Archives of Current Research International

14(2): 1-17, 2018; Article no.ACRI.37646
ISSN: 2454-7077

Population Dynamics of Microbial Communities in Mesotidal Estuarine Sediment of Iko River, Eastern Obolo, Akwa Ibom State, Nigeria

C. I. Udosen[1] and S. I. Umana[2*]

[1]Department of Microbiology, University of Uyo, Nigeria.
[2]Department of Biological Sciences, Akwa Ibom State University, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACRI/2018/37646
Editor(s):
(1) Amal Hegazi Ahmed Elrefaei, Division of Radioisotope Production, Hot Lab and Waste Management Center, Atomic Energy Authority, Egypt.
Reviewers:
(1) Augustine A. Unimke, University of Calabar, Nigeria.
(2) Evgeny Puchkov, Russian Academy of Sciences, Russia.
(3) V. Vasanthabharathi, Annamalai University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/24631

Original Research Article

Received 20th September 2017
Accepted 1st December 2017
Published 16th May 2018

ABSTRACT

The population dynamics of microbial communities in mesotidal estuarine sediment of Iko River Estuary, Nigeria were investigated using standard microbiological and analytical procedures. The results revealed that the abundance of culturable microbes in the estuary varied between both tides as well as with the different microhabitats. Tidal influence had little or no impact on the heterotrophic and pollution indicator bacterial communities of the estuarine sediment but its influence on the activities of autotrophic bacterial communities was remarkable. The study revealed that 22.64% and 22.28% abundance rates of total heterotrophic bacteria were obtained during the high and low tides respectively. The abundance rates of 5.03% and 17.53% recorded for nitrogen-fixing bacteria and sulphate reducing bacteria respectively during high tide were higher than 4.07% and 10.93% obtained during low tide. Bioaerosols concentrations were found to be higher during high tide than low tide. Geographic Information System (GIS) models of microbial communities revealed marked variation which ranged between tidal influences and locations. The

**Corresponding author: Email: usenyene@yahoo.com;*

model revealed high concentrations of microorganisms in the north-west zone during both tides, while fungi were highly concentrated in the north-east zone during high tide. High species richness was observed, but with little or no tidal influences and isolates included known pathogenic species. The total organic carbon content of the sediments during both tides were higher than an optimum value of 1.3%, with a high linear correlation (r = 0.777) between heterotrophic bacteria load and organic content level of the sediment during low tide indicating a relatively high content of organic matter in the sediments can increase the bacterial load. The study therefore calls for effective environmental monitoring.

Keywords: Mesotidal, estuarine, sediment, pollution, population dynamics and microbial.

1. INTRODUCTION

The answer to many bio-geograghical questions by microbiologists has brought about a recent resurgence in interest in microbial biogeography. This resurgence has been led to the advancements in molecular tools that allow us to survey uncultivated microbes in environment and a growing recognition that microbial taxa are the most biologically diverse taxa on earth.

However, we do know that a wide variety of microbial taxa exhibit bio-geographical patterns, microbial communities are not homogeneous across habitat- types, and within a given habitat microbial diversity can vary between locations separated by millimeters to thousands of kilometers. If microbial biogeography did not exist, there would be no spatial or temporal heterogeneity in microbial communities and global patterns in microbial communities and global patterns in microbial diversity could be predicted by studying the microbial community in a single location at a single point in time [1].

Microbes inhabit a wide range of habitats from hot springs to the deep subsurface and it is highly unprobable that we would observe. Similar bio-geographical patterns exist across the full range of possible microbial habitats. It is also likely that all microbial taxa share similar bio-geographical pattern as the term “microbe’’ encompasses a broad array of taxa e.g bacteria, fungi, archaea, viruses and protists. Those are phylogenetically distinct and distinct with respect to their morphologies, physiologies, and life histories. Among these, bacterial biogeography is the most studied microbial dispersal and colonization. The key process shaping microbial biogeography and macro-ecological pattern is the dispersal of plants and animals [2]. The extent of microbial dispersal is currently under debate. According to Finlay [3] who argued that any organism less than 1mm in size is likely to be ubiquitous due to an essentially unlimited capacity for long distance dispersal. This speculation is primary based on the assumption that the high local abundance of microbes (the large member of individuals per unit area) increase the probability that individual microbes may travel a long distance and successfully colonize a remote location simply by chance [4]. If we combined a high probability of dispersal with the ability to survive the long distance transport, we would expect few geographic constraints on microbial distribution [5].

Microbes dominate the ocean in terms of abundance, diversity and metabolic activity [6]. Marine bacteria mediate fluxes of matter and energy and have a critical role in driving the major biogeochemical cycles [7]. Although microbes play functional role in the ecosystem, very little is known about the factors structuring marine community distribution. Microbial communities have been described as stratified with depth [8], and depth has until recently been considered as the main factors explaining differences in marine microbial community composition [9]. Light availability (irradiance) is thought to be the main abiotic factor structuring communities in the euphotic zone [10]. Dark ocean communities, however, are not homogenous [11], suggesting that other key factors besides irradiance influence vertical microbial community structure. Latitude has recently been proposed as an important factor determining surface microbial diversity [12]. But other factors may control bacterial communities in the deep dark ocean. The dark ocean comprises the water below 500 m, including the mesopelagic (200–1000 mm depth) and bathypelagic (1000-4000m depth) zones and represents the largest biome on earth 70% of the global ocean’s volume. Sun irradiance does not reach deeper waters but, nevertheless, accumulating data suggest that they harbour diverse and active microbial communities [11].

Pollution of the aquatic ecosystem is a difficult problem because it is produced as a product of goods and services that are valuable to us. All the pollutants found in surface water may invariable be present in aquatic sediment. Because (i) sediment can smother bottom – dwelling plants and animals such as oyster and clams, (ii) suspended sediments make the water cloudy so less light is available for underwater grasses. (iii) sediment can carry high concentrations of certain toxic materials that can contaminate water systems, (iv) sediments also carry nutrients particularly phosphorus, which increase nutrient pollution and eutrophication development in aquatic ecosystems and (v) accumulation of sediments can fill parts and waterways, its pollution has always been of serious concern [13].

This may seem strange, but sediment is sometimes considered a pollutant too. Sediment is considered a form of pollution when there is too much of it. Excess sediment damages river environment by smothering the organisms that live on the bottom. Sediment blocks sunlight, which means that algae cannot grow (by photosynthesis). Remember, the main sources of food in aquatic systems are algae and detritus. When these are covered with sediment, they are no longer accessible to the organisms that eat them. This affects the other animals in the food web, some directly and some indirectly [13].

Erosion causes excess sediment. Erosion is the loss of soil and gravel from the surface of land. It occurs when soil sediments are exposed and swept away by wind or water. Soil is most likely to erode where there is no vegetation covering it, securing it to the ground. Erosion result in excess sediment that gets carried into our waterways by rain, water or wind. Excess sediment reduces the productivity of aquatic plant and animal communities. Sedimentary pollution is a major cause of the decreased quality of fisheries throughout the world. When excess sediment harms detritus, algae, oysters and fish, all other organisms in the ecosystem are affected too. [14].

Most of the transported sediments will be deposited in the joint of river mouths in lakes and in coastal areas, and in wetland of floodplain type, where the decrease in flow velocity and the presence of vegetation promotes sedimentation. The effect of salt water in estuarine mixing is to further enhance sediment removed by flocculation of clay particles. Transfer of the sediment from the water column to the water bottom has important consequences both for the quality of the water and the properties of the bottom deposits. Many contaminants are transported in storm-water runoff, which can enter and pollute streams, lakes, wetlands and ground water; and subsequently the sediments. The transport of trace metals and other contaminants associated with solid matter, in surface waters of small watershed, is dependent on the ability of the water to transport the carrier phrases. Thus the size distribution of the sediment matter has been suggested to determine the transport properties of sediment-bound contaminants [15] such as coliform bacteria (sewage), metals, hydrocarbons and organochlorines. Precisely, polluted soil and sediment has health, industrial and environmental impact on humans [16].

Though earlier in [17], Baas-Becking posited that historical factors such as isolation and environment could not be the forces determining microbial distributions, but rather that “everything is everywhere that the environment select”. The small size, short generation time and abundance of many microbial species have allowed them to be easily dispersed in air, water, sediment and on bodies of animals and thus spreading them all over. Many microbes lie dormant for long time until conditions improve, or until the environment selects them, thus creating what has been termed “seed bank” of microbes where all microbes are in all environments at the same time, only lying in wait for environmental conditions to favour their proliferation. Therefore, microbes dominate all conservable environments in terms of abundance, diversity and metabolic activity and mediate fluxes of matter and energy and have critical roles in driving the major biogeochemical cycles.

Microbes have an essential community distribution in aquatic systems but scientific understanding of microbial biogeography is particularly low even though the diversity and composition of microbial communities is thought to have direct influence on a wide range of ecosystem processes. This research work is therefore focused on providing information on the population dynamics of microbial communities in mesotidal estuarine sediment Iko River, Eastern Obolo, Nigeria. The data generated will provide information and update knowledge on Changes in the microbial loads of the tidal ecosystem during low and high tides.

2. MATERIALS AND METHODS

2.1 Study Area

The Iko River Estuary is a brackish ecosystem located in Eastern Obolo Local Government Area of Akwa Ibom State. Akwa Ibom State is located within the petroleum belt of the Niger Delta region of Nigeria. Iko River is located in the Eastern part of the Niger Delta (Plate 1). The river has a shadow depth ranging from 4.0 meters to 7.0 meters at flood and ebb tides and an average width of 16 meters. Iko River takes its rise from the Qua Iboe River Catchment and drains directly into the Atlantic Ocean at the Bight of Bonny. The Bight of Bonny has many adjoining tributaries and creeks, and part estuary, which opens into the Atlantic Ocean. The shore line of Iko River is characterized by soft-dark mud flats, usually exposed during low tide, mangrove swamps with mangrove trees, shoals and sand beaches. The river has a semi-diurnal tide and has a length of more than 30 km.

Sediments in Iko River Estuary become well sorted composed of mainly coarse quartz sand, shell debris, faucal pellets, silts and clay [18]. This assortment of sediments when trapped within the luxuriant mangrove prop roots are impregnated with decaying mangrove leaves and branches constitutes mud flats, with characteristic feature of Iko River shore-lines.

2.2 Sample Collection

Sediment samples were collected from four [4] strategic positions during the low and high tides level. This was carried out using Van-veen grab Sampler [19] and stored in clean containers. The samples which were properly labelled stored in ice-packed coolers were immediately transferred to the laboratory for microbiological and physicochemical analysis.

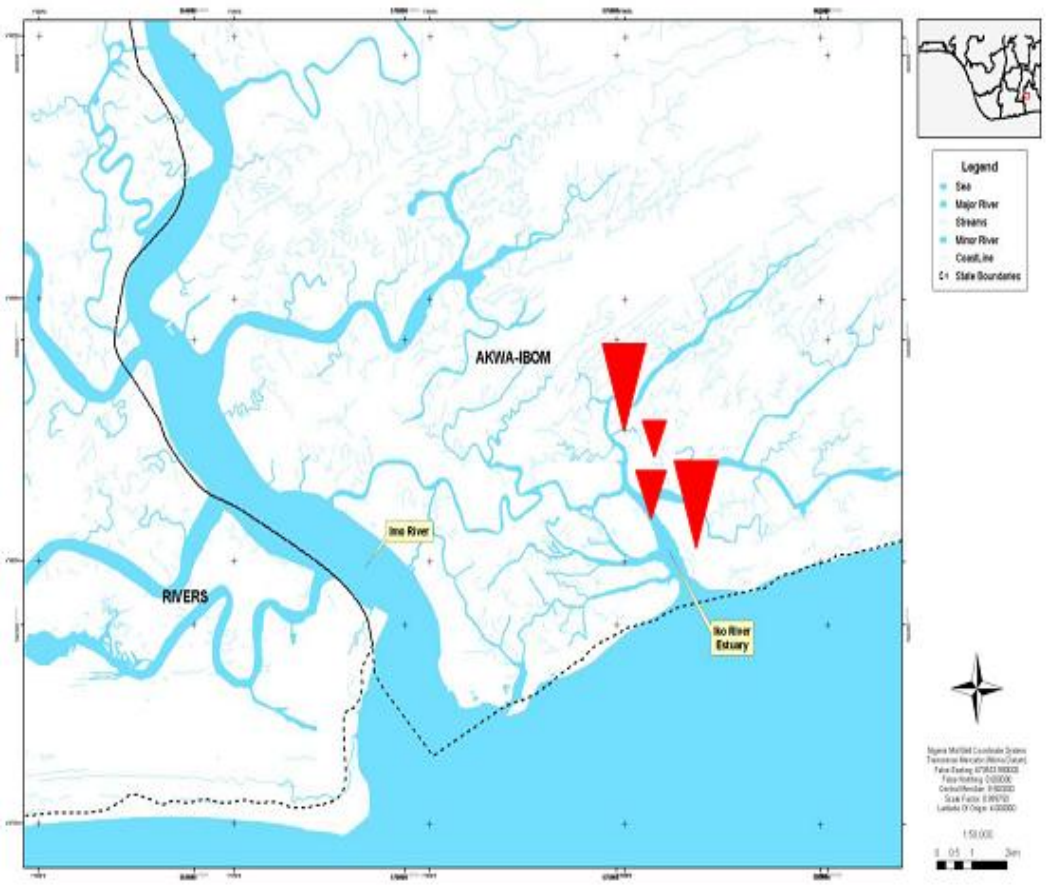


Plate 1. Sampling sites on the map of Iko river estuary

2.2 Microbiological Studies

2.2.1 Serial dilution of sediment samples

Serial dilution of sediment samples was done according to the method of Cheesbrough [20]. Precisely 10 g of sediment samples were measured and introduced into conical flasks containing 90ml of sterile distilled water. These were shaken for even distribution and thereafter 1ml of the aliquot was aseptically transferred into sterile test tubes containing 9ml of diluents to give a dilution of 10^{-1}. This was repeated until a seventh dilution factor was attained.

2.2.2 Estimation of loads, characterization and identification of microbial communities in sediment samples

Standard microbiological techniques described by Harrigan and McCance [21] were employed for the microbiological analysis of sediment samples.

The total heterotrophic bacterial and fungal counts in sediment samples were estimated by the pour plate method using Nutrient agar (NA) and Sabouraud Dextrose Agar as the analytical media respectively. The density of actinomycetes in sediment samples collected from the estuary were also enumerated after 7 days of incubation at 28ºC ± 2ºC using acidified Nutrient agar /Starch nitrate agar [22].

The numbers of sulphate reducers in the sediment samples were determined by the pour plate technique at 28ºC ± 2ºC after 7 days of incubation using compounded media as the analytical medium. On the other hand, the densities of nitrate reducers were enumerated using Nitrate agar after incubation at 28ºC ± 2ºC for 48 hours while the population of phosphates solubilizing bacteria (PSB) in the samples were estimated by the method of Lu and Huang [23]. In this case, the diluted samples were introduced into compounded medium and incubated for 3 days at 28ºC. Strains that produced clear zones around colonies were considered as phosphate solubilizing bacteria.

Using the same method, the coliform count, fecal coliform (*Escherichia coli*), *Salmonella* and *Shigella*, *Vibrio*, *Staphylococcus aureus*, *Pseudomonas*, *Clostridium* and fungi were estimated using McConkey agar (MA), Eosine Methylene Blue agar (EMB), Salmonella-Shigella agar (oxide), Thiosulphate – Citrate – Bile salts – Sucrose agar (TCBS), Staphylococcus medium (No. 110), Pseudomonas isolation agar, Re-enforced Clostridial agar and Sabouraud Dextrose Agar (SDA) as analytical media respectively APHA, [24]. The media were fortified with 50 µg/ml of streptomycin and 100 µg/ml cycloheximide/ 50 µg/ml benomyl respectively, for the selective enumeration and isolation of fungi and bacteria.

The bacterial plates were incubated for 24 hours at 28ºC in a Gallenkamp incubator and fungal plates at room temperature (28ºC ± 2ºC) for four days. Microbial colonies that emerged on the incubated plates after 24 hours were enumerated with the aid of a Quebec Colony counter and recorded as colony forming units (cfu) per gram of the sediment sample.

The colonies obtained from the samples were characterized using standard procedure as described by *Bergey's Manual of Determinative Bacteriology* [25]. The colonies were subjected to Gram's stain and various biochemical tests such as motility test, catalase test, urease test, coagulase test, citrate test, hydrogen sulphide test, sugars utilization test and MR-VP test. Fungal isolates were identified according to the method of Barnett and Hunter (1987).

2.3 Determination of Spatial Variations in the Bio-aerosol Loads of the Estuarine Environment

Geographic information system (GIS) was adopted to perform dynamic modeling of the bio-aerosols distribution pattern. This involves establishing the spatial variations through a period of time. To achieve the goal, the GIS-based pollution mapping which uses interpolation techniques such as distance weighting and kriging was employed [26].

2.4 Determination of the Physiochemical Properties of Sediments Sample

Physicochemical parameters of the sediment samples were determined using standard analytical procedures recommended in 1998 by APHA.

2.5 Data Analysis

The data collected were subjected to correlation matrix analysis to establish relationships between the microbial groups. Simple percentage was also used to express the

frequency of occurrence of microbial isolates where necessary.

3. RESULTS

3.1 Microbiological Properties of Sediment during Low and High Tides

The densities of heterotrophic and autotrophic bacteria in the pelagic sediment of the estuarine ecosystem during high and low tides are presented in Fig. 1. The result revealed heterotrophic bacteria as dominant bacterial group during both tides; 2.15 x 10^6±0.043 cfu/g during low tide and 2.13 x 10^5±0.045 cfu/g during high tide. The densities of hydrocarbon utilizing bacteria in the sediment were remarkable. During low tide, the density recorded was 1.98 x 10^3 ± 0.320 cfu/g while 1.70 x 10^2 ± 0.118 cfu/g was obtained during high tide. The density of sulphur reducing bacteria in the sediment was also remarkable during both tides with low tide having higher count (1.48 x 10^3±0.083 cfu/g) than 1.48 x 10^2±0.110 cfu/g during high tide. The densities of nitrate reducing bacteria and phosphate solubilizing bacteria during low and high tides were also recorded. Fungal densities in the sediment were higher during high tide (1.24 x 10^4±0.139 cfu/g) than low tide (8.20 x 10^3±0.246 cfu/g). The densities of *Pseudomonas* and Actinomycetes were relatively high during low tide than high tide. The distribution pattern of the microbial communities in the sediment during the low and high tides are shown in Figs. 2 and 3 respectively.

On the other hand, the densities of pollution indicator bacteria in the sediment samples of the estuarine ecosystem during the low and high tides are respectively presented in Fig. 4. The results show that the estuarine sediment haboured higher numbers of coliform during low tide (1.63 x 10^4±0.046 cfu/g) than high tide (1.58 x 10^3±0.054 cfu/g). The density of faecal coliform was higher in sediment during low tide (1.30 x 10^4±0.061 cfu/g) than high tide (1.00 x 10^2±1.784 cfu/g). Few viable cells of *Clostridium* were observed and the loads were lower during the low (1.35 x 10^1±0.061 cfu/g) than high (1.01 x 10^2±0.046 cfu/g) tides. The result also showed that the sediment was also heavily polluted with *Salmonella* and *Shigella* species. The densities of *Vibrio* sp and *Staphylococcus* sp encountered were also remarkable. The distribution pattern of the pollution indicator bacteria in sediment during the low and high tides are represented in Figs. 5 and 6 respectively.

3.2 Microbial Diversity of Iko River Estuarine Environment

The sediment samples had 28 bacterial and 11 fungal isolates (Tables 1 and 2) respectively. The most predominant bacteria were *Bacillus cereus* and *Actinomyces* sp with percentage prevalence of 100%. Among the fungi isolated from the estuarine environment, *Penicillium expansum* (50%), *Aspergillus terrus* (37.5%) and *Aspergillus fumigatus* (62.5%) were the most predominant.

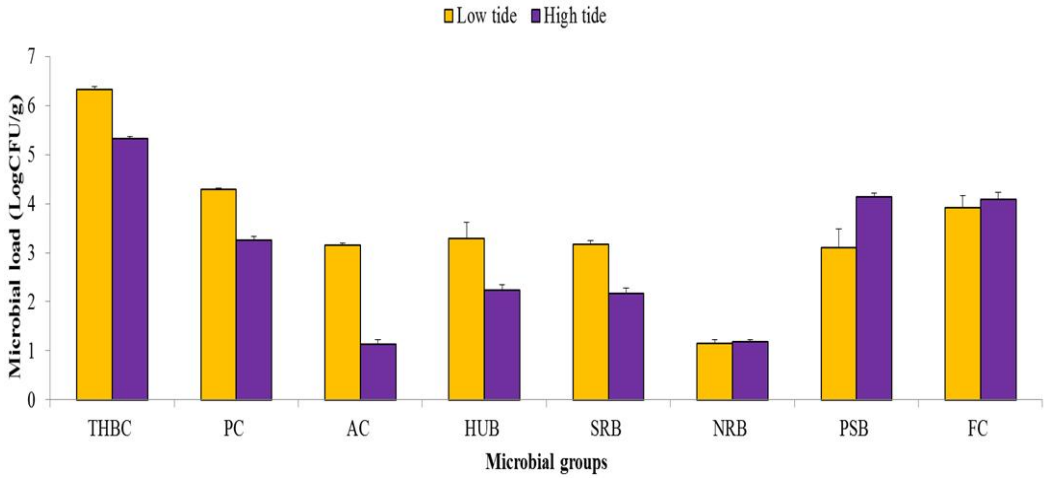


Fig. 1. Heterotrophic, autotrophic bacteria and fungal load in sediment during low and high tide

Key: THB = Total heterotrophic bacteria, PC = Pseudomonas count, AC = Actinomycetes count, HUB = Hydrocarbon utilizing bacteria, FC = Fungal count, SRB = Sulphate reducing bacteria, NRB = Nitrate reducing bacteria, PSB = Phosphate solubilizing bacteria

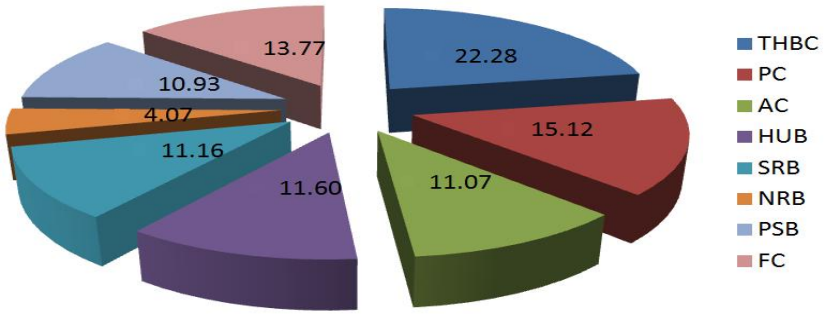


Fig. 2. Abundance (%) of heterotrophic and autotrophic bacteria communities in the sediment during low tide

Key: THB = Total heteotrophic bacteria, PC = Pseudomonas count, AC = Actinomycetes count, HUB = Hydrocarbon utilizing bacteria, FC = Fungal count, SRB = Sulphate reducing bacteria, NRB = Nitrate reducing bacteria, PSB = Phosphate solubilizing bacteria

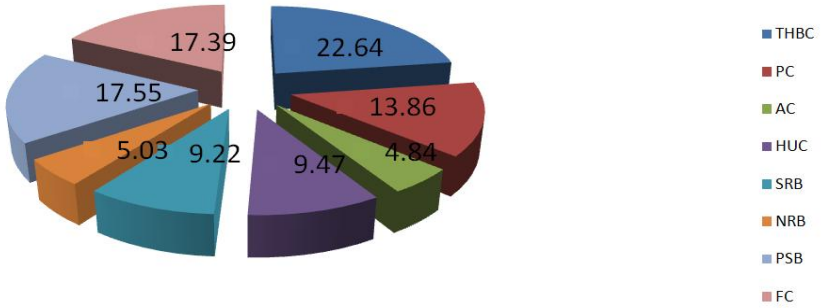


Fig. 3. Abundance (%) of heterotrophic and autotrophic bacteria communities in the sediment samples during high tide

Key: THB = Total heteotrophic bacteria, PC = Pseudomonas count, AC = Actinomycetes count, HUB = Hydrocarbon utilizing bacteria, FC = Fungal count, SRB = Sulphate reducing bacteria, NRB = Nitrate reducing bacteria, PSB = Phosphate solubilizing bacteria

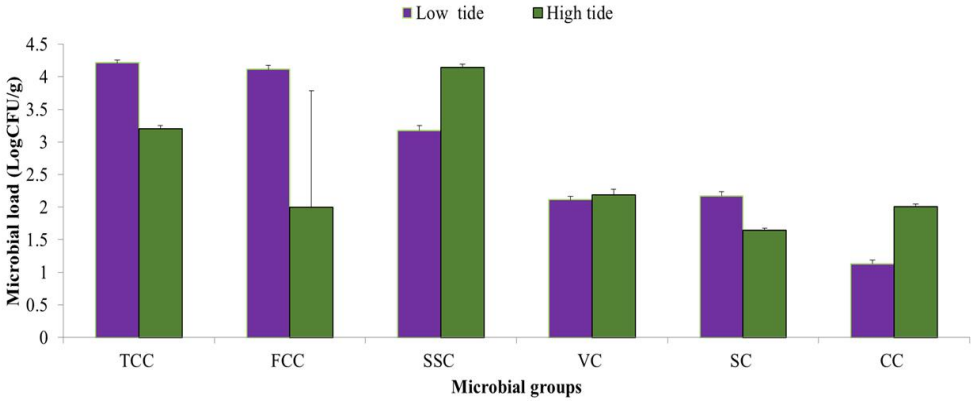


Fig. 4. Pollutant indicator bacteria load of sediment during low and high tide

Key: TCC = Total coliform count, FCC = Faecal coliform count, SSC = Salmonella shigella count, VC = Vibrio count, SC = Staphylococcus count, CC = Clostridium count

3.3 Spatial Variations in the Microbial Loads of the Estuarine Sediment

GIS model of spatial distribution of heterotrophic bacteria in sediment during the low and high tides is presented in Fig. 7. The pattern of distribution of bacteria in sediment was not definite. During low tide, the brown band shows high heterotrophic bacteria concentrations in the North-East of the estuarine environment. An evenly moderate is indicated by the green and blue bands towards North West. During high tide,

there were high bacteria loads in sediment (blue band) in the North West, while low to moderate and high concentrations (green to yellowish brown bands) were noticed in North East zone. The pattern of distribution of fecal coliform bacteria in sediment was not definite. During low tide, the blue band shows high fecal coliform concentrations in the North-East of the estuarine environment. An evenly moderate is indicated by the red and pink towards North East. During high tide, the loads of fecal colifom was high (blue band) in both the North West and North East zones (Fig. 8). On the other hand, the GIS model of spatial distribution of fungi in sediment during the low and high tides is presented in Fig. 9. The analysis shows that during low tide, the whitish-brown bands indicate high fungal concentrations in the North-East and North West of the estuarine environment. During high tide, the loads of fungi was high (whitish band) in the North West zone.

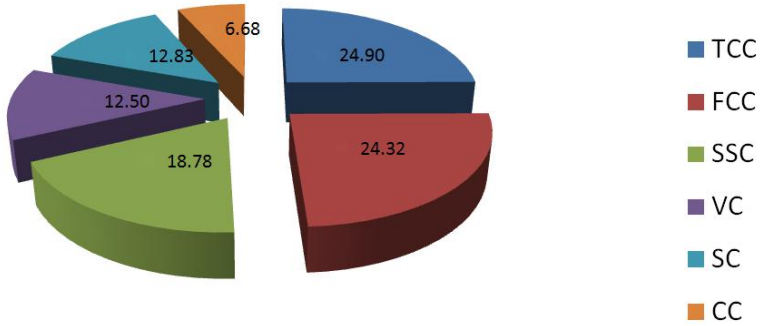


Fig. 5. Abundance (%) of pollution indicator bacteria in the sediment sample during low tide
Key: TCC =Total coliform count, FCC = Faecal coliform count, SSC =Salmonella shigella count, VC =Vibrio count, SC = Staphylococcus count, CC =Clostridium count

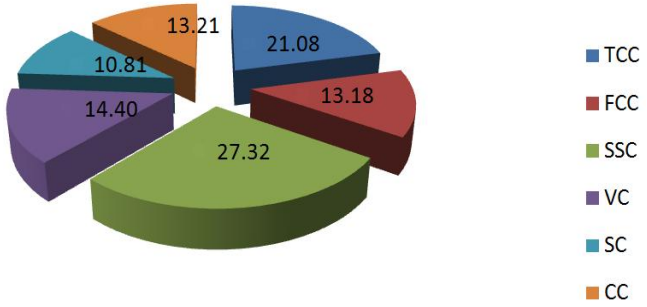
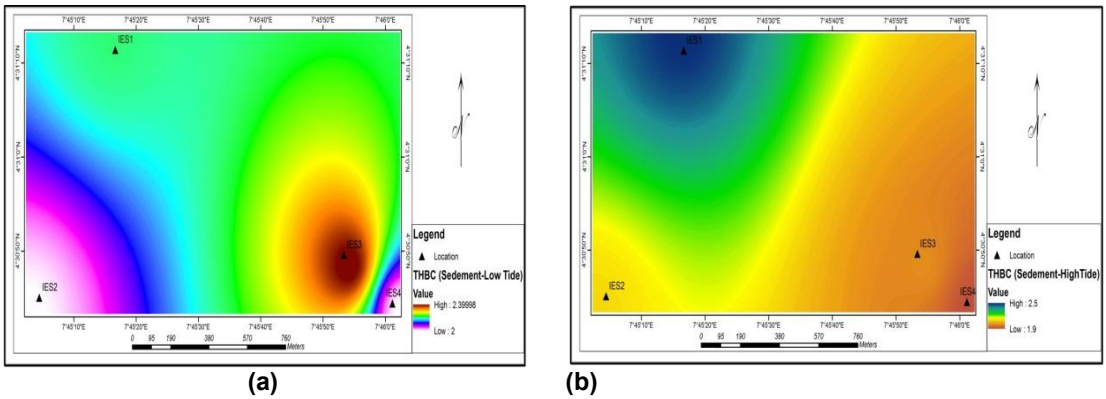


Fig. 6. Abundance (%) of pollution indicator bacteria in the sediment samples during high tide
Key: TCC =Total coliform count, FCC = Faecal coliform count, SSC =Salmonella shigella count, VC =Vibrio count, SC =Staphylococcus count, CC =Clostridium count



(a) **(b)**

Fig. 7. Spatial distribution of heterotrophic bacteria in sediment during (a) low tide and (b) high tide

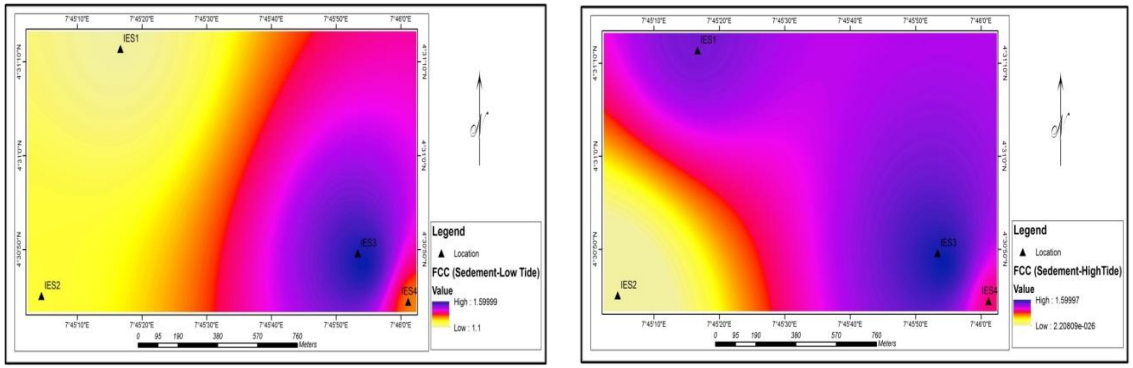
Table 1. Occurrence and distribution of the diverse species of bacteria in sediment samples from Iko River estuarine environment during low and high tides

	Low tide				High tide				
Organisms	**IES-1**	**IES-2**	**IES-3**	**IES-4**	**IES-1**	**IES-2**	**IES-3**	**IES-4**	**Prevalence rate (%)**
Micrococcus sp	+	+	+	+	+	+	+	-	87.5
Bacillus megaterium	-	-	+	+	-	+	-	-	37.5
Bacillus subtilis	+	+	+	+	+	-	-	-	62.5
Bacillus cereus	+	+	+	+	+	+	+	+	100.0
Streptococcus sp	-	-	-	-	+	-	-	-	12.5
Chromatium sp	-	+	-	-	+	-	-	+	37.5
Staphylococcus aureus	-	-	-	-	+	-	-	-	12.5
Klebsiella sp	+	+	+	+	+	+	-	+	87.5
Citrobacter sp	+	+	+	-	+	-	+	-	62.5
Enterobacter sp	+	-	-	-	-	+	-	-	25.0
Salmonella sp	+	-	-	-	-	-	-	-	12.5
Shigella sp	-	+	+	-	-	-	-	-	25.0
Vibrio sp	+	-	-	-	-	+	-	-	25.0
Vibro haemolyticus	-	+	+	+	-	-	+	+	62.5
Staphylococcus albus	+	+	+	+	-	-	+	+	75.0
Actinomycetes sp	+	+	+	+	+	+	+	+	100.0
Escherichia coli	+	+	+	-	+	-	-	-	50.0
Pseudomonas aeruginosa	+	+	+	-	+	+	+	+	50
Pseudomonas putida	+	+	-	-	-	+	-	+	87.5
Pseudomonas fluorescens	-	+	-	-	+	-	-	-	25.0
Clostridium perfringens	+	+	+	+	-	-	+	+	75.0
Desulfovibrio sp	-	-	-	+	-	-	-	-	12.5
Nitrobacter sp	-	-	-	+	-	+	+	-	37.5
Nitrosomonas sp	-	-	-	-	+	-	-	+	25.0
Proteus sp	-	-	+	-	-	+	+	-	37.5
Serratia sp	+	+	+	-	-	+	-	-	50.0
Lactobaccillus sp		-	-	-	-	-	+	-	12.5
Species Richness (27)	15	16	15	12	13	13	11	10	

Tables 2. Occurrence and distribution of the diverse of the diverse species of fungi in sediment samples from Iko River estuarine environment during low and high tides

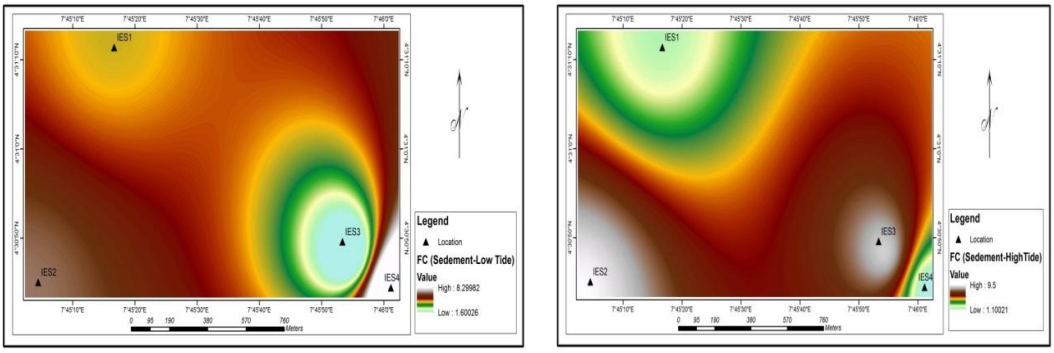
	Low tide				High tide				
Organisms	**IES-1**	**IES-2**	**IES-3**	**IES-4**	**IES-1**	**IES-2**	**IES-3**	**IES-4**	**Prevalence rate (%)**
Aspergillus niger	-	-	-	+	-	-	-	+	25.0
Aspergillus fumigates	-	-	-	-	-	-	+	-	12.5
Aspergillus terrus	-	-	+	-	+	+	-	-	37.5
Aspergillus glaucus	+	-	-	-	-	-	-	-	12.5
Rhizopus stolonifera	-	+	-	-	-	-	-	-	12.5
Penicillium expansum	-	+	-	-	-	-	-	-	12.5
Penicillium frequentant	+	-	-	-	-	-	-	-	12.5
Eurotium sp	-	-	-	-	-	-	-	+	12.5
Absidia sp	-	-	-	-	-	-	-	+	12.5
Alternaria sp	-	-	-	-	+	-	-	-	12.5
Geotrichum candidum	-	-	-	-	-	-	+	-	12.5
Species Richness (11)	2	2	1	1	2	1	2	3	

Key: IES - Iko Estuary Station



(a) **(b)**

Fig. 8. Spatial distribution of faecal coliform in sediment during (a) low tide and (b) high tide



(a) (b)

Fig. 9. Spatial distribution of fungi in sediment during (a) low tide and (b) high tide

3.4 Physicochemical Properties of the Iko Estuary Sediment

Data presented in Tables 3 and 4 indicate the physicochemistry of sediment obtained from Iko River Estuary during the low and high tides respectively. The temperature values of the sediment during low and high tides were 26.12±0.333ºC and 25.52±0.406ºC respectively. The results also revealed that the sediment tends to also be slightly acidic with mean pH value of 6.05±0.134 at low tide and 5.22±0.171 at high tide. The results also revealed low salinity levels at low (3.86±0.095%) and high (4.30±0.219%) tides. The redox potential levels were high during the low and high tides. Lower concentration of total nitrogen (<0.5%) was recorded with increased concentration of organic carbon and available phosphate at both tides (≥8.45 mg/kg). The result also revealed appreciable concentration of hydrocarbons in the estuarine sediment. Levels ranging from 139.68±0.073 mg/kg and 154.5±0.034 mg/kg were recorded for sediments during the low tide and high tide respectively. Fig. 10 represent different sediment particle sizes with sand having the largest size followed by clay and silt with the smallest particle size revealing that the sediment was predominantly sandy.

4. DISCUSSION

Natural environments are usually inhabited by a diverse population of microorganisms. These include a wide range of physiological and nutritional types [27]. Findings from this study on the abundance and distribution of microbial communities in the Iko River estuarine sediment during low and high tides have revealed variation in microbial population dynamics between tidal influences. The results have revealed the rich microbial assemblage and diversity in the estuarine sediment. Analysis has shown that the sediments harboured higher heterotrophic bacterial loads during low tides. However statistical analysis (at 95% confidence limit, p = 0.05) showed a positive correlation (r = 0. 796, p < 0.05) between bacterial loads during low tide.

Table 3. Some physicochemical attributes of the estuarine sediment samples during low tide

Parameter	IES-1	IES-2	IES-3	IES-4	Mean	SD
Temperature (ºC)	26.61	25.75	26.22	25.88	26.12	0.333
pH	6.22	5.88	6.13	5.96	6.05	0.134
Redox Potential (mV)	299.1	301	202.4	233.3	258.95	0.073
Salinity (%)	3.77	4.01	3.79	3.88	3.86	0.095
Organic Carbon (%)	9.2	8.9	9.1	8.8	9.00	0.158
Total Nitrogen (%)	0.46	0.45	0.46	0.44	0.45	0.008
Available P (mg/kg)	6.33	5.92	6.22	6.45	6.23	0.197
Conductivity (ms/cm)	74.9	63.9	55.09	66.05	64.99	0.047
THC (mg/kg)	170.2	122.4	148.5	105.6	139.68	0.073
Particle Size (%)						
Sand	55.20	56.30	54.20	56.20	55.48	0.853
Silt	16.30	16.50	16.20	17.30	16.58	0.432
Clay	28.50	27.20	29.60	26.50	27.95	1.193

Table 4. Some physicochemical attributes of the estuarine sediment samples during high tide

Parameter	IES-1	IES-2	IES-3	IES-4	Mean	SD
Temperature (ºC)	25.22	25.32	26.22	25.32	25.52	0.406
pH	5.07	5.32	5.03	5.44	5.22	0.171
Redox Potential (mV)	268.1	244	198.1	244.8	238.75	0.048
Salinity (%)	4.43	4.23	3.98	4.56	4.30	0.219
Organic Carbon (%)	8.8	8.6	7.9	8.5	8.45	0.335
Total Nitrogen (%)	0.44	0.46	0.43	0.45	0.45	0.011
Available P (mg/kg)	8.99	7.96	8.06	8.77	8.45	0.443
Conductivity (ms/cm)	76.9	64.88	57.99	76.16	68.98	0.051
THC (mg/kg)	165.4	147.3	138.5	166.8	154.5	0.034
Particle Size (%)						
Sand	57.20	57.10	56.00	58.00	57.08	0.711
Silt	16.00	15.50	17.20	16.10	16.20	0.620
Clay	26.80	27.40	26.80	25.90	26.73	0.536

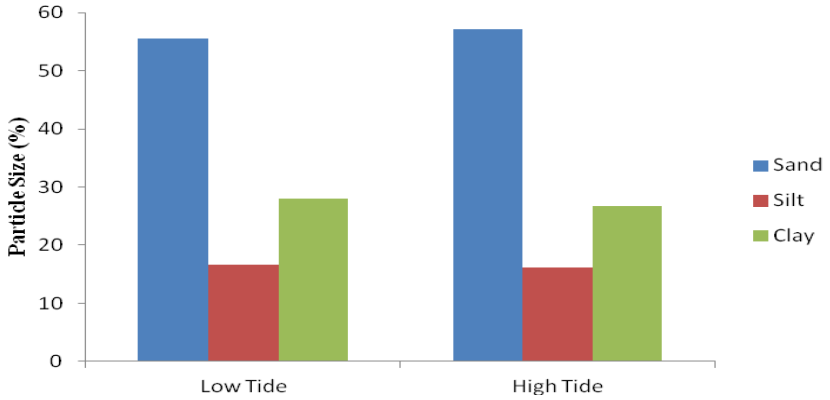


Fig. 10. Trends of different sizes of particles present in Iko river sediment during low and high tide

This implies that during low tide, the loads of bacteria in sediment can contribute remarkably to the air bacterial loads. The densities of *Pseudomonas* sp and Actinomycetes were relatively high in the sediment. Although comparatively high numbers of oil degrading bacteria were observed and suggestive of the presence of petroleum hydrocarbons in the environment as a result of either natural or anthropogenic activities involving oil production in the area.

The high densities of heterotrophic bacteria obtained for the estuarine sediment are in accord, but slightly lower than values reported in Australia where the numbers ranged from 2.0 x 10^8 cells to 3.6 x 10^{10} cells g^{-1} dry weight of sediment [28]. Heterogeneous microbial communities are of major importance in microbial world because of the considerable advantages gained by members of the population. It has been stated that heterotrophic activities among microorganisms permit them to obtain many of the benefits of multicellular life. Interaction between microorganisms permits activities such as co-metabolism and cross feeding, while diverse populations are less affected by environmental change and can recover from adverse conditions more rapidly than ecosystem of less diversity [27]. The wide heterotrophic activity of sediment microorganisms is of very considerable importance in the remediation of aquatic system after pollution with hydrocarbons and other organic chemicals [27,29]. Actinomycetes were also obtained from estuarine sediment. Their low count in the aquatic system may be because Actinomycetes live predominantly aerobically, i.e. they need oxygen for their metabolism [30]. Generally, actinomycetes grow on fresh substrates more slowly than other bacteria and fungi but are known to possess strong ability to degrade natural substances such as chitin or cellulose.

Autotrophic bacterial groups including sulphate reducing bacteria, nitrogen fixing bacteria and

phosphate solubilizing bacteria were also encountered in the estuarine sediment. Sulfate occurs widely in seawater, sediment, or water rich in decaying organic material. Sulfate-reducing bacteria (e.g. *Desulfovibrio* sp) are common in anaerobic environments where they aid in the degradation of organic materials [30]. The toxic hydrogen sulfide is a waste product of sulfate-reducing bacteria; its rotten egg odour is often a marker for the presence of sulfate-reducing bacteria in nature [30]. Sulfate-reducing bacteria are responsible for the sulfurous odours of salt marshes and mud flats. Much of the hydrogen sulfide will react with metal ions in the water to produce metal sulfides. These metal sulfides, such as ferrous sulfide (FeS), are insoluble and often black or brown, leading to the dark color of sludge [31].

Nitrogen fixing (nitrifying or denitrifying) organisms are autotrophs, and use carbon dioxide as their carbon source for growth. Some possess the enzyme, urease, which catalyzes the conversion of the urea molecule to two ammonia molecules and one carbon dioxide molecule. Free-living nitrogen fixers such as *Pseudomonas, Klebsiella, Nocardia, Bacillus, Micrococcus* and *Enterobacter* sp and confirmed diazotrophs like *Nitrosomonas and Nitrobacter* were isolated from the estuarine sediment. They are known to assimilate the carbon dioxide released by the reaction to make biomass via the Calvin Cycle, and harvest energy by oxidizing ammonia (the other product of urease) to nitrite [32].

Phosphate solubilizing bacteria (PSB) are beneficial bacteria capable of solubilizing inorganic phosphorus from insoluble compounds [33]. P-solubilization ability of rhizosphere microorganisms is considered to be one of the most important traits associated with plant phosphate nutrition. It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids, through which their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms. *Pseudomonas putida* strains isolated from Iko river sediment in this study are highly efficient insoluble phosphate solubilizers. Recently, researchers at Colorado State University demonstrated that a consortium of four bacteria synergistically solubilize phosphorus at a much faster rate than any single strain alone. However, there is a limit on the amount of phosphate which can be added to the environment due to the issue of eutrophication [34].

Coliform bacteria are enteric bacteria that are used as indicators of the likelihood of the presence of bacterial pathogens. Although faecal coliforms themselves are usually not harmful to humans, their presence indicates the presence of faecal wastes which may contain pathogens [35]. The high incidence of coliforms observed for the estuarine sediments samples may be attributed to human impact and a pointer to the inherent risk of disease outbreak if the contaminated water is deliberately or accidentally consumed. This assertion is confirmed by the equally high densities of *Escherichia coli*, *Salmonella* and *Shigella*, *Vibrio* in the estaurine sediment. This finding is in agreement with the report that reduction in faecal coliforms often correlates with reduction in *Salmonella* species and other pathogenic microorganisms [35]. Humans and animals could be exposed to the pathogens directly by coming in contact with contaminated sediments and water or indirectly by consuming or drinking water or seafood contaminated by the pathogens. The pathogenicity of the suspected isolates was, however, not determined in the present study.

Microorganisms are ubiquitous in the atmosphere with concentrations of bacterial cells typically exceeding 100 million m^3 of air over land. Numerous studies have suggested that the presence of microbes in the atmosphere may impact cloud development, atmospheric chemistry and microbial geography [36,37]. A sound knowledge of bacterial concentrations and distributions in the atmosphere is needed to evaluate these claims. With the advent of microbial generation sequencing techniques, scientists have uncovered the details of biodiversity and biogeography of a largely unknown ecosystem, the air. The discoveries prove that airborne microbes do much more than just ride the wind transmitting disease [35] The results of this study have revealed fairly high microbial loads in the estuarine atmosphere. The results have shown that bacteria and fungi were detected in all the stations investigated. For both the low tide and high tide samples analyzed, the values of mesophilic aerobic bacteria obtained by the sedimentation technique were more than APHA's (1998) standard (30 cfu/15 mins) for settling technique although the estimated values per volume of air were below values reported elsewhere. Sullivan [38] reported mean bacterial and fungal loads of 4344 cfu/m^3 and 4121 cfu/m^3

respectively for the atmosphere of an outdoor environment in Upper Silesia. This was attributed to contamination from the soil surface, since higher concentrations of bacteria were present when dust was raised [39].

The results of the research have further confirmed that indigenous microflora of an environment developed in spatially organized physicochemical gradients [27]. It is the existence of physicochemical gradient that permits the development and coexistence of a heterogenous population of microorganisms. The microbial population is organized either horizontally or vertically depending on the direction of the gradient. Analysis of the spatial distribution of the populations of some microbial communities using the GIS model has revealed marked variation in the distribution of microbial communities between tidal influences and microhabitats. This is in agreement with previous report that microbial population changes can result from periodic or non periodic events affecting either the physicochemistry of the environment as a whole, or the gradients within a given environment [27]. The same authors reported that physicochemical effects may be both; (i) direct through the immediate effects on a given part of the population as exemplified by the high densities of hydrocarbon utilizing bacteria recorded for sediment as index of the hydrocarbons loads of the sediment and (ii) indirect through the effect on interactions between members of the community.

The research results have that among the bacterial isolates, the most predominant bacteria were *Bacillus cereus* and *Nocardia* sp with percentage prevalence of 100% while in air, *Nocardia* sp and *Pseudomonas aeruginosa* were the most predominant (100%) bacteria. Among the fungi isolated from the estuarine environment, *Penicillium expansum* (50%), *Aspergillus terrus* (37.5%) and *Aspergillus fumigatus* (62.5%) were the most predominant. These organisms are broadly present in nature, including soil, cereal grains, hay and other plant material or foodstuff [40]. Many of the microorganisms isolated from this study are known to be capable of overcoming the deleterious effects of petrogenic wastes in sediment. Though direct degradation of hydrocarbon by the estuarine organisms was not carried out in this study, research has shown that, *Pseudomonas aeruginosa* is implicated in the utilization of hydrocarbons as sole carbon and energy source [41].

Witzel [42] and Akpan [43] opined that the natural waters diverse in their physical, chemical and biological characteristics. The physicochemical attributes of the sediment samples from the Iko River Estuary have revealed typical tropical estuarine water body. The particle size arrangement of sediment determines the texture of the sediment, while sediment texture determines the organic chemical contaminants absorption capacity and water fertility [44]. The particle size distribution of the sediments of Iko River gave 55.48±0.853% sand, 16.58±0.432% silt and 27.95±1.193% clay during the low tide, while the results gave 57.08±0.711% sand, 16.20±0.620% silt and 26.73±0.536% clay during the high tide. The predominance of sand in the sediments is in agreement with the observation by Ekwere et al. [18], that in the sediments of the Bight of Bonny, which is in the eastern flank of the Niger Delta, sand and sandy silt are predominant by the classification of Folk [45]. They observed that the strong tides and long shore drift tend to influence the distribution pattern of the sediment fractions. The low content of clayey materials which are known to be good scavengers for metallic and organic contaminants indicates that Iko River sediments are not likely to be important sinks for metallic pollutants entering the water body. This implies that the absence of a sediment sink (low clay) will enhance the pollutants remaining mostly in suspension or in solution in the river, and of course, increasing the lifetime of the pollutants in the water column obviously increases their accessibility to the biota.

The total organic carbon content of the sediments during both tides were higher than an optimum value of 1.3%, with a high linear correlation (r = 0.777) between heterotrophic bacteria load and organic content level of the sediment during low tide indicating a relatively high content of organic matter in the sediments can increase the bacterial load. This may be due to the diversified sources of organic matter in Iko River sediments, which are mixture of marine and freshwater sediments and could have been derived from decomposition of marine organisms, terrestrial inputs from surface discharge/runoff and oil spillages from bunkering activities [46].

The total hydrocarbon content (THC) of the sediment is a measure of the hydrocarbon content of the sediment. Sources of hydrocarbon accumulation in an environment include natural

sources (e.g. plant and animal matter, oil seeps); the atmosphere; accidents during transportation, storage, or use of petroleum products, inland oil exploration and exploitation, as well as municipal/industrial wastes. The mean total hydrocarbon content of the sediment from Iko River was 139.68±0.073 mg/kg during low tide and 154.5±0.034 mg/kg during the high tide. The values for both seasons were below 200 mg/kg total hydrocarbon concentration set for moderately polluted areas in the bottom sediments of Arabian Gulf [47]. In comparison to this study, the natural background levels of total hydrocarbon in bottom sediments of the Arabian Gulf were found to be 10 to 50 mg/kg and the concentration above this level was attributed to inputs from anthropogenic sources such as oil spills, oil slicks and seepage [48].

The pH range of the sediment (6.5 to 8.5) was below the acceptable range, optimal aquatic productivity (6.5 to 9.0) and within liveable range of 5.5 to 10 [42]. On the contrary, pH ranges of 6.2 - 7.5 and 6.0 - 8.5 have previously been reported in the Cross River [49] and Andoni River [50] respectively, all within the Niger Delta area of Nigeria. The pH values obtained are characteristic of tidal brackish water environment [51,52]. Significant decrease in pH values were also observed during the high tide, in sampling stations closer to the sea due to increased in salt intrusions from the ocean, while input of humic materials from the associated swamps and creeks, dilution of ionic concentrations by rain water and poor buffering capacity of flood waters [43], thereby causing a general drop in pH throughout the system. The pH values of rain can be as low as 5.0 due mainly to dissolved CO_2 [53]. Levels below 6.0 may be corrosive.

5. CONCLUSION

The Iko River estuarine sediment harbours distinct microbial populations of ecological and biogeochemical importance with the physicochemical attributes that show tidal variations and could affect aquatic biota along the food chain. The use of modern molecular tools would reveal the communities' relationship and biogeography of the estuarine sediment. However, the present results have shown that the Iko River estuarine environment is polluted by indiscriminate disposal of industrial effluent, oil spillage, gas flaring, disposal of domestic waste and fecal matter by inhabitants of the area.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fierer N, Jackson R. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Science, USA. 2006;103;626-631.
2. Lomolino M, Riddle B, Brown J. Biogeography. 3rd ed. Sunderland, Massachusetts: Sinauer Association. 2006; 98-105.
3. Finlay BJ. Global dispersal of free-living microbial eukaryote species. Science. 2002;296:1061-1063.
4. Fenchel T. Microbiology: Biogeography for bacteria. Science. 2003;301(5635):925-926.
5. Martiny JBH, Bohannan BJM, Brown JH, Colwell RK, Fuhrman JA, Green JL, Horner-Devine MC, Kane M, Krumins JA, Kuske CR, Morin PJ, Naeem S, Øvreås L, Reysenbach A, Smith VH, Staley JT. Microbial biogeography: Putting micro-organisms on the map. Nature Reviews Microbiology. 2006;4:102-112.
6. Azam F, Malfatti F. Microbial structuring of marine ecosystems. Nature Reviews Microbiology. 2007;5:782-791.
7. Karl DM. Nutrient dynamics in the deep blue sea. Trends in Microbiology. 2002;10: 410-418.
8. Karner MB, De Long EF, Karl DM. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature. 2001; 409:507-510.
9. Pham SV, Leavitt PR, McGowan S, Wissel B, Wassenaar LI. Spatial and temporal variability of prairie lake hydrology as revealed using stable isotopes of hydrogen and oxygen. Limnology and Oceanography. 2008;54(1):101-118.
10. Giovannoni SJ, Stingl U. The importance of culturing bacterioplankton in the 'Omics' age. Natural Review Microbiology. 2007;5: 820-826.
11. Teira E, Aken HV, Veth C, Herndl G. Archaeal uptake of enantiomeric amino acids in the meso- and bathypelagic waters of the north Atlantic. Limnology and Oceanography. 2006;51(1):60-69.
12. Hewson I, Fuhrman JA. Richness and diversity of bacterioplankton species along

an estuarine gradientin moreton bay, Australia. Applied and Environmental Microbiology. 2004;70;3425-3433.
13. Mathevan PM. Hydro biological investigation on the intertidal diatoms of the Cuddalore Uppanar estuary India. Ph.D. Thesis, Annamalai University, India, 1994;159.
14. Jonsson B, Jonsson N. Partial migration: Niche shift versus sexual maturation in fishes. Reviews in Fisheries Biology. 1993; 3:348-365.
15. Yannarell AC, Triplett EW. Within- and between-lake variability in the composition of bacterioplankton communities: Investigations using multiple spatial scales. Applied and Environmental Microbiology. 2004;70:214-223.
16. Franklin RB, Blum LK, Mc Comb AC, Mills AL. A geostatistical analysis of small-scale spatial variability in bacterial abundance and community structure in salt marsh creek bank sediments. FEMS Microbiology Ecology. 2002;42:71-80.
17. Baas-Becking LGM. Geobiologie of Inleiding Tot de Milieukunde. Van Stockkum and Zoon, The Hague; 1934.
18. Ekwere J, Akpan EB, Ntekim EEU. Geochemical studies of sediments in qua iboe estuary and associated creeks, Southern Nigeria. Tropical Journal of Applied Science. 1992;2:91-95.
19. Loving DH, Rantala RT. Manual for the geochemical analysis of marine sediments and suspended particulate matter. Earth Science Review. 1992;32(4):235-283.
20. Cheesbrough M. District laboratory practice in tropical countries. (Part II). Cambridge Universities. 2004;80-81.
21. Harrigan WF, Mc Cance ME. Laboratory Methods in Foods and Diary Microbiology. London: London Academic Press. 1990; 210.
22. Essien JP, Udosen ED. Distribution of actinomycetes in oil contaminated ultisols of the Niger Delta (Nigeria). Journal of Environmental Science. 2000;12:296-302.
23. Lu C, Huang B. Isolation and characterization of *Azotobacter* from pure *Rhizosphere*. African Journal of Micro-biology. 2010;7(6):773-779.
24. APHA. Standard methods for examination of water and wastewater. 18th ed. Hanover, Maryland: EPS Group, Inc; 1998.
25. Bergey DH, Holts JG. Bergey's manual of determinative bacteriology. 9th ed. Philadelphia: Lipincott Williams and Wilkins Publication; 1994.
26. Kunzli N, Jerrett M, Mack WJ, Beckerman B, La Bree L, Gilliland F. Ambient air pollution and atherosclerosis in Los Angeles. Environmental Health Perspectives. 2005;113:201-206.
27. Varnam AH, Evans MG. Environmental Microbiology. ASM Press, U.S.A. 2000;7-155.
28. Alongi DM. Zonation and seasonality of benthic primary production and community respiration in tropical mangrove for gests. Oecologia. 1994;98:320-330.
29. Essien EA, Umoren SA, Essien EE, Udoh AP. Preparation and evaluation of *Cucumeropsis manii.* Naud. Seed oil metallic soaps as driers in gloss paint. Journal of Material and Environmental Science. 2012;3(3):477-484.
30. Dexter E. Operating room utilization: Information management systems. Current Opinions in Anaesthesiology. 2003;16(6): 619-22.
31. Ernst-Detlef S, Mooney HA. Biodiversity and Ecosystem Function. Springer Study Edition; 1993.
32. Marsh KL, Sims GK, Mulvaney RL. Availability of urea to autotrophic ammonia-oxidizing bacteria as related to the fate of 14C- and 15N-labelled urea added to soil. Biology and Fertility of Soils. 2005;49(1):51-60.
33. Chen YP, Rekha PD, Arun AB, Shen FT, Lia WA, Young CC. Phosphate solubilizing bacteria from subtropical soils and their *Tricalcium phosphate* Solubilizing abilities. Applied Soil Ecology. 2006;34:33-41.
34. Park B, Bola A, Megharaj M, Naidu R. Bioremediation approaches for organic pollutants: A critical perspective. Environment International. 2011;37(8): 1362-1375.
35. Farrell JB. Fecal pathogens control during composting. In: Science and engineering of composting: Design, environmental, microbiological and utilization aspects edit. Hoitink HAJ, Keenerg HM. 1993;282-300.
36. Brodie EL, De Santis TZ, Parker JP, Zubietta IX, Piceno YM, Anderson GL. Urban aerosols harbor diverse and dynamic bacterial populations. Procedures for Natural Academic Science. 2007;104: 299-304.
37. Burrows SM, Elbert W, Lawrence MG, Poschl U. Bacteria in the global atmosphere: Part I – review and synthesis

of literature data for different ecosystems. Atmospheric Chemistry and Physics. 2009; 9:9263-9280.
38. Sullivan JJ. Air microbiology and dairy processing. Australian Journal of Dairy Technology. 1979;34:133-138.
39. Jones AM, Harrison RM. The effect of meteorological factors on atmospheric bioaerosols concentration- a review. Science of the Total Environment. 2004; 326:151-180.
40. Burge HA, Pierson DL, Groves TO, Strawn KF, Mishra SK. Dynamics of airborne fungal population in a large office building. Current Microbiology. 2000;40(1):10-16.
41. Kanaly RA, Harayama S. Biodegradation of higher molecular weight polycyclic aromatic hydrocarbons by bacteria. Journal of Bacteriology. 2000;182(8): 2059-2067.
42. Witzel RG. Limnology: Lakes and river ecosystems. 3rd edn. Academic Press Philadelphia: Saunders. 1983;860.
43. Akpan ER. Influence of meteorological and hydrographic factors in water quality of Calabar River, Nigeria. Tropical Journal of Environmnetal Research. 1992;23:107-111.
44. Donahue DJ, Linick TW, Jull AJT. Isotope-ratio and background corrections for accelerator mass spectrometry radio-carbon measurements. Radiocarbon. 1990;32(2):135.
45. Folk RL. Petrology of sedimentary rocks. UK: Academic Press. 1974;78.
46. Demaison GJ, Moore GT. Anoxic environments and oil sources bed genesis. American Association of Petroleum Geologist Bullettin. 1980;64(8):1179-1209.
47. Massoud MS, Al-Abdali F, Al-Ghadban AN. The status of oil pollution in the Arabian gulf by the end of 1993. Environment International. 1998;24(1/2):11-22.
48. Literathy P, Halder O, Samhan S, Morel G. Experimental studies on biological and chemical oxidation of dispersed oil in seawater. Water Science and Technology. 1989;21:845-856.
49. Lowenberg U, Kunzel T. Investigation on the hydrology of the lower cross river, Nigeria. Animal Research Development. 1992;35:72-75.
50. Ansa EJ Studies of the benthic macro-fauna of the Andoni flats in the Niger Delta area of Nigeria. Ph.D. Thesis University of Port Harcourt, Port Harcourt, Nigeria; 2005.
51. Dublin-Green CO, Awobamise AA, Ajao EA. Large marine ecosystem project for the gulf of guinea. Coastal Profile of Nigeria Global Environment Facility (GEF) and United Nations Industrial Development Organization. 1997;2-47.
52. Ajao EA, Fagade SO. The ecology of *Neritina glabrata* in Lagos Lagoon, Nigeria. Archives of Hydrobiology. 2000;119(3): 339-350.
53. Udoessien EI. Basic principles of environmental science. Etiliew Inter-national Publishers, Uyo, Nigeria. 2003; 339.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24631