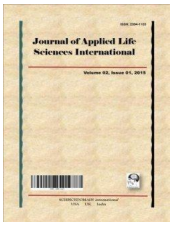


Journal of Applied Life Sciences International

17(3): 1-14, 2018; Article no.JALSI.37644
ISSN: 2394-1103

Diversity and Population Dynamics of Microbial Groups in Pelagic Column of Iko River, Eastern Obolo L.G.A, Akwa Ibom State, Nigeria

C. I. Udosen[1], S. I. Umana[2*], J. P. Essien[1], M. P. Bassey[2] and M. P. Uko[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 all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2018/37644
Editor(s):
(1) J. Rodolfo Rendón Villalobos, Department of Technological Development, National Polytechnic Institute, México.
Reviewers:
(1) Dorota Porowska, University of Warsaw, Poland.
(2) Hanan E. S. Ali, Egyptian Petroleum Research Institute, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/24641

Original Research Article

Received 4th September 2017
Accepted 11th November 2017
Published 16th May 2018

ABSTRACT

The diversity and population dynamics of microbial groups in pelagic column of Iko River Estuary, Nigeria were investigated using standard microbiological and analytical procedures. The results revealed that the densities of culturable microbes in the estuary were influenced by tidal regimes. Heterotrophic bacteria were the most abundant in the pelagic column with 26.39% and 29.01% occurrences during the low and high tides respectively. The study revealed variations in the loads of heterotrophic and autotrophic bacteria in the pelagic column of the estuarine ecosystem during the low and high tides respectively. The values recorded showed that the Protists community was dominated by heterotrophic bacteria during the low and high tides with the later harboring a much higher total heterotrophic count of 2.25 $x10^5$±0.023 cfu/ml as against 1.95 $x10^5$±0.260 cfu/ml obtained during the low tide. Geographic Information System (GIS) models of microbial communities revealed marked variation which ranged between tidal influences and locations. A high fecal coliform concentration in the North-East of the estuarine environment is a pointer to the unsanitary status of the estuarine environment. The mean temperature of the epilimnion revealed characteristic mesophilic temperature ranges with narrow spatial variations. The findings revealed that tidal bars and flats in shallow mesotidal estuary are subject to the action

**Corresponding author: E-mail: usenyene@yahoo.com;*

of tidal currents and waves. These complex events give rise to large variations in microbial communities in pelagic column which may be harnessed for effective environmental monitoring.

Keywords: Population dynamics; pelagic; Iko river; diversity and microbial groups.

1. INTRODUCTION

The question has been "why do organisms live where they do" [1]. 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.

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 1 mm 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 (1000-4000 m 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]. These communities contain potentially novel phylogenetic diversity and metabolisms but their role in the oceans remains poorly understood.

The oceans are not uniform, but made up of regionally formed water masses with distinct temperature and salinity characteristics, which moves around the globe over different spatial scales [13]. This thermohaline circulation has global significance for life on earth and communities of large plankton such as irrdarians are known to be structured by water masses [14]. Microbes are also influenced by the hydro-geography of the ocean, but recent evidence suggests a link between microbial community composition and water masses [15]. Water masses could be a key factor that explains microbial biogeography, since different waters habour different bacterial communities.

Although microbes have an essential community distribution in aquatic systems, 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 cataloging the diversity of microbes and documenting how microbial communities are affected by specific environmental changes or disturbances and how the composition and diversity of microbial communities can be influenced by a wide range of biotic and abiotic factors in the mesotidal estuarine environment of Iko River Estuary located in the Bight of Bonny in Eastern Obolo – Nigeria. The data generated will provide information and update knowledge on how microbial communities are structured across a scale along the tidal influenced brackish ecosystem.

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. 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.

The hydrology of Iko River is directed by tides, although seasonal influences which are related to the climatic regime are evident. Iko River is directly influenced by processes in the Atlantic coastal waters [16].

2.2 Sample Collection

Surface Water samples were collected from four (4) strategic positions during the low and high tides level. At each sampling point, the water samples were collected with the aid of sterile 1-litre capacity clean plastic containers that were pre-rinsed with the river water before sampling from top 10 cm depth of the estuarine water body.

2.3 Microbiological Studies

Serial dilution of water sample was done according to the method of Cheesbrough [17]. Precisely 10ml of the estuarine water sample was introduced into 90ml of distilled water, well shaken for even distribution and serially diluted. Standard microbiological techniques described by Harrigan and McCance [18] were employed for the microbiological analysis water samples.

The total heterotrophic bacterial and fungal counts in water 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 water samples collected from the estuary were also enumerated after 7 days of incubation at 28°C ± 2°C using acidified Nutrient agar /Starch nitrate agar [19].

The numbers of sulphate reducers in the water samples were determined by the pour plate technique at 28°C ± 2°C 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 28°C ± 2°C for 48 hours while the population of phosphates solubilizing bacteria (PSB) in the samples were estimated by the method of Lu and Huang [20]. In this case, the diluted samples were introduced into compounded medium and incubated for 3 days at 28°C. 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 [21]. 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 28°C in a Gallenkamp incubator and fungal plates at room temperature (28°C ± 2°C) 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 millilitre water samples.

The colonies obtained from the samples were characterized using standard procedure as described by *Bergey's Manual of Determinative Bacteriology* [22]. 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 Barnett and Hunter [23].

2.4 Determination of Spatial Variations in the Pelagic Load of the Estuarine

Geographic information system (GIS) was adopted to perform dynamic modeling of the pelagic 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 [24].

2.5 Determination of the Physiochemical Properties of Water Sample

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

2.6 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 the Pelagic Column during Low and High Tides

The results presented on Fig. 1 revealed variations in the loads of heterotrophic and autotrophic bacteria in the pelagic column of the estuarine ecosystem during the low and high tides respectively. The values recorded showed that the Protists community was dominated by heterotrophic bacteria during the low and high tides with the later harboring a much higher total heterotrophic count of 2.25 $x10^5$±0.023 cfu/ml as against 1.95 $x10^5$±0.260 cfu/ml obtained during the low tide. No *Pseudomonas* was isolated from the pelagic column water samples obtained from stations one (IES-1) and three (IES-3) during the low tide. The densities of hydrocarbon utilizing bacteria in the water sample were remarkable. During low tide, the density oil degrading bacteria recorded was 8.88 $x10^3$ ±0.031 cfu/ml while 3.03 $x10^3$ ± 0.065 cfu/ml was obtained during high tide. Few viable cells of *Clostridium* were observed and loads were lower (7.0 $x10^1$ ± 2.076 cfu/ml) during the low than high (9.0 $x10^1$ ± 1.765 cfu/ml) tides. The densities of *Pseudomonas* sp, *Actinomycetes* and fungi encountered in pelagic column were relatively remarkable. The distribution pattern of the microbial communities in the pelagic column during the low and high tides are shown in Figs. 2 and 3 respectively.

The results presented in Figure 4 shows the densities of pollution indicator bacteria in the pelagic column of the estuarine ecosystem during the low and high tides respectively. The result showed that the pelagic column is highly polluted with coliform bacteria during the low and high tides but the high tide (2.70 $x10^4$±4.4314 cfu/ml) harboured more numbers bacteria than the low tide (1.45 $x10^3$±3.1614 cfu/ml). No faecal coliform and *Clostridium* were isolated from the pelagic column water samples obtained from stations one (IES-1) and three (IES-3) during the low tide with *Clostridium* also being absent at stations two (IES -2) and three (IES-3) of the pelagic column water during high tide.

Few viable cells of *Clostridium* were observed and the densities were lower during the high (3.35 $x10^1$ ± 2.076 cfu/ml) than low (7.0 $x10^1$ ± 1.765 cfu/ml) tides. The densities of coliform, faecal coliform, salmonella and shigellae, *Vibrio* sp, *Staphylococcus* sp encountered in pelagic column were also remarkable. Their distribution pattern in the pelagic column during the low and high tides are also shown in Figs. 5 and 6 respectively.

3.2 Microbial Diversity of Iko River Pelagic Column

The cultural, morphological and biochemical attributes of the bacterial and fungal isolates from the pelagic column revealed 21 bacterial (Table 1) and 11 fungal (Table 2) isolates. The

result revealed little or no variation in diversity between low and high tides. The research findings have revealed higher bacterial loads and diversity in sample.

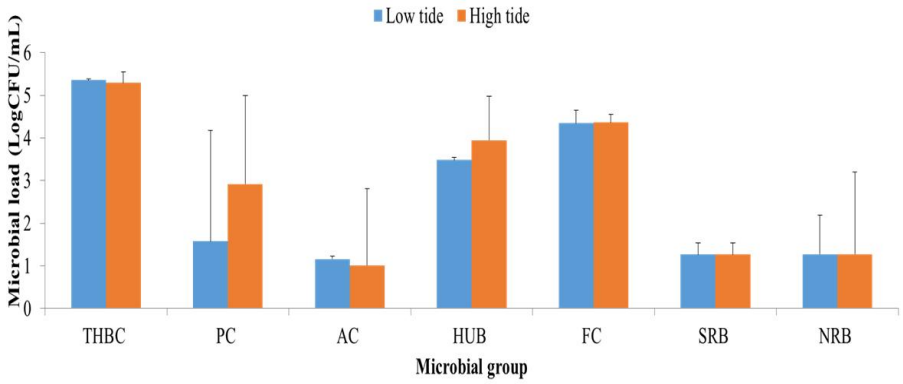


Fig. 1. Heterotrophic bacteria, autotrophic bacteria and fungal loads of the pelagic column during low and high tide

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

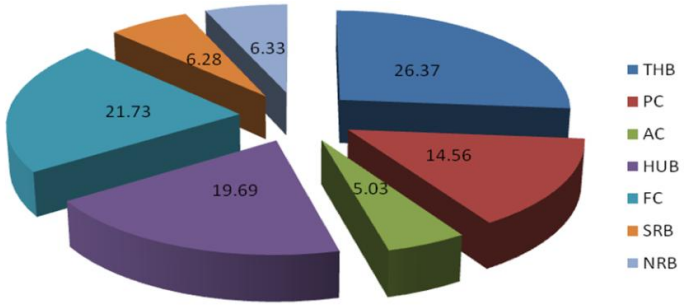


Fig. 2. Abundance (%) of heterotrophic and autotrophic bacteria communities in the pelagic column during low tide

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.

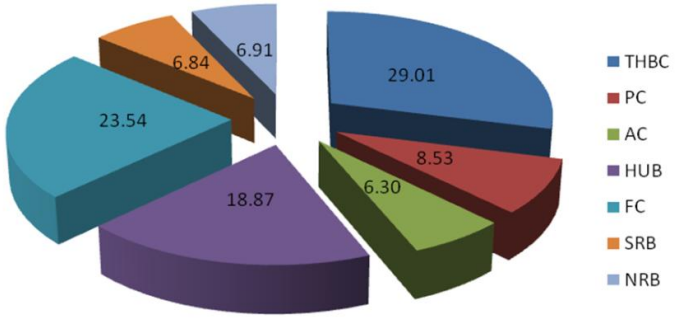


Fig. 3. Abundance (%) of heterotrophic and autotrophic bacteria communities in the pelagic column during high tide

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

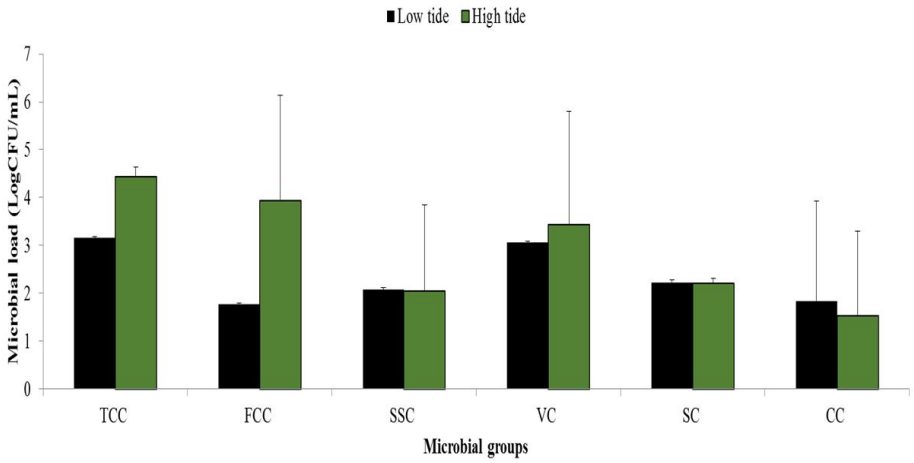


Fig. 4. Pollutant indicator bacterial loads of the pelagic column during low and high tide
Key: TCC = Total coliform count, FCC = Faecal coliform count, SSC = Salmonella shigella count, VC = Vibrio count, SC = Staphylococcus count, CC = Clostridium count

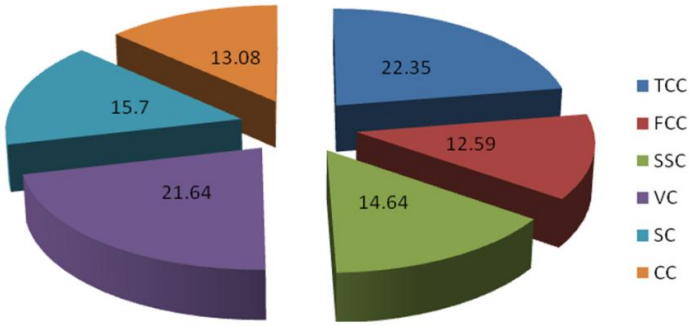


Fig. 5. Abundance (%) of pollution indicator bacteria in the pelagic column during low tide
Key: TCC = Total coliform count, FCC = Faecal coliform count, SSC = Salmonella shigella count, VC = Vibrio count, SC = Staphylococcus count, CC = Clostridium count

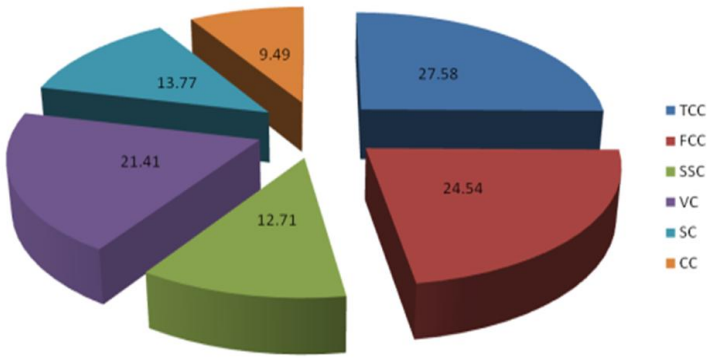


Fig. 6. Abundance (%) of pollution indicator bacteria in the pelagic column during high tide
Key: TCC = Total coliform count, FCC = Faecal coliform count, SSC = Salmonella shigella count, VC = Vibrio count, SC = Staphylococcus count, CC = Clostridium count

Table 1. Occurrence and distribution of the diverse species of bacteria in water samples from Iko river estuary during low and high tides

Organisms	Low tide				High tide				Prevalence rate (%)
	IES-1	IES-2	IES-3	IES-4	IES-1	IES-2	IES-3	IES-4	
Micrococcus sp	+	+	+	+	+	+	-	+	87.5
Bacillus subtilis	+	+	+	+	+	+	-	+	87.5
Bacillus cereus	+	-	-	-	+	+	+	+	62.5
Streptococcus sp	+	-	-	-	-	-	+	+	37.5
Chromatium sp	+	-	-	-	+	-	+	+	50.0
Staphylococcus aureus	+	+	+	+	+	-	+	+	87.5
Klebsiella sp	+	+	+	+	+	+	+	+	100
Citrobacter sp	+	+	+	-	-	+	+	+	75.0
Enterobacter sp	+	-	+	+	-	-	-	-	37.5
Salmonella sp	+	+	-	-	+	-	+	-	50.0
Shigella sp	+	+	+	-	+	-	+	+	75.0
Vibrio cholera	+	-	+	+	-	+	-	-	50.0
Vibro haemolyticus	+	+	+	+	+	+	-	+	87.5
Staphylococcus albus	+	+	+	+	+	+	+	+	100
Actinomycetes sp	+	-	+	+	+	+	+	+	87.5
Escherichia coli	-	+	+	-	+	+	+	+	75.0
Pseudomonas aeruginosa	-	+	-	+	+	+	-	-	50.0
Pseudomonas fluorescens	-	+	-	+	-	-	-	-	25.0
Clostridium sp	-	+	-	-	+	+	+	+	62.5
Proteus sp	-	-	+	+	+	+	-	-	50.0
Serratia sp	-	-	+	-	+	-	-	+	37.5
Species Richness (21)	15	13	14	12	16	13	12	15	

Key: IES - Iko Estuary Station

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

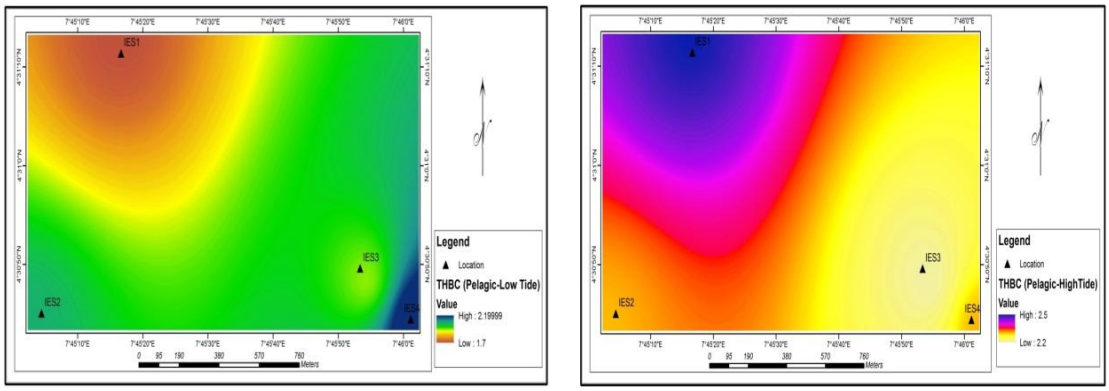
Organisms	Low tide				High tide				Prevalence rate (%)
	IES-1	IES-2	IES-3	IES-4	IES-1	IES-2	IES-3	IES-4	
Aspergillus flavus	-	-	-	+	+	-	-	+	37.5
Aspergillus niger	+	+	-	-	+	+	-	-	50.0
Aspergillus fumigates	-	+	-	-	-	+	-	-	25.0
Aspergillus terreus	-	-	+	-	-	-	+	-	25.0
Rhizopus stolonifer	-	+	+	-	+	-	-	-	37.5
Penicillium expansum	-	+	-	+	+	-	+	-	50.0
Candida albicans	-	-	-	-	-	-	+	-	12.5
Candida tropicalis	-	+	-	-	-	-	-	-	12.5
Eurotium sp	+	-	+	-	-	-	+	-	37.5
Absidia sp	-	-	-	-	-	-	-	+	12.5
Geotrichum candidum	+	-	-	-	-	-	-	-	12.5
Species Richness (11)	3	5	3	2	4	2	4	2	

Key: IES - Iko Estuary Station

3.3 Spatial Variations in the Microbial Loads

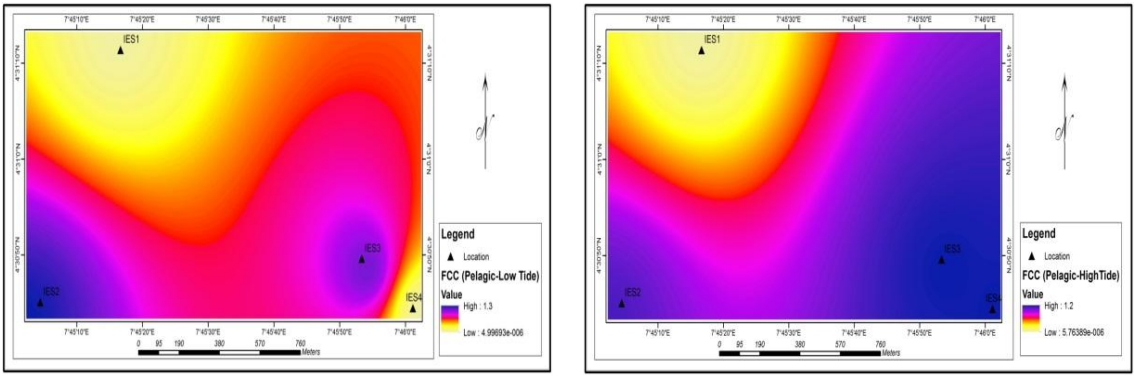
GIS model of spatial distribution of heterotrophic bacteria in water during the low and high tides is presented in Fig. 7. During low tide, the blue band shows high heterotrophic bacterial concentrations in the North-East zone of the estuarine environment, while the yellowish brown band signifies lower bacterial concentrations in the North West zone. During high tide, the blue colour shows high heterotrophic bacterial concentrations in the North-West of the estuarine environment, while the yellow colour band signifies lower bacterial concentrations. The middle pink band shows moderate bacterial load. For fecal coliform in water (Fig. 8) the results show that during low tide, the blue band shows high fecal coliform concentrations in the North-West zone of the estuarine environment, while the pinkish-blue band signifies low fecal colifom concentrations. During high tide, the blue band shows high fecal coliform concentrations in the North-East of the estuarine environment, while the yellow band signifies lower fecal coliform concentrations. The middle pink band shows moderate fecal coliform load.

GIS model of spatial distribution of fungi in water during the low and high tides is shown in Figure 9. The pattern of fungal spread in surface water was not definite. During low tide, the sky-blue band shows high fungal concentrations in the North-West of the estuarine environment. An evenly moderate to high loads distribution was indicated by the green band in the North East, while the brownish -white band signifies low fungal loads. During high tide, the distribution was also not definite, as low to moderate and high concentrations were noticed in all the zones.



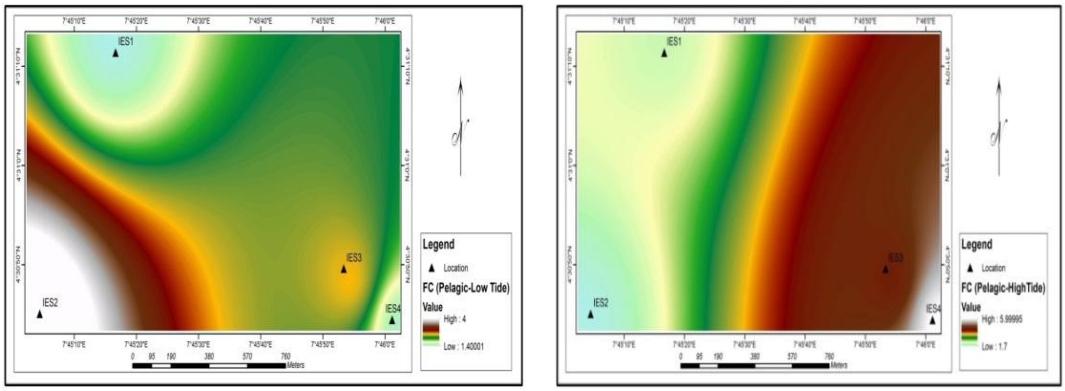
(a) (b)

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



(a) (b)

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



(a) (b)

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

3.4 Physicochemical Properties of the Estuarine Water

The result of the physicochemical properties of the Iko estuary water during low tide and high tide are presented in Tables 3 and 4 respectively. The results revealed no significant difference in temperature and pH of the water during both tides but a higher redox potential level was obtained during high (238.75±0.048 mV) than low (238.75±0.048) tides. The estuarine water was slightly acidic with unexpectedly low salinity levels at the time of survey. High levels of conductivity, alkalinity and hardness were observed at both tides whereas mean total dissolved solids (TDS), dissolved oxygen (DO), biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) were respectively 12.15±2.831 mg/L, 6.34±0.378 mg/L, 8.13±0.150 mg/L and 10.39±0.418 mg/L during low tide and 66.48±25.46 mg/L, 5.86±0.09 mg/L, 8.33±2.12 mg/L and 13.72±0.75 mg/L during high tide. Nitrite, nitrate and phosphate levels in the water body were low (<0.5mg/L) during both tides. However, the level of sulphate was lower during low (165.63±0.021 mg/L) than high (203.28±5.29 mg/L) tides.

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

Parameter	IES-1	IES-2	IES-3	IES-4	Mean	SD
Temperature (^oC)	24.1	25.02	24.44	24.12	24.42	0.333
pH	5.83	5.92	5.93	5.77	5.86	0.059
Redox Potential (mV)	41.30	42.33	43.01	44.18	42.71	1.047
Salinity (%)	2.26	2.44	3.7	2.44	2.71	0.576
Conductivity (ms/cm)	39.14	54.26	37.1	36.18	41.67	6.572
Alkalinity (mg/L)	225	222	301	202	237.5	0.053
Hardness (mg/L)	2000	1411	2023	3011	2111.25	0.117
Total Dissolved Solids ;TDS (mg/L)	10.57	17.01	11.02	9.99	12.15	2.831
Dissolved Oxygen; DO (mg/L)	6.92	6.44	5.99	6.02	6.34	0.378
Biochemical Oxygen Demand; BOD_5 (mg/L)	8.22	8.27	8.14	7.88	8.13	0.150
Chemical Oxygen Demand; COD(mg/L)	10.19	10.08	10.18	11.11	10.39	0.418
Nitrite (mg/L)	0.088	0.117	0.078	0.092	0.094	0.014
Nitrate (mg/L)	1.21	0.98	1.17	1.33	1.17	0.126
Phosphate (mg/L)	1.445	1.875	1.331	0.982	1.41	0.319
Sulphate (mg/L)	178.3	156.9	160.9	166.4	165.63	0.021

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

Parameter	IES-1	IES-2	IES-3	IES-4	Mean	SD
Temperature (°C)	24.3	25.75	24.66	24.89	24.90	0.53
pH	5.83	6.12	6.46	6.23	6.16	0.23
Redox Potential (mV)	81.3	72.67	78.8	69.8	75.64	4.61
Salinity (%)	5.64	4.67	8.7	5.63	6.16	1.52
Conductivity (ms/cm)	169.4	154.6	166.2	168.6	164.7	5.949
Alkalinity (mg/L)	1025	997	1011	1021	1013.50	10.81
Hardness (mg/L)	17000	14671	15023	16533	15806.75	981.77
Total Dissolved Solids; TDS (mg/L)	84.6	77.5	81.2	22.6	66.48	25.46
Dissolved Oxygen; DO (mg/L)	5.83	6.01	5.77	5.82	5.86	0.09
Biochemical Oxygen Demand; BOD_5 (mg/L)	11.13	6.89	5.77	9.54	8.33	2.12
Chemical Oxygen Demand; COD(mg/L)	13.99	14.42	12.45	14.00	13.72	0.75
Nitrite (mg/L)	0.042	0.051	0.048	0.044	0.046	0.003
Nitrate (mg/L)	0.249	0.222	0.217	0.236	0.231	0.013
Phosphate (mg/L)	0.325	0.336	0.298	0.322	0.320	0.014
Sulphate (mg/L)	201.6	198.9	212.3	200.3	203.28	5.29

4. DISCUSSION

The aquatic ecosystem is usually inhabited by a diverse population of microorganisms. The results of this study have revealed the rich microbial assemblage and diversity in the estuarine environment. Analysis has shown that the pelagic column harboured higher heterotrophic bacterial loads during high tides. Low numbers of oil degrading bacteria were observed in the surface water.

The variation in fungal loads of the estuarine pelagic column between tides was not high. The results revealed high density of fungi in pelagic column followed in both tides. There was also a strong relationship (r = 0.853) in the fungal loads of surface water between tides. On the other hand, the densities of pollution indicator bacterial communities such as coliforms, faecal coliforms, *Salmonella* and *Shigella* species as well as *Vibrio* sp and *Staphylococcus aureus* were relatively high. Their occurrences are indicative of environmental contamination. The high presence of faecal coliform shows the estuarine water body is seriously impacted with fresh human fecal matter [25].

The high densities of heterotrophic bacteria obtained for the estuarine are in accord. 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 [26]. The wide heterotrophic activity of water microorganisms is of very considerable importance in the remediation of aquatic system after pollution with hydrocarbons and other organic chemicals [26,27]. Actinomycetes were also obtained from estuarine water. Their low count in the aquatic system may be because Actinomycetes live predominantly aerobically, i.e. they need oxygen for their metabolism [28]. 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. 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 [28]. 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 [28]. 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 [29].

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 were isolated from the estuarine. 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 [30].

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 [31]. The high incidence of coliforms observed for the pelagic water 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 water. This finding is in agreement with the report that reduction in faecal coliforms often correlates with reduction in *Salmonella* species and other pathogenic microorganisms [31]. 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.

Natural waters are known to be diverse in their physical, chemical and biological characteristics [32]. The physicochemical attributes of the surface water from the Iko River Estuary have revealed typical tropical estuarine water body. The mean temperature of the epilimnion revealed characteristic mesophilic temperature ranges with narrow spatial variations. Mean temperatures of 24.42±0.33°C was recorded during low tide and 24.90 ± 0.53°C during high tide, as compared to the WHO limit of 25°C for surface waters (WHO, 1984) (26.6 - 27.8°C). The pH also exhibited narrow amplitude of variation (6.8 - 7.4) and reflected that of unpolluted aquatic ecosystem. The pH levels recorded were within the WHO [33] recommended range of 6.5 - 8.50 for fishing. Total suspended solids (TSS) were slightly high and much higher in during high tide (243.2 mg/l). The high TSS in the water bodies is probably due to the high organic matter load in suspension. According to FAO/EIFAC [34], TSS of 25 – 80 mg/l in European waters would not harm fishery, but waters with TSS of 80 - 100mg/l is unlikely to support a good freshwater fishery in temperate environment. No comparative studies of the effect of TSS on tropical water bodies are available. Conductivity levels were generally high in all the samples (range: 164.7±5.949 ms/cm at high tide and 41.67±6.572 ms/cm at low tide respectively) indicating high ionic richness. Total hardness (alkalinity) was highly variable (1.51 - 61.61 mg/l) and did not indicate any significant contribution from causative ions. Dissolved oxygen in water samples was high with ranges between 6.34±0.378 mg/L during the low tide and 5.86±0.09 mg/L during high tide, indicating high oxygenation. These values are higher than the WHO limit of 5 .0 mg/l for surface waters. Thus, the values indicated an oxidized environment that retard the activities of the sulphate reducing and nitrogen fixing microorganisms. The DO level of 5.0 mg/l or above is recommended for fish and other aquatic life forms [35]. In general, DO profile is known to influence the stocking density, diversity and productivity of aquatic ecosystems [36]. The total hydrocarbons and oil and grease levels in water samples from the estuarine ecosystem were negligible, <0.01 indicating remarkably low hydrocarbon input. The mean concentrations of nitrite and nitrate, phosphate and sulphate in the pelagic column were 0.094±0.014 mg/L, 1.17±0.126 mg/L, 1.41±0.319 mg/L 165.63±0.021 mg/L respectively during the low tide and 0.046±0.003 mg/L, 0.231±0.013 mg/L, 0.320±0.014 mg/L 203.28±5.29 mg/L respectively during the high tide and were within their respective WHO limit.

Changes in water physicochemistry occurs regularly, some of which are episodic from run-off with resultant effect on the pH and alkalinity and decrease in buffering capacity of the water bodies. Such changes in water quality generally influence the diversity of aquatic biota including

the survival and productivity of microbiota. In general, the physicochemical attributes of the surface water biotopes were generally low and apparently within the limits allowed by the regulatory agencies [37]. This notwithstanding, the variation may affect the population, survival and distribution, as well as culminating in synergistic or antagonistic effects on the microbial communities.

The result of this study showed significant differences in salinity between stations ($p < 0.05$), because the estuary is highly vulnerable to external perturbations and mixing of freshwater with marine (Atlantic Ocean). There was moderate linear positive correlation (0.556) between salinity and bacterial load. Similar observations were made by Lowenberg and Kunzel [38], for the Cross River while Abowei and George [39] and Abowei [40], differ in their reports of no significant difference in salinity between sampling stations, ($p < 0.05$), along Okpoka Creek and Nkoro River, respectively; largely due to the fact that water at these sampling stations were from same source (linear in nature). Salinity changes in estuaries are also mainly controlled by freshwater discharge and precipitation. The high freshwater discharge during the high tide is responsible for the absence of spatial variation in estuary in Eastern Obolo. During the low tide, rainfall recedes and discharges from most creeks cease thereby increasing marine influence and salt water intrusion in salinity towards the sea. Lowenberg and Kunzel [38] reported a change of salinity from 0.5 psu during the high tide to 12 psu during the low tide.

5. CONCLUSION

This study on microbial population dynamics and diversity in a mesotidal estuarine ecosystem of Iko River Estuary has revealed the rich microbial assemblage and diversity of the tropical estuarine environment. Results obtained from this study have shown that Iko River estuary is laden with diverse species of microorganisms including groups of pathogens of both human and animal significance. The study has specifically revealed the:

i. High level of contamination with faecal matter as depicted by the high densities of *E. coli* in the estuarine water during both tides.
ii. There is potential risk of drinking water as well as consuming fish and other aquatic produce from the estuary contaminated with harmful microbial agents.
iii. Risk of increased rate of food contamination with food-borne pathogens due to their possible presence in the atmosphere.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Green JL, Bohannan BJM. Spatial scaling of microbial biodiversity in scaling biodiversity (Storch, D. and Marquet, P. A. and Brown, J. H. Cambridge: Cambridge University Press. 2006;155.
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 microorganisms 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, DeLong 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. Broecker WS. The great ocean conveyor. Oceanography. 1991;4(2):78-89.
14. Hosia A, Stemmann L, Youngbluth M. Distribution of Net-collected Planktonic Cnidarians along the Northern Mid-Atlantic Ridge and their Associations with the Main Water Masses. Deep Sea Research Part II: Tropical Studies in Oceanography. 2008;55(1):106-118.
15. Galand PE, Casamayor EO, Kirchman DL, Lovejoy C. Ecology of the rare microbial biosphere of the Arctic Ocean. Proceedings of the National Academy of Sciences, USA. 2009;106:22427-22432.
16. 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.
17. Cheesbrough M. District laboratory practice in tropical countries. (Part II). Cambridge Universities. 2004;80-81.
18. Harrigan WF, McCance ME. Laboratory methods in foods and diary microbiology. London: London Academic Press. 1990; 210.
19. Essien JP, Udosen ED. Distribution of actinomycetes in oil contaminated ultisols of the Niger Delta (Nigeria). Journal of Environmental Science. 2000;12:296-302.
20. Lu C, Huang B. Isolation and characterization of azotobacter from pure rhizosphere. African Journal of Microbiology. 2010;7(6):773-779.
21. APHA. Standard methods for examination of water and wastewater. 18th ed. Hanover, Maryland: EPS Group, Inc. 1998;50.
22. Bergey DH, Holts JG. Bergey's manual of determinative bacteriology. 9th ed. Philadelphia: Lipincott Williams and Wilkins Publication. 1994;56 70.
23. Barnett HL, Hunter BB. Illustrated genera of imperfect fungi. New York: Macmillan Publishing Company. 1987;70-80.
24. Kunzli N, Jerrett M, Mack WJ, Beckerman B, LaBree L, Gilliland F. Ambient air pollution and atherosclerosis in Los Angeles. Environmental Health Perspectives. 2005;113:201-206.
25. Prescott LM, Harley JP, Klein DA. Microbiology. 6th Edition. McGraw-Hill Co. New York, London. 2005;60-79.
26. Varnam AH, Evans MG. Environmental microbiology. ASM Press, U.S.A. 2000; 100-155.
27. 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.
28. Dexter E. Operating room utilization: Information management systems. Current Opinions in Anaesthesiology. 2003;16(6): 619-22.
29. Ernst-Detlef S, Mooney HA. Biodiversity and ecosystem function. Springer Study Edition; 1993.
30. 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.
31. Farrell JB. Fecal pathogens control during composting, In: Science and Engineering of Composting: Design, Environmental, Microbiological and Utilization Aspects edit. H. A. J. Hoitink and H. M. Keenerg. 1993;282-300.
32. Akpan ER. Influence of meteorological and hydrographic factors in water quality of Calabar River, Nigeria. Tropical Journal of Environmnetal Research. 1992;23:107-111.
33. WHO. Guidelines for drinking water quality, health criteria and other information. Vol.2. Geneva Publication. 1984;306.
34. FAO/EIFAC. Report of the 3rd Session of the EIFAC Working Party on Fish Farm Effluent. Tihany, Hungary. FAO, Rome. 1992;117.
35. Aderomoti CMO. Standard methods for water and effluent analysis. Ibadan: Foludex Press Ltd. 1996;22-23.
36. Fagade SO, Adebisi AA, Ugwumba OA. Strategies for the establishment of profitable fish farm in Nigeria. Procceedings of the National Academy of Science, FISON. 1986;1:326-338.
37. FEPA, Federal Environmental Protection Agency (Ammendment) Degree No. 59 of

1992. Laws of the Federation of Nigeria; 1991.
38. Lowenberg U, Kunzel T. Investigation on the hydrology of the lower cross river, Nigeria. Animal Research Development. 1992;35:72-75.
39. Abowei JFN, Goerge ADI. Some physical and chemical characteristics in Okpoka Creek, Niger Delta, Nigeria. Research Journal of Environmental and Earth Sciences. 2002; 1(2):45-53.
40. Abowei JFN. Salinity, dissolved oxygen, ph and surface water temperature conditions on Nkoro River, Niger Delta, Nigeria. Advanced Journal of Food Science and Technology. 2010; 2(1):36-40.

© 2018 Udosen et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24641