Original Research Article

MICROBIOLOGICAL PROPERTIES AND POPULATION DYNAMICS OF ATMOSPHERE IN MESOTIDAL ESTUARINE OF IKO RIVER, AKWA IBOM STATE, NIGERIA

ABSTRACTS

The microbiological properties and population dynamics of atmosphere in mesotidal estuarine of Iko River 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. Their abundance varied between both tides as well as locations. Proportionately more fungal isolates were found in the estuarine air. There was a significant positive correlation ($r = 0.717$, $p < 0.05$) between the total heterotrophic bacteria in the atmosphere and wind speed, and between the fungi in the atmosphere and wind speed ($r = 0.799$) during high tide, indicating that increase in wind speed resulted in a corresponding effect in heterotrophic bacterial and fungal counts during high tide. A comparison of the relation between atmospheric temperature and microbial load showed little or no correlation ($r = 0.30$). The results of the air quality attributes of Iko Estuary during low tide and high tide showed that the quality of air in the estuarine environment was affected. The air quality in the estuarine environment was relatively "clean" and wholesome as most criteria gaseous pollutants (HCN, NO_2, SO_2) except CO and SPM were below detectable limits and within the FMENV and WHO acceptable limits. However, the recorded levels of CO in some parts of the fishing settlement were above Federal Ministry of Environment (FMEnv) limits of 10.0 - 20.0ppm for daily average of 8 hourly values in Nigeria. The levels of atmospheric contaminants varied between low and high tides. The 2.0 ppm level of SPM recorded during high tide is higher than the FMEnv limits of 0.25ppm and is dangerous. The study has revealed significant emission of CO from the fish smoking activity which is common in the settlements. Geographic Information System (GIS) models of microbial communities revealed marked variation which ranged between tidal influences and microhabitats. The 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 findings revealed that tidal bars and flats in shallow mesotidal estuary are subject to the action of tidal currents and waves. These complex events give rise to large variations in microbial communities in estuarine microhabitats which may be harnessed for effective environmental monitoring.

Keywords: Atmosphere, Microbiological properties, Estuarine, Iko River, Mesotidal, and Population Dynamics

1.0 Introduction

Attempts have been made to describe and explain spatial patterns of biological diversity and how these patterns change over time (1). The question has been “why do organisms live where they do” (2). 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 (3).

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 (1). The extent of microbial dispersal is currently under debate. According to Finlay (4) 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 (5). 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 (*6*).

Despite this long and rich history of study little is known about the biology of the atmosphere relative to aquatic and terrestrial habitats. Technical limitations have hindered the study of the air. Low densities of micro-organisms in the air can make even sensitive molecular analysis difficult because of the small amount of biological material present in the air. Additionally, the lack of standardization in air collection and sample processing methods complicate comparisons across studies (7). Owing to this lack of methodological standardization, it is unclear whether large difference in density estimates among studies can be attributed to biological variation (8). Conceptual limitations also continue to impact the advancement of our understanding of life in the atmosphere. Most of what is known about airborne microorganisms is based on the assumption that the atmosphere is a conduit for the dispersal of microbes rather than a dynamic habitat where microorganisms actively metabolized and reproduced in the atmosphere.

Microorganisms may belong to one of these groups, those that are not metabolically active and actively reproducing. Microbes can form inactive propagules (e.g. spores) that disseminates through the atmosphere, however, for these organisms the atmosphere would not be a ''habitat'' in the conventional sense. Microbes that remain metabolically active in the atmosphere but rarely reproduce are organisms for which the atmosphere serves only as accidental dispersal mechanism. Despite past assumptions, residents of the atmosphere are likely to exist, and that the atmosphere can act as habitat for microbial life. Sources of information shows that large portions of the atmosphere have environmental characteristics consistent with other microbial habitats; that biogeochemical cycling mediated by microbes occurs in the atmosphere, that at least some microbes found in the atmosphere are metabolically active, and that residence times of microbes in the atmosphere are long enough that actively reproducing residents could exist (9).

The atmosphere is not the most extreme microbial habitat. By several measures (pH, temperature, ultraviolet (UV) radiation, resources and water availability) the atmosphere appears to be less extreme than many other microbial habitats. The pH of clouds and rainwater ranges from 3 to a narrower range than that found in many microbial habitats. Microbes have adapted to a much wider range of pH condition that occur in air, from highly acidic conditions near pH 0 to extremely alkaline conditions up to pH – 11 (10). Temperature can vary widely throughout the atmosphere, but includes ranges that are suitable for microbial life in the lower atmosphere up to 20km above the earth's surface; average temperature decrease with altitude and range from an average of 15^{o}C (at sea level) to -56^{o}C (at 20km). Many micro-organisms are capable of growth at temperatures near and below 0^{o}C with some communities reported to be metabolically active at temperatures as low as -18^{o}C (11)

Resource availability in the atmosphere is not necessarily lower than that of many terrestrial or aquatic environment in clouds and rainwater concentrations of nutrients (e.g. sulphate and nitrate) reach levels typical of oligothrophic lakes (12). Numerous potential carbon sources are found in both clouds and the atmosphere, including carboxylic acids and alcohols at concentrations up to 1mg-1 (12) as well as hydrocarbons at concentrations up to 4mg-1 (9) in addition to available resources for supporting heterotrophic metabolism the air provides a suitable habitat for phototrophs. Pigmented micro-organisms found in the atmosphere could be using pigments for photosynthesis. Gene sequences from putative photoautotrophs have been amplified from air samples (13) although to our knowledge, no photoautotrophs have been isolated from atmosphere.

Biogeochemical cycling may occur in the atmosphere: if metabolically active microbes are present in the atmosphere, they should leave chemical "footprint" of their metabolisms. For example, microbes are intimately involved in biogeochemical transformations, and evidence for such transformations in the atmosphere would support the hypothesis of a resident microbiota. Nitrogen cycling in clouds (including mineralization and nitrification) has been demonstrated (14), suggesting the presence of metabolically active microbes. There is some evidence for carbon cycling in clouds, although it is not as clear cut as the case for nitrogen, for example bacteria have been isolated from clouds that are able to use organic compounds commonly found in clouds water, including acetate, formate, succinate, lactate, formaldehyde and methanol as carbon sources (15). Bacterial end products of these metabolic reactions are also commonly found in cloud water suggesting that these microbes are actively transforming these compounds in clouds.

Although the majority of aerobiology has focused on community level abundance patterns, culture-based research has provided a foundation for exploring taxa-level patterns, the study of taxa-level distributional pattern, such as species geographical range, is central to biogeography. Culture-based work has begun to address fundamental questions about the upper boundary of microbial geographical ranges in the atmosphere. lsolated cultures of the common mould, *Penicillium notatum*, have been collected at an altitude of 77km, and the bacteria *Micrococcus albus* and *Mycobacterium luteum* at an altitude of 70km. Culture-based studies have been used to

understand the link between atmospheric environmental conditions and occurrence of particular microbial species, for e.g the occurrence of *Micrococcus* has been shown to correlate with the concentration of air borne particulate matter this might explain why air borne *Micrococcus* species are commonly dominant in urban environments (16). Finally, culture based studies can help identify ubiquitous species that are likely to have large geographical range sizes, spore-forming organisms such as *Bacillus* species and other Gram-positive, tend to dominate culture-dependent surveys of air-borne microbial diversity and thus may have large geographical ranges (16).

Despite our knowledge and understanding of microbial diversities and interactions in our ecosystems as well as vast amounts of literatures available, much has not been done on the diverse microbial communities inhabiting the Iko River Estuarine atmosphere and their interactions with the biotic and abiotic components, thus called for this study.

MATERIALS AND METHOD

2.1 Description of Study Area

The Iko River Estuary (Figure 1) 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 30km.

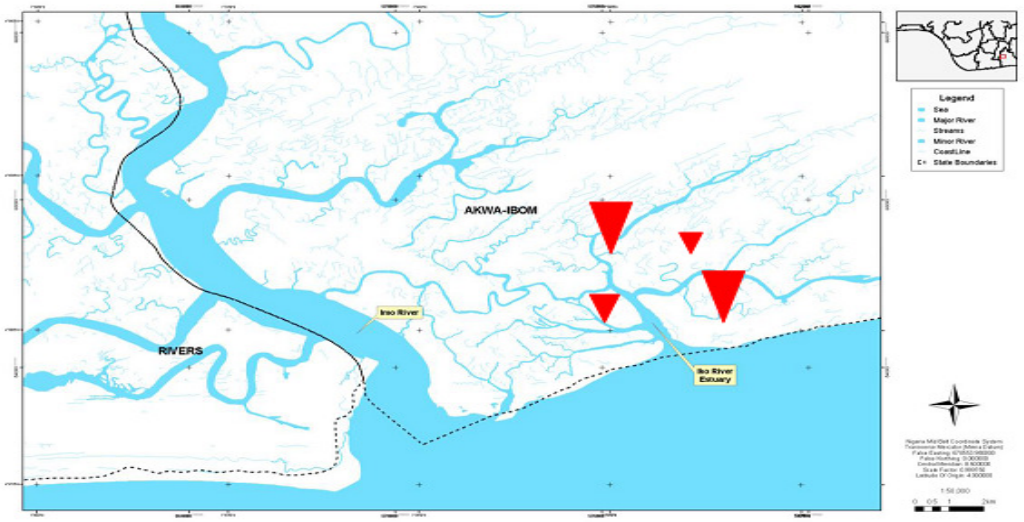


Figure 1: Sampling sites on the Map of Iko River Estuary

2.2 Air Sampling and Quality Analysis

The air sampling was conducted during high and low tide regimes of the estuarine ecosystem. Precisely four stations designated IES-1, IES -2, IES-3 and IES-4 respectively located at the coast of the Iko River Estuary. The coordinates of the sampling stations are presented in Table 1.

The monitors were mounted at 1.5m above the ground level so that the pollutants were measured at about the sitting and breathing zone (17).

Table 1: Coordinates of air sampling stations

Station	Northing	Easting
IES-1	04°31'13.2"	007°45'16.4"
IES-2	04°30'43.4"	007°45'02.4"

IES-3	04°30'48.5"	007°45'54.2"
IES-4	04°30'43.9"	007°46'02.4"

2.2.1 Meteorological and Air Quality Measurements and Data Acquisition

In choosing sampling location, special preference was given to the accessibility of sampling station, availability of open space with good configuration free from shed and meteorological consideration of upward and downward directions, Areas with minimal local influence from vehicular movement was selected. For quality analysis, special attention was also given to sampling collection and analytical procedure with respect to the sensitivity and stability of equipment used, re-calibration of equipment and re-producibility of results.

The existing meteorological and climatic data from MPN's QIT meteorological station are used for the write-up. However, additional field data were collected for Atmospheric pressure, relative humidity, temperature, wind speed and wind direction. The measurements were taken at the different sampling (IES-1, IES -2, IES-3 and IES-4) stations. The measurements of the various meteorological parameters were carried out using *in situ* portable pieces of equipment as listed below.

2.2.2 Gaseous Emission Data Acquisition

Air quality studies were also carried out. The levels of gaseous emissions were determined at the 4 stations in the study area during low and high tides and its spatial boundaries in the upwind and downwind directions. The equipment used were highly sensitive digital meters held at arm's length of the body.

The parameters determined and equipment used were: Sulphur dioxide (SO_2) – (SO_2 gas monitor Gasman model 19648H); Nitrogen dioxide (NO_2) – (NO_2 gas monitor, Gasman); Hydrogen sulphide (H_2S) – (H_2S gas monitor, Gasman); Carbon monoxide (CO) – (CO gas monitor, Gasman); Chlorine – Cl_2 gas monitor, Gasman model 19812H; Hydrogen cyanide – (HCN gas monitor, Gasman model: 19772); Suspended Particulate Matter (Haz – Dust TM 10µg/m^3 Particulate monitor); Volatile Organic Carbon (VOC) – A multi RAE Plus (PGM – 50); and Radiation – (Radiation alert (R) Monitor 4). All gaseous concentrations were recorded in parts per million (ppm).

Air quality was examined at sampling stations in the onward and leeward directions at a distance of 1.5m above ground level. Highly sensitive digital portable meters were used for the measurements of NO_2, SO_2, H_2S, Ci_2, HCN, NH_3, SPM and CO.

2.3 Bio-aerosol Analysis of the Estuarine Environment

The microbiological air quality at the study stations was determined using settle plate culture technique, also known as sedimentation technique. This is based on deposition of viable particle on the surface of a solid medium per a given exposure time, as proposed by APHA (18). The numbers of aerobic count (mesophilic aerobic bacteria), total coliform, faecal coliform, *Staphylococcus aureus* and fungi (yeast and molds) in the atmosphere were determined using Nutrient agar (NA), MacConkey agar (MA), Eosine Methylene Blue agar (EMB), Staphylococcus medium (SM), Pseudomonas medium (PM) Starch Nitrate Agar and Sabouraud Dextrose Agar (SDA) as analytical media respectively (18). The media were fortified with 50µg/ml of streptomycin and 100µg/ml cycloheximide-50µg/ml benomyl respectively for the selective enumeration and isolation of fungi and bacteria.

The media were aseptically prepared in Postgraduate (PG) Laboratory of the Department of Microbiology, University of Uyo and contained in sterile Petri dishes before transportation to the study sites for air analysis.

For the settling technique, open 9cm diameter Petri dishes containing 20ml of appropriate culture media (NA, MCA, EMB, SM, SNA and SDA) were distributed at each sample station using 4 ft high wooden platforms and exposed for 15 minutes.

At the end of exposure, the Petri dishes were closed, transported to the laboratory and then incubated at 28ºC for 2days for aerobic bacteria, coliforms, *Staphylococcus aureus* and *E.coli*, and at 28 ± 2 ºC (room temperature) for 4 days for fungi. After incubation the colonies on culture plates were separately counted with the aid of a Quebec colony counter and the results recorded as cfu/15 minutes (19).

The numbers of microorganisms in the estuarine atmosphere expressed as CFU/m3 were estimated according to Polish standard PN89/2-04088/08 (20) as:

$CFU/m^3 = a.1000/pt \times 0.2$ - - - - - Equation 1

Where,

a= number of colonies on the Petri dish

p= surface measurement of the Petri dish used

t= time of Petri dish exposure

The colonies obtained from the samples were characterized using standard procedure as described by *Bergey's Manual of Determinative Bacteriology* (21). 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 (22).

2.4 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 (23).

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

RESULTS

3.1 Microbial Diversity of Iko River Estuarine Atmosphere

The estuarine atmosphere had 12 bacterial (Table 2) and 15 fungal (Table 3) isolates. Proportionately more fungal isolates were found in the estuarine air. The occurrence and distribution of the isolates however varied with the locations. *Nocardia* sp and *Pseudomonas aeruginosa* were the most occurring bacterial isolates (100%) while *Aspergillus fumigates* was the most occurring fungal isolate with occurrence rate of 62.5%.

Table 2: Occurrence and distribution of the diverse species of bacteria in the estuarine atmosphere 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	Occurrence rate (%)
Micrococcus sp	-	+	+	-	-	+	-	-	37.5
Bacillus subtilis	+	-	+	-	+	-	-	+	50.0
Bacillus cereus	-	-	-	-	+	-	-	-	12.5
Streptococcus sp	+	-	+	+	+	-	+	-	62.5
Staphylococcus aureus	+	+	-	+	+	-	+	+	75.0
Citrobacter sp	-	+	+	+	+	+	-	-	62.5
Enterobacter sp	+	-	+	-	-	-	-	+	37.5
Staphylococcus albus	-	+	+	-	+	+	-	+	62.5
Nocardia sp	+	+	+	+	+	+	+	+	100.0
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	100.0
Serratia sp	-	+	_	+	-	-	-	-	25.0
Klebsiella sp	-	-	-	-	+	-	+	-	25.0
Species Richness (12)	6	7	8	6	9	5	5	6	

Key: IES - Iko Estuary Station

Table 3: Occurrence and distribution of the diverse species of fungi in the estuarine atmosphere 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	Occurrence rate (%)
Aspergillus flavus	-	+	-	+	-	-	+	+	50.0
Aspergillus niger	+	+	-	-	+	-	-	-	37.5
Aspergillus fumigates	+	-	-	+	+	+	-	+	62.5
Aspergillus terrus	-	-	+	+	-	-	+	-	37.5
Rhizopus stolonifer	-	+	+	-	+	-	-	+	50.0
Penicillium expansum	-	-	+	-	+	-	+	-	37.5
Candida albicans	-	-	-	-	-	-	+	-	12.5
Candida tropicalis	-	+	+	-	-	-	-	-	25.0
Eurotium sp	+	+	-	-	-	-	-	-	2.5.0
Absidia sp	-	-	-	-	-	+	-	+	25.0
Mucor sp	+	-	+	-	+	+	-	-	50.0
Cladosporum sp	+	-	-	-	-	-	-	-	12.5
Verticillium sp	-	+	_	-	-	-	-	-	12.5
Fusarium sp	-	-	-	+	-	-	+	-	25.0
Trichoderma sp	-	+	-	-	-	+	-	-	25.0
Species Richness	5	7	5	4	5	4	5	4	

Key: IES - Iko Estuary Station

3.2 Air quality, Meteorology and Noise Levels in Iko Estuarine Environment during Low and High Tides

The results of the air quality attributes of Iko Estuary during low tide (Table 4) and high tide (Table 5) showed that the quality of air in the estuarine environment was affected. The mean values of the air attributes during low tide and high tides were respectively 0.15±0.05 and 0.10±0.00 ppm for NO_2, 0.28±0.08 and 0.15±0.05 ppm for SO_2, 0.38±0.04 and 0.38±0.08 ppm for H_2S, 13.0±3.24 and 11.75±2.17 ppm for CO, 2.13±0.74 and 3.75±1.30 ppm for NH_3, 0.28±0.04 and 0.25±0.11 ppm for Cl_2, 0.15±0.05 and 0.1±0.00 ppm for SPM and 0.75±1.09 and -1.05±0.09 ppm for HCN. The values obtained varied between low and high tides. VOC was not detected in the atmosphere of most station except at station IES-3 during the high tide. H_2S, CO and NH_2 values were remarkable showing that the estuarine air was unwholesome. The results also showed that the values NH_3 were remarkably high. However, the levels of NO_2, SO_2 and HCN were within the permissible limits.

The results also showed that mean temperature, relative humidity (RH), pressure and wind speed were 24.13±0.65 ^{O}C, 74.50±2.03%, 738.75±0.25 mmHg and 0.48±0,04 m/s and 24.63±0.41^{O}C, 69.03±1.87%, 738.75±0.56mmHg, and 0.68±0.20 m/s respectively during the low and high tides. Slightly higher values of noise were recorded in the estuarine environ during high tide. The noise level measured in decibel (dB) revealed a mean minimum value of 51.28±8.15 dB and 57.35±3.28 dB during low and high tides respectively and maximum of 88.23±3.03 dB and 88.98±3.35 dB during low and high tides respectively.

Table 4: Air quality, meteorology and noise levels in iko estuarine environment during low tide

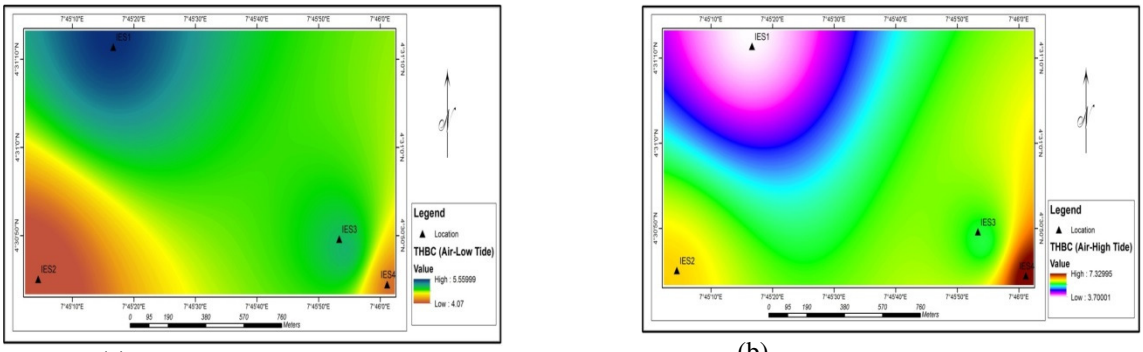
Parameter	IES-1	IES-2	IES-3	IES-4	Mean	SD	WHO limit
NO_2 (ppm)	0.1	0.2	0.1	0.2	0.15	0.05	200µg/m^3/hr
SO_2 (ppm)	0.2	0.4	0.2	0.3	0.28	0.08	350µg/m^3
H_2S (ppm)	0.3	0.4	0.4	0.4	0.38	0.04	
CO (ppm)	9.0	13	18.0	12.0	13.0	3.24	10 µg/m^3
VOC (ppm)	-	-	-	-	-	-	
NH_3 (ppm)	1.0	2.5	2.0	3.0	2.13	0.74	
Cl_2 (ppm)	0.2	0.3	0.3	0.3	0.28	0.04	
SPM (ppm)	0.1	0.2	0.2	0.1	0.15	0.05	50 µg/m^3
HCN (ppm)	-1.0	2.0	1.0	1.0	0.75	1.09	
Temperature (^{o}C)	23.5	25.0	23.5	24.5	24.13	0.65	
RH (%)	76	77.0	72.5	72.5	74.50	2.03	
Pressure (mmHg)	738.5	739	739	738.5	738.75	0.25	
Wind Speed (m/s)	0.5	0.4	0.5	0.5	0.48	0.04	
Noise (dB (A)) Min.	53.1	37.7	55.0	59.3	51.28	8.15	
Max.	84.9	85.5	91.4	91.1	88.23	3.03	

Table 5: Air quality, meteorology and noise levels in iko estuarine environment during high tide

Parameter	IES-1	IES-2	IES-3	IES-4	Mean	SD	WHO limit
NO_2 (ppm)	0.1	0.1	0.1	0.1	0.10	0.00	200µg/m^3/hr
SO_2 (ppm)	0.1	0.2	0.1	0.2	0.15	0.05	350µg/m^3
H_2S (ppm)	0.4	0.3	0.5	0.3	0.38	0.08	
CO (ppm)	12.0	9.0	11.0	15.0	11.75	2.17	10 µg/m^3
VOC (ppm)	-	-	0.04	-	-	-	
NH_3 (ppm)	5.0	2.0	5.0	3.0	3.75	1.30	
Cl_2 (ppm)	0.4	0.1	0.2	0.3	0.25	0.11	
SPM (ppm)	0.1	0.1	0.1	0.1	0.1	0.00	50 µg/m^3
HCN (ppm)	-1.0	-1.2	-1.0	-1.0	-1.05	0.09	
Temperature (^{o}C)	24.5	25.0	25.0	24.0	24.63	0.41	
RH (%)	67.5	67.0	70.0	71.6	69.03	1.87	
Pressure (mmHg)	739.50	738.51	739	738	738.75	0.56	
Wind Speed (m/s)	0.5	1.0	0.7	0.5	0.68	0.20	
Noise (dB(A)) Min.	56.5	55.5	62.9	54.5	57.35	3.28	
Max.	89.6	94.1	87.0	85.2	88.98	3.35	

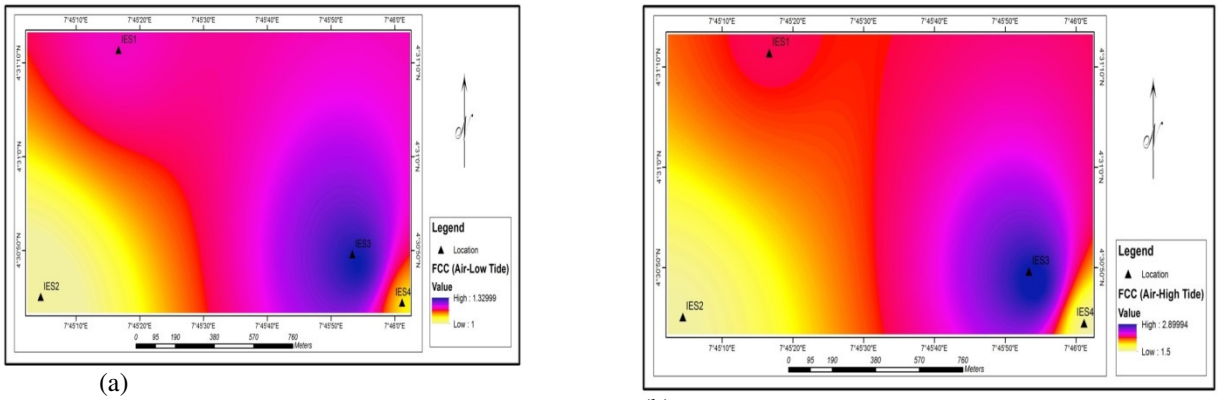
3.3 Spatial Variations in the Microbial Loads of the Estuarine Atmosphere

GIS model of spatial distribution of heterotrophic bacteria in the estuarine atmosphere during the low and high tides is presented in Figure 2. The results show high (blue band) heterotrophic bacterial concentrations in the North-West of the estuarine environment, while the yellowish brown band signifies lower bacterial concentrations. During high tide, the orange brown band shows high heterotrophic bacterial concentrations in the North-East of the estuarine environment, while the pink band signifies lower bacterial concentrations in the North East of the estuarine environment. Analysis also showed high concentrations (blue band of fecal coliform in the North East zones of the atmosphere during the low and high tides (Figure 3), while fungal loads (whitish brown band) were evenly distributed in the estuarine atmosphere during low tide but higher (bluish pink band) in the North West zone during the high tide (Figure 4).



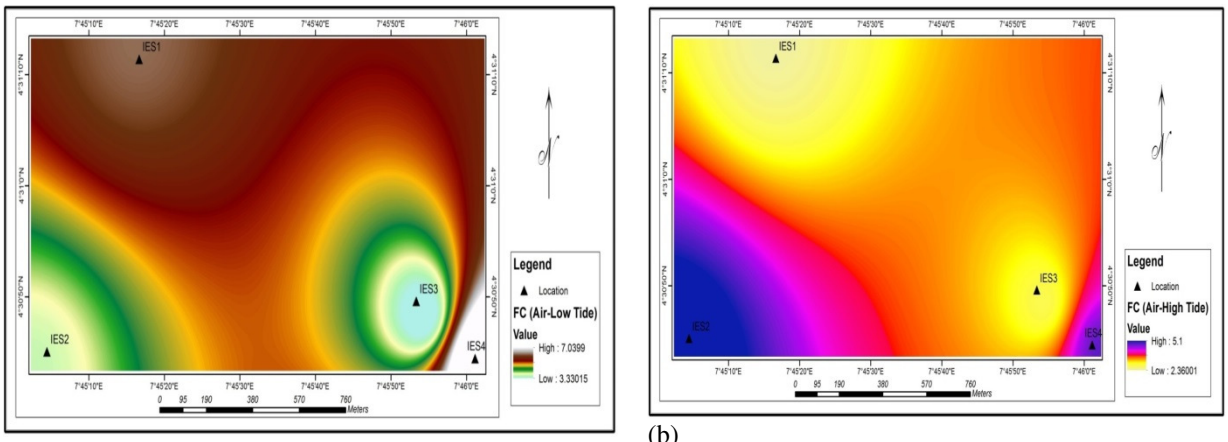
(a) (b)

Figure 2: Spatial distribution of heterotrophic bacteria in air during (a) low tide and (b) high tide



(a) (b)

Figure 3: Spatial distribution of faecal coliform bacteria in air during (a) low tide and (b) high tide



(a) (b)

Figure 4**:** Spatial distribution of fungi in air during (a) low tide and (b) high tide

4.0 Discussion

Diverse population of microorganisms inhabits the natural environments. These include a wide range of physiological and nutritional types (24). Findings from this study on the abundance and distribution of microbial communities in the Iko River estuarine atmospher 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 environment.

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 (8, 13). 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 (25) 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 (18) standard (30 cfu/15 mins) for settling technique although the estimated values per volume of air were below values reported elsewhere. Sullivan (26) 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 (27).

In this study more microbes were encountered in air during high tide than low tide and this may attribute to the upwelling effect of tidal currents and the associated waves. Bioaerosols are airborne particles, solid or liquid. They can be large molecules or volatile compounds. They contain living organisms. They will vary in size from a fraction of a micron to around 100 microns. As with inert "dust" particles all bioerosols are governed by the laws of gravity, and will be affected by air movements being transported by turbulence and diffusion. This could forcefully discharge microbial cells especially fungal spores from entrapped conditions. In addition to APHA (18), there are other recommendations for microbiological counts in the air of tourist and food processing areas. Kang and Frank (1989) recommended 180-360 cfu/m^3 for mesophilic aerobic bacteria and 70-430 cfu/m^3 for yeasts and molds. Higher values were recorded in the present study. This was expected because the area proximity to enormous vegetation and apparently poor sanitary condition of the nearby riparian forest.

The mean numbers of mesophilic aerobic bacteria and fungi as well as that of the indicator bacteria in the atmosphere of the estuarine environment shows that the fungi were more abundant. This may be attributed to the aerodynamic behavior of the fungal aerosols, affecting the deposition of yeasts and molds on solid media surface exposed to air. The aerodynamic behavior of theses aerosols is different for each microbial group and is influenced by their physical and biological characteristics, diameter of the particle, humidity, temperature, ventilation and human activities in the study area as well as the gravitational and electrostatics forces (28). For example, particles with diameter equal or higher than 10mm are able to move vertically between 30 and 60 cm per minute while particles of lower diameter take longer to move the same distance if there are no interferences from other factors,

such as ventilation and personnel activity at site (26). Similarly, the spore dimensions influence the deposition on surfaces. Continuous dumping of wastes at the studied site would certainly affect the initial sources of the bio-contaminant (plants and soil) and distort their distribution dynamics leading to higher number of bio-aerosols in the air.

Air will often contain micro-organisms such as viruses, bacteria, and fungi. None of these actually live in the air, the atmosphere tends to kill off most of them. However, they are frequently transported attached to other particles, such as skin flakes, soil, dust, or dried residues from water droplets. Aggregation of cells into clumps can enhance the survival whilst airborne. Bacterial cells when they become airborne normally rapidly die – within a few seconds, due to evaporation of water associated with the particle. Thus with higher humidity higher bioaerosol levels can prevail. Airborne fungal cells (yeasts, moulds, spores) can remain viable for much longer periods, even at low relative humidity and high or low temperature extremes.

Epidemiological studies have shown that high concentration of microorganisms in the air can be allergenic; however sometimes even very low concentrations of some particulars microorganisms can cause serious diseases (28). Among the microorganisms isolated from the estuarine atmosphere, *Staphylococcus aureus*, *Streptococcus* sp, *Bacillus* sp, *Escherichia coli* and *Pseudomonas aeroginosa* are known to be pathogenic. *Staphylococcus aureus* is normally part of the skin flora. About 20% of the human populations are long-term carriers of *Staphylococcus aureus*. *Staphylococcus aureus* are known to form aggregates in nature, so they tend to give higher colony counts and also because of the possible breaking up of the clusters (29). The bacterium has also been reported on the body of many mammals. *Staphylococcus aureus* is associated with a wide range of illness ranging from minor skin infections such as pimples, impetigo, boils (furuncles), cellutitis, folliculitis, carbuncles, scaled skin syndrome and abscesses to life threatening diseases such as pneumonia, meningitis, oseteomyelitis, endocarditic, toxic shock syndrome (TSS), chest pain and sepsis (30). On the other hand, *Streptococcus pyogenes* is associated with sore throat. *Streptococcus pneumoniae* is associated with Pneumococcal pneumonia pink eye, meningitis, endocarditis and other respiratory tract diseases (30). *Bacillus* endospores have a usual resistance to chemical and physical agents and this makes them predominant in the soil habitat and explains their aerial distribution. Species of *Enterococcus*, e.g. *E. faecalis* can cause endocarditis as well as bladder, prostate and epidermal infections (30). *Escherichia coli* is a Gram negative, facultatively anaerobic, rod-shaped bacterium that is an indicator of recent faecal contamination. Most *E. coli* strains are harmless but some serotypes can cause serious food poisoning in humans and are occasionally responsible for product recalls due to food contamination (31). The presence of this organism in the atmosphere indicates high level of faecal contamination in the environment. However, they are released into the atmosphere when the faecal matter is disturbed (31).

Aspergillosis is the name given to a wide variety of diseases caused by infection by fungi of the genus *Aspergillus*. The majority of cases occur in people with underlying illnesses such as tuberculosis or chronic obstructive pulmonary disease (COPD), but with otherwise healthy immune systems. Most commonly, aspergillosis occurs in the form of chronic pulmonary aspergillosis (CPA), aspergilloma or allergic bronchopulmonary aspergillosis (32). People with deficient immune systems – as patients undergoing hematopoietic stem cell transplantation, chemotherapy for leukaemia, or AIDS – are at risk of more disseminated disease. Acute invasive aspergillosis occurs when the immune system fails to prevent *Aspergillus* spores from entering the bloodstream via the lungs. Without the body mounting an effective immune response, fungal cells are free to disseminate throughout the body and can infect major organs such as the heart and kidneys.

The most frequently encountered fungus was *Aspergillus fumigatus* - a ubiquitous organism that is capable of living under extensive environmental stress. It is estimated that most humans inhale thousands of *Aspergillus* spores daily, but they do not affect most people's health due to effective immune responses (28).

The results of the research have further confirmed that indigenous microflora of an environment developed in spatially organized physicochemical gradients (24). 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. 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 (24). 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.

Variation in distribution of microbial communities noticed may be of serious implications to the existence of microbes in air, it has significant health implications as the information may be used to predict exposures to harmful air contaminants. The spatial distribution of the bio-aerosols revealed that bacteria and fungi were found in high concentrations in the north-west zone of the estuarine environment during both tides, while fungi were highly concentrated in the north-east during high tide. Varnam and Evans (24) reported that variation in distribution in bio-aerosol concentrations are caused by variation in temperature, moisture availability and hours of daylight. They further affirmed that cultivable bacteria are more prevalent in dry season than wet season and in some regions may be influenced by the dry, dusty conditions and associated agricultural or human activities apparent in dry season in contrast to wet conditions with snow cover during the wet season. Typically, a higher environmental temperature, wind speed and relative humidity favour microbial growth (33). This study was carried out during the wet season and accordingly, it is suggested that the wind speed-influenced variation in tidal currents was majorly the factor that causes the spatial distribution difference in the microbial concentrations in the estuarine atmosphere. This finding shows that, although microorganisms are ubiquitous in the environment, their identities and concentrations are not consistent as they fluctuate according to geographical location, climate events, seasons, and human activities (34).

The study area was predominated by Gram positive and Gram negative rods. The atmosphere harboured more diverse species of fungi. The results revealed diverse microbial species. Some of the species encountered in this ecosystem have previously been reported by Udotong et al., (35).

The research results have that among the bacterial isolates, *Nocardia* sp and *Pseudomonas aeruginosa* were the most predominant in air with 100% prevalence rate. Among the fungi isolated from the estuarine environment, *Penicillium expansum*, *Aspergillus terrus* and *Aspergillus fumigatus* were the most predominant. These organisms are broadly present in nature, including soil, cereal grains, hay and other plant material or foodstuff (36).

The air quality in the estuarine environment was relatively "clean" and wholesome as most criteria gaseous pollutants (HCN, NO_2, SO_2) except CO and SPM were below detectable limits and within the FMENV and WHO acceptable limits. However, the recorded levels of CO in some parts of the fishing settlement were above Federal Ministry of Environment (FMEnv) limits of 10.0 - 20.0ppm for daily average of 8 hourly values in Nigeria. The levels of atmospheric contaminants varied between low and high tides. The 2.0 ppm level of SPM recorded during high tide is higher than the FMEnv limits of 0.25ppm and is dangerous. It is apparent that there was significant emission of CO from the fish smoking activity which is common in the settlements. Similarly, the study has shown that there is potential risk of contamination with H_2S, NH_3 and HCN although the values were within the FMEnv recommended limits. The concentrations of gaseous pollutants recorded within the estuarine represent background levels or baseline conditions of the area. The low concentrations of gaseous pollutants obtained in the study area during this study may be due to the absence of industrial activities in the area that would generate such materials as well as influence of rains which dilute and dissolve pollutants in the atmosphere.

Maximum Noise level in the Iko river estuary environment was also beyond the permissible limits of 90dB (A) for 8 hourly period at some stations during both tides. The results from this study revealed a range of 37.7 dB - 94.1 dB during both tides. This shows that in some stations the noise values recorded were higher than the threshold limits of 90dB (A) recommended by FMEnv for 8 hourly exposure period and 85dB recommended by WHO, although these values at some stations are below the 44.0dB recommended for Agricultural cropland. According to the EPA protective noise level, it shows that Iko estuary environment was noisy and this can be attributed to human activities in the estuary and activities of the gas plant from the nearby oil and gas industries. However, there was no potential source for noise increases, since the settlement is situated in a large riparian forest.

Many atmospheric factors influence the way air pollution is dispersed, including wind direction and wind speed, type of terrain and heating effects. To better understand how atmosphere processes can affect ground level pollution, atmospheric conditions can be described simply as either stable or unstable, where the stability is determined by wind (which stirs the air) and heating effects (which cause convection currents). Atmospheric stability affects pollution released from ground level and elevated sources differently. The present study has revealed variation in the atmosphere quality of the estuarine environment during low and high tides creating an unstable atmospheric condition. In unstable conditions, ground level pollution is readily dispersed thereby reducing ground level concentrations. Elevated emissions, however, such as those released from a chimney, are returned more readily to ground level, leading to higher ground level concentrations. However in stable Conditions there is less atmospheric mixing and therefore higher concentrations around ground level sources, but better dispersal rates, and therefore lower ground level concentrations, for elevated plumes. Bioaerosols coexist with gaseous substances in the air, in particular volatile organic compounds whether perceived as odorous or not. Synergistic effects could thus result from the presence of bioaerosols or particles (dust or water droplets) in odorous air. Ossowska-Cypryk (37) reported on the current state of knowledge on the subject and indicated that dusts may concentrate certain odorous compounds, for example volatile organic acids or ammonia, which help to exacerbate respiratory irritation caused by

the dusts. However, there is very little known about additive or synergistic effects of odorous volatile organic compounds that are irritants and/or toxic and the various bioaerosols (37).

Poor air quality can also affect vegetation. Sulphur dioxide, one of the main components of acid rain, has direct effects on vegetation. Changes in the physical appearance of vegetation are an indication that the ability of many plants to photosynthesise (generate food from sunlight, water and carbon dioxide) is impaired by the concentration of sulphur dioxide. Harm caused by sulphur dioxide is first noticeable on the leaves of the plants. For some plants injury can occur within hours or days of being exposed to high levels of sulphur dioxide. Leaves in mid-growth are the most vulnerable, while the older and younger leaves are more resistant. Damage to coniferous needles can be seen in the extreme colour difference between the green base and the bright orange-red tips. It may also increase the severity of many fungal diseases.

There was a significant positive correlation ($r = 0.717$, $p < 0.05$) between the total heterotrophic bacteria in the atmosphere and wind speed, and between the fungi in the atmosphere and wind speed ($r = 0.799$) during high tide, indicating that increase in wind speed resulted in a corresponding effect in heterotrophic bacterial and fungal counts during high tide. A comparison of the relation between atmospheric temperature and microbial load showed little or no correlation ($r = 0.30$).

5.0 CONCLUSION

The Iko River estuarine environment 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 environment. 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. This study has revealed that the estuarine air is heavily contaminated with microorganisms. The level and quality of contamination however varied with the sample locations and tidal influences. GIS modeling of the spatial distribution of microbial contaminants in the atmosphere has revealed that both bacteria and fungi exist in high concentrations in the north-west of the study area, while fungal contaminants occurred in high concentrations in the north-east section of the estuarine environment. The accumulation of fungal spores in the air can affect the health status of the estuarine environment inhabitants. The findings revealed that tidal bars and flats in shallow mesotidal estuary are subject to the action of tidal current and waves. These complex events give rise to large variations in microbial communities in estuarine atmosphere which may be harnessed for effective environmental monitoring.

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