BIOAEROSOLS LOAD, SPATIAL DISTRIBUTION AND QUALITY IN THE OUTDOOR AIR ENVIRONMENT OF UYO URBAN IN AKWA IBOM STATE, NIGERIA

ISSN: 2141 – 3290
www.wojast.com

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ABSTRACT

This research was conducted to survey airborne microorganisms (bacteria and fungi), and assess their spatial distribution in the outdoor air of Uyo urban, Akwa Ibom State, Nigeria using the sedimentation technique and Geographic Information System (GIS) and ARC map software respectively. Air quality analysis of the outdoor environment was performed simultaneously using standard air sampling devices. The mean bacterial counts recorded for the urban center were 1.4×10^3 cfu/m^3 and 2.01×10^3 cfu/m^3 during the wet and dry seasons respectively as compared to the mean fungal counts of 9.9×10^2 cfu/m^3 and 1.78×10^3 cfu/m^3 respectively. This revealed higher levels of bacterial contamination in open areas. The bio-aerosol loads of bacteria were generally found to be lower than fungi in residential areas. Although observed only in the dry season sampling, the mean fungal counts also out-numbered the bacterial counts in the secretariat complex atmosphere. These findings have shown that regardless of the season, bacteria were detected in all the outdoor air samples. The prevalent bacterial isolates encountered were *Micrococcus, Staphylococcus, Bacillus* and *Pseudomonas,* while the fungal genera included: *Aspergillus, Candida, Fusarium, Rhizopus* and *Penicillium. Escherichia coli* were encountered in the open market and urban center stations revealing the unsanitary status of the environment. Many of the microorganisms isolated have been implicated in various human ailments and their effects may be curtailed by adopting improved wastes management approaches in Uyo City

INTRODUCTION

Bio-aerosols are particles that are living or originate from living organisms (ACGIH, 1999). They are particles of biological origin suspended in the air and include bacteria, fungi, spores, pollens, and other whole cells and cell fragments (USEPA, 2004). Airborne microorganisms originate from different natural sources such as soil, animals, and humans. Man-made activities such as sewage treatment plants, animal rendering, fermentation processes and agricultural activities emit microorganisms into the air (Abdel-Hameed, 2009). Airborne microorganisms have been shown to vary throughout the day and season depending on various environmental factors such as: type of vegetation, air pollution, human activities, meteorological and seasonal climatic factors and periodicity of the emission sources (Abdel-Hameed, 2009).

Bio-aerosols in indoor and outdoor environments have been found to cause adverse health effects (Douwes *et al*., 2000; Den Boer *et al*., 2002). Airborne bacteria and fungi can be toxigenic, allergenic and/or infectious (Burrell, 1991).

Bio-aerosols are ubiquitous in the atmosphere. Their identities and concentrations are not consistent as they fluctuate according to geographical location, climate events, seasons, and

human activities (Shaughnessy *et al*., 1999). Atmospheric air is less favourable for microbial survival as airborne microorganisms are subjected to certain conditions which can inhibit their survivability. Many parameters are being potentially damaging and lethal for microbial organisms. These include solar radiation, changing temperatures, relative humidity, atmospheric pollution and free radicals (Sinha *et al*., 2000; Maier *et al*., 2000; Levetin and Dorsey, 2006, Karra and Katsivella, 2007 and Mansour *et al*., 2012). Bacteria and most enteric viruses survive longer at high Relative Humidity (RH), such as those occurring during the night. High RH delays droplet evaporation and retards organism die-off. Relative humidity has a relatively low significant effect on airborne bacteria. The bacteria concentration in the atmosphere also increases with increasing RH which is in agreement with other reports (Paya-Vicent and Suarez-Fernandez, 1984). Paya-Vicent and Suarez-Fernandez (1984) also revealed that the concentration of bacteria may increase either with the increase or decrease in RH; because the higher levels of RH favor the viability whereas the lower RH favors the spore release in greater number. Wind velocity also has a very good positive correlation with bio-aerosol concentrations signifying that concentrations increase with increasing wind velocity while low wind speeds reduce biological aerosol transmission (Paya-Vicent and Suarez-Fernandez, 1984, Levetin and Dorsey, 2006, Karra and Katsivella, 2007; Mansour *et al*. 2012).

It has been reported that bio-aerosol concentrations are more prevalent in dry season than wet season. Di-Giorgio, Krempff, Guiraud, Binder, Tiret and Dumenil (1996) observed that seasonal patterns in bio-aerosol concentrations are influenced by temperature, moisture availability and hours of daylight. The same authors affirmed that cultivable bacteria are more prevalent in dry season than wet season in some regions. This they attributed to the dry, dusty conditions of the dry season and associated agricultural or human activities during the period. This study aims at evaluating the bio-aerosols load, spatial distribution and quality in the outdoor air environment of Uyo urban in Akwa Ibom State, Nigeria.

METHODOLOGY

Study Area

The study area, Uyo is the capital city of Akwa Ibom State in Nigeria. It is located in equatorial West Africa, which comprises the region lying between latitude 5^0 North of the equator and longitude 8^o on the Atlantic Coast of Africa. Tropical wet and semi-hot equatorial climate with high solar radiation that is mostly diffused due to cloud cover heavy precipitation, light winds and low atmospheric pressure are the major climatic characteristics of the study area. The area falls into the Equatorial Monsoon (Udosen, 2006). Although temperatures are moderated by the cloud cover and by the generally damp air, mean annual temperatures are as high as 24^0C-32^0C with little variation in monthly means. The lowest monthly temperatures (25-26^0C) are recorded in the rainy season months of June to September while the highest temperatures (27 - 28.5^0C) are recorded in February and March. Rain falls every month of the year with a short dry spell in the months of January to March in some parts. Highest temperatures are observed between March and April and lowest between July and September.

A total of 30 samples were collected from five locations namely Urban Center, Housing Estate, Local Residence, Open Market and Secretariat Complex (Fig. 1) for bio-aerosols analysis during the wet and dry seasons.

Analysis of Bio-aerosols Load

Sedimentation or settle plate method described by Downes and Ito (2001) was adopted. In this technique, pre-sterilized Petri dishes containing the appropriate media were left open and exposed in the open for 20 minutes on 4ft high wooden platforms.

To estimate the densities of bacteria, fungi and enteric bacteria, Nutrient Agar (NA), Saboraud Chloramphenicol Agar (SCA) and MacConkey Agar (MA) respectively, were aseptically prepared and used as the analytical media. After exposure, NA and MA plates were incubated

at 37°C for 48 hours while the SCA plates were incubated at room temperature (28± 2°C) for 96 hours. After the incubation, the colonies on culture plates were separately counted and recorded (Pasquarella *et al*., 2000).

Fig. 1: Street Map of Uyo-Urban showing the sample locations

The number of microorganisms in the atmosphere expressed as CFU/m^3 was estimated according to Polish standard PN89/2-04088/08 (Friberg *et al*., 1999) as:

$$cfu/m^3 = \frac{a \times 1000}{P \times t \times 0.2}$$

a = the number of colonies on the Petri plate
p = the surface measurement of the of the plate used
t = the time of exposure of the Petri plate.

Determination of Spatial Variations in the Bio-aerosol Loads of the Sample Locations

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 (Kunzli *et al*., 2005).

Characterization of Microbial Isolates

The different bacterial and fungal isolates obtained from the primary plates were aseptically sub-cultured by streaking onto freshly prepared Nutrient Agar (NA) and Saboraud's Dextrose Agar (SDA) plates respectively. To derive pure microbial isolates, the inoculated NA plates were incubated at 37°C±2°C for 24 hours while the SDA plates were incubated at 28± 2°C for 96 hours. The pure culture of the isolates were streaked on prepared sterile set agar slant in stock bottles and kept in the refrigerator at 4°C to 6°C for identification.

The pure bacterial isolates were grouped into recognizable taxonomic units and characterized to their generic level using standard procedures. The isolates were examined for colonial morphology, cultural; Gram's staining reaction and biochemical characteristics according to the methods of MacFadden (1980) and Cowan (1985). Similarly the pure fungal isolates were characterized to their generic level according to the taxonomic schemes of Cowan (1985), Domsch *et al.* (1980), Samson *et al* (1984), and Barnett and Hunter (1987). Identification was based on morphological characteristics of the colony including colony diameter, colour, exudate and colony texture, which is primarily used to establish the genera.

RESULTS AND DISCUSSION

The results of the bio-aerosol loads of the outdoor locations analyzed are presented in Table 1. The mean bacterial counts recorded for the urban centre were 1.4×10^3 cfu/m^3 and 2.01×10^3 cfu/m^3 during the wet and dry seasons respectively as compared to the mean fungal counts of 9.9×10^3 cfu/m^3 (wet) and 1.78×10^3 cfu/m^3(dry). The bio-aerosol loads of bacteria were generally found to be lower than fungi in residential areas such as the Housing Estate and Local Residential Units (Figures 2 and 3). Although observed only in the dry season sampling, the mean fungal counts also out-numbered the bacterial counts in the Secretariat Complex atmosphere. These findings have shown that regardless the season; bacteria were detected in all the outdoor air samples. However, their densities and occurrence in all the urban centre and open market sample stations were significantly higher than that of fungi. This finding is in agreement with the observations by Pastuszka *et al*. (2000). Pastuszka *et al*. (2000) 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 (Jones and Harrison, 2004).

Table 1: Mean viable counts of microorganisms encountered in outdoor ambient air of Uyo urban

Sampling Location	Coordinates of Sampling Location		Bacterial Counts (CFU/m^3)		Fungal Counts (CFU/m^3)	
	Northing	Easting	Wet	Dry	Wet	Dry
Urban Center (UC)	05°02^{I}04.4^{II}	007°59^{I}41.9^{II}	1.39×10^3	2.02×10^3	9.94×10^2	1.78×10^3
Housing Estate (HE)	05°00^{I}50.3^{II}	007°57^{I}02.2^{II}	4.82×10^2	9.64×10^2	1.05×10^3	1.27×10^3
Local Residence (LR)	05°01^{I}33.2^{II}	007°55^{I}33.0^{II}	4.22×10^2	1.48×10^3	7.23×10^2	1.54×10^3
Open Market (OM)	05°01^{I}01.7^{II}	007°55^{I}27.4^{II}	1.39×10^3	2.47×10^3	1.32×10^3	2.05×10^3
Secretariat Complex (SC)	05°01^{I}20.0^{II}	007°54^{I}21.2^{II}	7.23×10^2	1.02×10^3	6.93×10^2	1.08×10^3

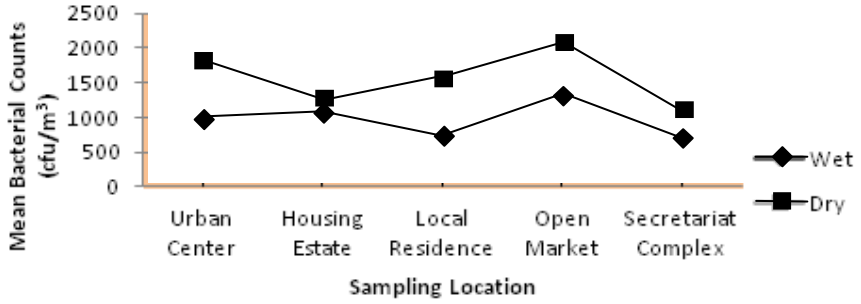

Fig. 2: Variation in bacteria loads in the outdoor stations

Fig. 3: Variation in fungal loads in the outdoor stations

The spatial distribution of the bio-aerosols illustrated in Figures 4 - 7 revealed that bacteria and fungi were found in high concentrations in the north-west of the study area in both seasons, while fungi were highly concentrated in the north-east during wet season.

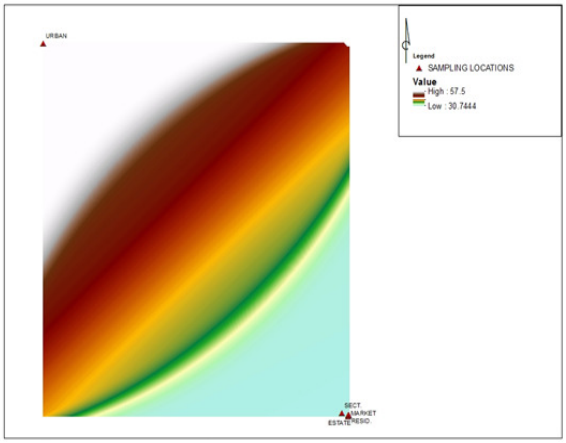

Figure 4: Spatial Distribution of Bacteria during the Wet Season

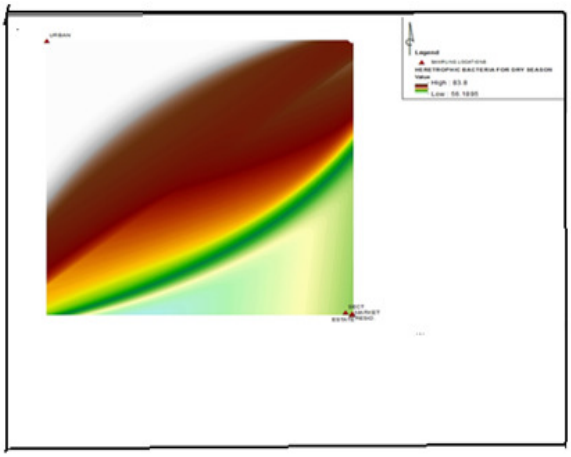

Figure 5: Spatial Distribution of Bacteria during the dry season

Fig. 4: GIS model of Spatial Distribution of bacteria during wet season: The North-West point of the study area shows high spatial concentration of bacteria during wet season. The white-brown colour is portraying high bacterial concentrations, while sky blue-green colour in the North-East location indicates lower concentrations of bacteria.

Fig. 5: GIS model of Spatial Distribution of Bacteria during the dry season: The brown-white colour shows high bacterial concentrations in the North-West of Uyo urban area, while the green-blue colour band signifies lower bacterial concentrations. The middle light brown colour band shows moderate bacterial load.

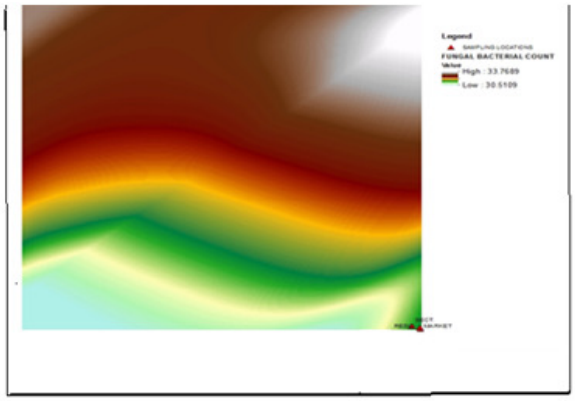

Figure 6: Spatial Distribution of Fungi during the wet season

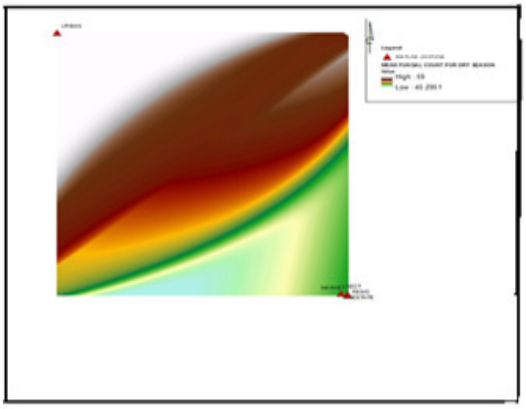

Figure 7: Spatial distribution of fungi during the dry season

Fig. 6: GIS model of Spatial Distribution of Fungi during the wet season: The fungal isolates are highly concentrated in the north eastern part of Uyo urban. The white-brown colour band up to the middle part of the model portrays high fungal concentrations. The light brown area of the model shows moderate fungal concentrations. The green-blue colour bands down south shows lower concentrations.

Fig. 7: GIS model of Spatial Distribution of Fungi during the dry season: The fungal distribution concentrations tilt towards North-West of the study location. The white-brown colour bands indicate high fungal concentrations. The area with light brown shows moderate concentrations, while green and sky blue bands indicate lower fungal concentrations.

Di-Giorgio, *et al,* (1996) have reported that seasonal patterns 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 and relative humidity favour microbial growth (Ren *et al.,* 2001). Accordingly, it is suggested that the temperature and relative humidity were important factors causing the seasonal difference in the microbial concentrations in the Uyo urban atmosphere.

Five bacterial genera were constantly found in the outdoor air including *Staphylococcus*, *Proteus*, *Bacillus*, *Escherischia*, *Micrococcus*; others were *Pediococcus*, *Pseudomonas*, *Serratia*, *Salmonella*, and *Shigella*. On the other hand, the prevalent fungal genera encountered were *Aspergillus, Fusarium, Geotricum, Verticillium, Cladosporium and Penicillium*. Others including *Absidia, Cephalosporium, Epicoccum, Botrytis, Moniliella, Eurotium,Diplodia, Rhizopus,Phoma, Candida, Alternaria, Scopulariopsis, Pichia, Trichoderma, Sacchromyces, Monilia*, and *Humicola* were present in very low numbers and varied according to locations and seasons.

The health significance of fungal isolates present has been reported Pasquarella, *et al*, (2000) and Burrel, (1991) and many of the isolates are associated with various human ailments: aspergillosis, cryptococcosis, histoplasmosis, blastomycosis, coccidioidomycosis, respiratory irritation, allergic reactions, etc. The isolates of *Escherischia coli* from the local residential centres and open market stations is an indicator that the atmosphere is contaminated with fecal matter (Pasquarella, *et al*, (2000). Airborne transmission of viable microorganisms is an important mode of disease transfer. Tuberculosis, acute respiratory diseases, all transmitted by viable bio aerosols, are the leading causes of death in the developing world (De-Evgrafov, 2002). Legionnaire's disease, pneumonia, and gastrointestinal diseases are also transmitted through the air, and comprise some of the most recognized pathogenic bio-aerosols.

Table 2: Distribution and Prevalence of Microbes in Outdoor Ambient Air of Uyo Urban during the Wet Season

Isolates	Sampling Location					% Prevalence
	UC	HE	LR	OM	SC	
Bacteria:						
Micrococcus nishinomiyaensi	-	-	+	-	-	20
Escherischia coli	-	-	+	+	-	40
Serratia marcescens	-	-	-	+	-	20
Bacillus subtilis	+	+	+	+	-	80
Staphylococcus aureus	+	-	+	+	-	60
Staphylococcus saprophyticu	*s*+	+	-	-	+	60
Shigella dysenteriae	-	-	-	+	-	20
Proteus vulgaris	-	-	+	+	-	40
Salmonella indica	-	-	+	-	-	20
Pediococcus acidilactici	-	-	+	-	-	20
Staphylococcus albus	+	-	+	+	+	80
Fungi:						
Aspergillus glaucus	+	+	+	+	+	100
Geotricum sp.	+	-	-	+	-	40
Verticillium sp.	-	+	+	+	-	60
Pichia sp.	-	+	-	-	-	20
Candida tropicalis	-	+	-	-	-	20
Phoma sorghina	-	+	-	-	-	20
Fusarium sp.	+	-	+	-	-	40
Aspergillus niger	+	-	-	+	-	40
Absidia sp.	+	-	-	-	+	40
Cladosporium carrion	+	+	+	-	-	60
Candida albicans	-	+	-	-	-	20

Rhizopus oligosporus	+	-	-	-	-	20
Alterneria alternate	-	+	-	-	-	20
Diplodia seriata	-	+	-	+	+	60
Aspergillus flavus	-	-	-	+	-	20
Botrytis cinerea	-	+	+	-	+	60
Epicoccum nigrum	+	+	-	-	+	60
Aspergillus fumigates	-	-	-	-	+	20
Eurotium sp.	+	-	+	+	-	60
Penicillium expansum	+	-	-	+	-	40
Humicola sp.	-	+	-	-	-	20
Cephalosporium sp.	+	-	+	-	-	40
Aspergillus clavatus	-	-	-	-	+	20
Sacchromyces cerevisiae	-	+	-	-	-	20
Scopulariopsis sp.	-	+	-	-	-	20
Penicillium italicum	-	+	-	+	-	40
Penicillium nalgiovense	-	-	-	-	+	20

Table 3: Distribution and Prevalence of Microbes in Outdoor Ambient Air of Uyo Urban during the Dry Season

Isolates	Sample Location					% Prevalence
	UC	HE	LR	OM	SC	
Bacteria:						
Staphylococcus saprophyticus	+	-	+	+	-	60
Pseudomonas aeruginosa	+	-	+	+	-	60
Staphylococcus albus	-	+	+	-	-	40
Salmonella indica	-	-	+	+	-	40
Proteus vulgaris	-	-	+	+	-	40
Bacillus subtilis	+	-	+	+	+	8
Escherischia coli	-	-	+	+	-	40
Staphylococcus aureus	+	+	+	+	-	80
Micrococcus roseus	+	-	-	+	-	40
Shigella dysenteriae	-	-	+	+	-	40
Fungi:						
Moniliella acetoabutens	-	-	+	+	+	60
Trichoderma viride	-	-	+	-	-	20
Penicillium italicum	-	-	-	+	+	40
Scopulariopsis sp.	-	+	-	-	+	40
Penicillium nalgiovense	-	-	-	+	+	40
Aspergillus clavatus	+	+	-	+	+	80
Monilia sp.	-	-	-	-	+	20
Eurotium sp.	+	-	-	-	+	40
Aspergillus fumigates	+	+	-	+	-	60
Diplodia seriata	-	-	-	+	-	20
Epicoccum nigrum	+	-	-	-	+	40
Aspergillus flavus	+	+	-	-	+	60
Alterneria alternate	-	-	+	+	+	60
Botrytis cinerea	+	-	-	-	-	20
Aspergillus niger	+	+	+	+	-	80
Candida albicans	-	+	+	-	-	40
Cladosporium carrionil	-	-	-	+	-	20
Absidia sp.	+	-	-	-	-	20
Fusarium sp.	-	+	-	+	+	60
Candida tropicalis	+	-	-	+	-	40
Geotricum sp.	-	-	+	-	+	40
Aspergillus glaucus	+	-	+	+	-	60

\+ Isolated, - Not isolated

CONCLUSION AND RECOMMENDATION

This study has revealed that the outdoor ambient air in Uyo Urban is laden with microbial contaminants. The level and quality of contamination however varied with the sample locations and season. 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 contaminant s occurred in high concentrations in the north-East section of Uyo Urban during wet season. The high incidence of the microbial isolates may be associated with indiscriminate disposal of solid wastes especially domestic one in the city centers and public drains. Improved waste management approaches may help in curtailing the discharge of microbial contaminants into the atmosphere.

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