pollutants and that the levels of hydrocarbon utilizing microorganisms generally reflect the degree of contamination of the ecosystem.

In general, the sediment shows high average density of heterotrophic and crude oil utilizing microorganisms than water. This may be due to the fact that microorganisms tend to attach and grow on the surface of sediments as reported by Xia *et al.* (2006). The humic substances and other elements in sediment phase could also serve as nutrients for microorganisms, stimulating their growth (Poeton *et al.,* 1999). The high densities of crude oil utilizing microorganisms in sediments than the water samples may be due to low heterotrophic activities of oil degraders and the presence of crude oil in the microhabitat (Amadi and Braid, 2003).

It indicates that the sediments harbor high degree of culturable microbial populations with low potential for oil degradation or high preference for other substrates.

Imo River estuary is recognized as a significant area of high microbial diversity. In this ecosystem, eleven (11) species of bacteria and eight (8) species of fungi were isolated, characterized and identified. The bacteria species were *Flavobacterium* sp, *Micrococcus* sp, *Vibrio* sp, *Pseudomonas* sp, *Salmonella* sp, *Serratia* sp, *Klebsiella* sp, *Escherichia coli*, *Staphylococcus aureus, Enterococcus* sp and *Bacillus* sp. The fungi species isolated were *Paecilomyces* sp, *Cladosporium* sp, *Penicillium* sp, *Humicola* sp, *Aspergillus* sp, *Saccharomyces* sp, *Monilia* sp and *Fusarium* sp. These organisms were isolated from all the microhabitats with predominant densities observed in the sediments. In the pristine site (Great Qua river), the predominant bacteria species were *Staphylococcus aureus*, *Enterococcus* sp, *Escherichia coli* and *Bacillus* sp, while the fungi species encountered in Great Qua river were *Penicillium* sp, *Aspergillus* sp, *Fusarium* sp and *Cladosporium* sp. The presence of these microorganisms in both sites may be due to the constant human activities in the environment. Other microorganisms encountered in the study site may be attributed to the level of pollution in the estuary.

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