International Research Journal of Microbiology (IRJM) (ISSN: 2141-5463) Vol. 6(2) pp. 012-019, August, 2015
Available online http://www.interesjournals.org/IRJM
DOI: http:/dx.doi.org/10.14303/irjm.2015.132

Full Length Research Paper

Prevalence of bio-aerosols in the outdoor air environment in Uyo Urban, Akwa Ibom state, Nigeria

[1*]Ambrose, I., [2]Nweke, C. O., [3]Umeh, S. C. I. and [4]Braide, W.

Department of Microbiology, Federal University of Technology, Owerri, Imo State
*Corresponding author's E-mail: Ukosco2014@gmail.com

ABSTRACT

Outdoor bio-aerosols quality in Uyo urban was evaluated for their prevalence using settle plate (culture-based) method. Five (5) locations were established for wet and dry season sampling which included Urban Center, Housing Estate, Local Residence, Open Market and Secretariat complex. Mean viable plate counts of heterotrophic bacteria ranged between 1.386 × 10^3 (wet season) and 2.018 × 10^3 (dry season) in Urban Center; 4.82 × 10^2 (wet season) and 9.64 × 10^2 (dry season) in Housing Estate; 4.22 × 10^2 (wet) and 1.476 × 10^3 (dry) in Local Residence; 1.386 × 10^3 (wet) and 2.470 × 10^3 (dry) in Open Market, and 7.23 × 10^2 (wet) and 1.024 × 10^3 (dry) in Secretariat Complex while the mean viable plate counts of heterotrophic fungi ranged between 9.94 × 10^2 (wet) and 1.777 × 10^3 (dry) in Urban Center; 1.054 × 10^3 (wet) and 1.265 × 10^3 (dry) in Housing Estate; 7.23 × 10^2 (wet) and 1.536 × 10^3 (dry) in Local Residence; 1.325 × 10^3 (wet) and 2.048 × 10^3 in Open Market, and 6.93 × 10^2 (wet) and 1.084 × 10^3 in Secretariat Complex. Bacteria isolated were *Micrococcus nishinomiyaensi, Staphylococcus aureus, Escherischia coli, Bacillus subtilis, Serratia marcescens, Staphylococcus saprophyticus, Shigella dysenteriae, Proteus vulgaris, Salmonella indica, Pediococcus acidilactici, Staphylococcus albus, Pseudomonas aeruginosa, Micrococcus roseus.* The fungi isolated included *Aspergillus glaucus, Geotricum sp., Verticillium sp., Pichia sp., Candida tropicalis, Phoma sorghina, Fusarium sp., Aspergillus niger, Absidia sp., Cladosporium carrionil, Candida albicans, Rhizopus oligosporus, Alterneria alternate, Botrytis cinerea, Aspergillus flavus, Epicoccum nigrum, Diplodia seriata, Aspergillus fumigates, Eurotium sp., Penicillium expansum, Sacchromyces cerevisiae, Monilia sp., Humicola sp., Cephalosporium sp., Aspergillus clavatus, Scopulariopsis sp., Penicillium italicum, Penicillium nalgiovense, Trichoderma viride* and *Moniliella acetoabutens. Bacillus subtilis* showed 80% prevalence in both wet and dry seasons. The only fungal isolate that showed 100% prevalence during wet season was *Aspergillus glaucus*. 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 Bio-aerosol concentration was found to be high in dry season than wet season. This is because of increase in suspended particulate matter in atmospheric air. The present study has provided evidence of microbial load in outdoor ambient air at different locations.

Keywords: Percentage prevalence, Bio-aerosol, outdoor, air environment.

INTRODUCTION

Bio-aerosols are defined as airborne particles of biological origin. Bio-aerosols include airborne bacteria, viruses, fungi, and other biological fragments such as airborne DNA fragments. Bio-aerosols were thought to exist in only minute concentrations in atmospheric aerosols. However, Jaenicke (2005) showed that the portion of bio-aerosols constituting ambient aerosols ranges from 5% to 80%.

Bio-aerosols play roles in atmospheric chemistry and physics by altering the chemistry of the atmosphere via microbiological degradation, modifying the chemical composition of other organic compounds upon collision or

contact, and driving the chemistry at environmental interfaces such as the air/snow interface (Ariya and Amyot, 2004; Lee, 2011). With this increased awareness of the roles of bio-aerosols in atmospheric components, bio-aerosols were originally notable due to their effects on health. Bio-aerosols have characteristics as biological materials, such as bacteria, fungi, viruses, plant debris, and so on (Lee, 2011).

Airborne microorganisms 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 favorable 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 such as 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). 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).

Temperature is a significant variation factor for airborne bacteria, which governs the rate of change of water vapor and the rate of change of heat between the surface and environment. It also affects the viability of airborne bacteria through the evaporation of their cellular water. Increased temperature can also reduce the viability of organisms in aerosols, mainly by accentuating the effects of relative humidity. Pronounced temperature effects do not appear until a temperature of 80°F (27°C) is reached. The release of bacteria will be increased with increasing temperature by reducing the binding force (Savery, 1984). Temperature can both increase and decrease bacterial bio-aerosol concentrations. Increasing temperature has been found to decrease the survival of bacterial bio-aerosols under experimental conditions (Handley and Webster l995).

Bacteria and most enteric viruses survive longer at high relative humidity, such as those occurring during the night. High RH delays droplet evaporation and retards organism die-off. Relative humidity (RH) has a relatively low significant effect on airborne bacteria. The bacteria concentration increases with increasing RH which is in good agreement with other reports (Paya-Vicent and Suarez-Fernandez, 1984). Paya-Vicent and Suarez-Fernandez (1984) revealed that the concentration of bacteria may increase either with the increase or decrease in RH; because the higher percent of RH favors the viability where as the lower percent of RH favors the spore release in greater number. Wind velocity has a very good positive correlation with bio-aerosol concentration signifying that the bio - aerosol concentration will increase with increasing wind velocity. Low wind speeds reduce biological aerosol transmission.

It was found that bio-aerosol concentrations are more prevalent in dry season than wet season. Di-Giorgio et al (1996) reported that seasonal patterns in bio-aerosol concentrations are caused by temperature, moisture availability and hours of daylight. They further affirmed that culturable bacteria are more prevalent in dry season than wet season in some regions due to dry, dusty conditions and associated agricultural or human activities in dry season in contrast to wet conditions with snow cover in wet season.

This study aims at evaluating the prevalence and distribution of bio-aerosols in the outdoor air.

GEOGRAPHICAL SETTING OF THE STUDY AREA

The study area is in equatorial West Africa, which comprises the region lying between latitude 5^0 North of the equator, and between 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 (25^0C) are recorded in the rainy season months of June to September while the highest temperatures (27.0^0C-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 between March and April and lowest between July and September.

A total of 30 samples were collected from 3 types of media exposed in duplicates each at 5 locations in Uyo metropolis, for wet and dry seasons sampling of bio-aerosols. The five locations are Urban Center, Housing Estate, Local Residence, Open Market and Secretariat Complex.

MATERIALS AND METHOD

The different procedures through which data used for this study were collected and analyzed are explained as follows:

Preparation of Media

Three types of media were used to isolate microorganisms from the outdoor air. These were Nutrient Agar (NA), Saboraud Chloramphenicol Agar (SCA) and MacConkey Agar (MA) for the isolation of

Figure 1: The Map Showing Uyo-urban, the Study Area

bacteria, fungi and enteric bacteria respectively.

Isolation of Microorganisms

Sedimentation (or settle plate) method described by Downes and Ito (2001). It is an apparatus-free method. The bio-aerosol was allowed to settle on uncovered Petri dishes in which an appropriate culture medium had been placed. The Petri dishes were left open for 20 minutes and were placed at a height of one (1) meter from the floor and at a distance of one (1) meter from the wall or any object. After exposure, the culture media were incubated according to the type of micro-organisms to be examined. After the incubation, the colonies were counted (Pasquarella *et al*., 2000).

The number of microorganisms expressed as CFU/m^3 was estimated for the Settle Plate Technique using Koch's sedimentation method according to Polish standard PN89/2-04088/08 as thus:

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

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 (Friberg *et al*., 1999).

Purification of Isolates and Maintenance of Pure Culture

Different isolates from the primary plates were aseptically sub-cultured by streaking onto prepared nutrient agar plates. Plates were incubated at 37^{O}C±2^{O}C for 24 hours.

These gave pure culture of isolated organism. The pure culture of the isolates were streaked on prepared sterile set agar slant in stock bottles and kept in the refrigerator at 4^{O}C to 6^{O}C for further test and identification.

RESULT AND DISCUSSION

RESULTS

The results of microbiological evaluation of bio-aerosols revealed different bacterial and fungal isolates found in outdoor ambient air (Tables 1-3 and Figures 2-7). There was a high incidence of microbial load in ambient air during dry season than in wet. The seasonal variation is due to the fact that more dust particles (during dry season) are air-borne on which viable microbial cells are attached.

Table 1: Seasonal Viable Counts of Microorganisms Isolated in Outdoor Ambient Air

Sampling Location	Coordinates of Sampling Location		Mean Viable Bacterial Counts (CFU/m^3)		Mean Viable Fungal Counts (CFU/m^3)	
	Northing	Easting	Wet	Dry	Wet	Dry
Urban Center (UC)	05°02'04.4"	007°59'41.9"	1.386×10^3	2.018×10^3	9.94×10^2	1.777×10^3
Housing Estate (HE)	05°00'50.3"	007°57'02.2"	4.82×10^2	9.64×10^2	1.054×10^3	1.265×10^3
Local Residence (LR)	05°01'33.2"	007°55'33.0"	4.22×10^2	1.476×10^3	7.23×10^2	1.536×10^3
Open Market (OM)	05°01'01.7"	007°55'27.4"	1.386×10^3	2.470×10^3	1.325×10^3	2.048×10^3
Secretariat Complex (SC)	05°01'20.0"	007°54'21.2"	7.23×10^2	1.024×10^3	6.93×10^2	1.084×10^3

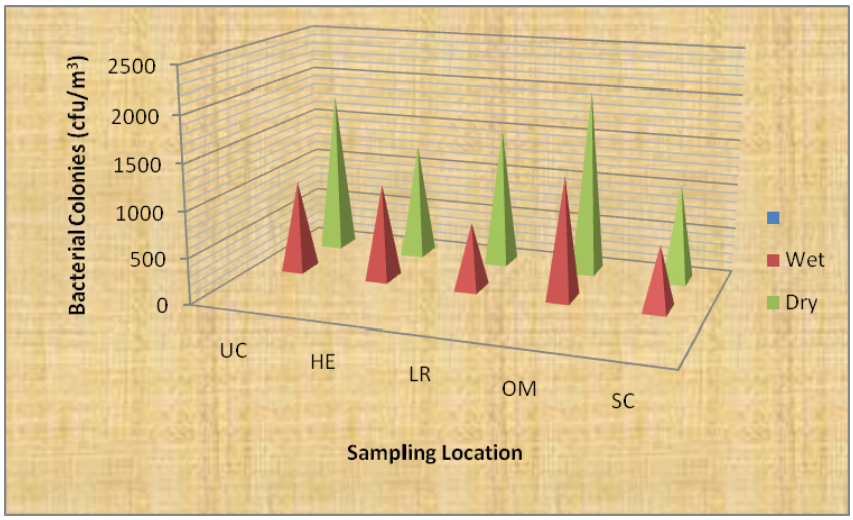

Figure 2: Seasonal Viable Counts of Bacteria Isolated from Outdoor Ambient Air

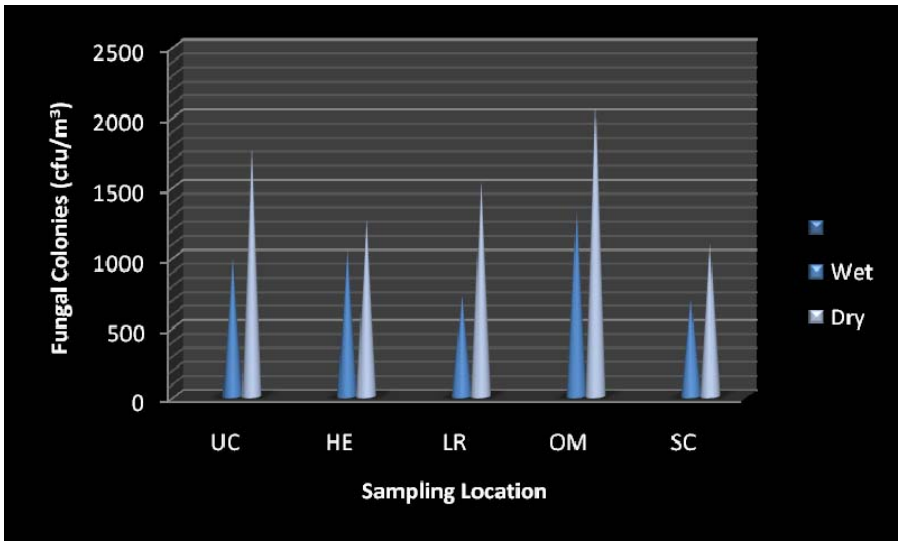

Figure 3: Seasonal Viable Counts of Fungi Isolated from Outdoor Ambient Air

Table 2: Distribution and Prevalence of Microorganisms isolated from Outdoor Ambient Air during 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 saprophyticus+	+	-	-	+		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

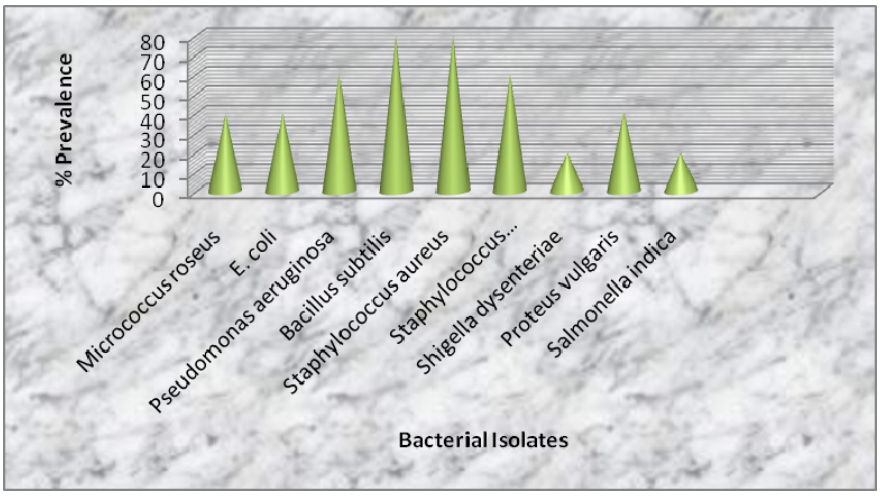

Figure 4: % Prevalence of Bacteria isolated from Outdoor Ambient Air during Wet Season

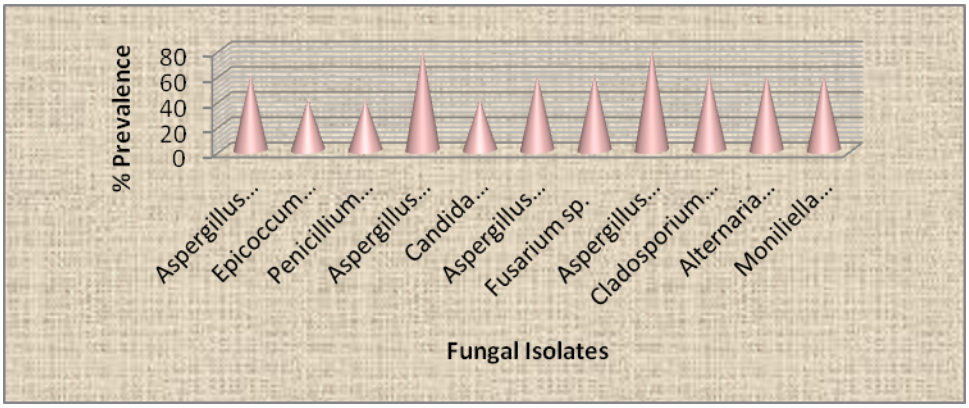

Figure 5: % Prevalence of Fungi isolated from Outdoor Ambient Air during Wet Season

Table 3: Distribution and Prevalence of Microorganisms isolated from Outdoor Ambient Air during 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	+	-	+	+	+	80
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

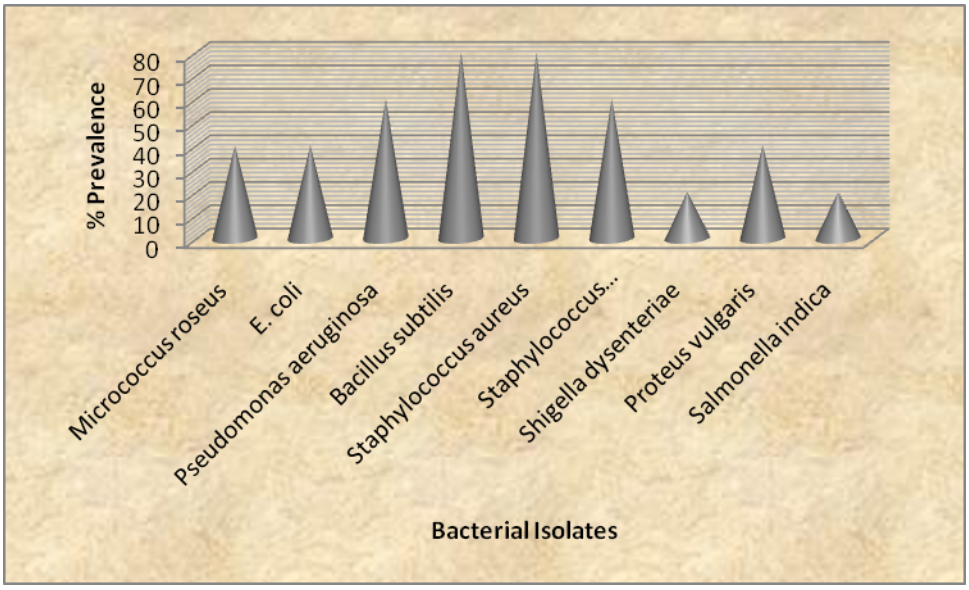

Figure 6: % Prevalence of Bacteria isolated from Outdoor Ambient Air during Dry Season

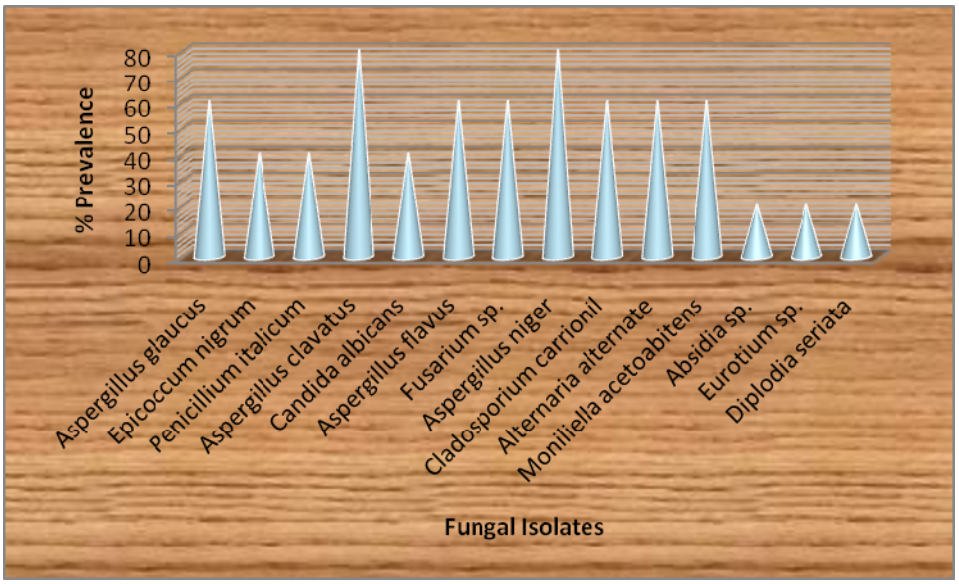

Figure 6: % Prevalence of Fungi isolated from Outdoor Ambient Air during Dry Season

DISCUSSION

Regardless the season, bacteria were detected (mean counts in CFU/m^3) in all the outdoor air samples. However, the occurrence of individual bacterial species and the outdoor bacterial concentrations and their seasonal distributions were significantly higher than the outdoor mean fungal concentrations in some locations. This is also supported by Pastuszka *et al*. (2000) with an outdoor total bacterial count (4344 *cfu*/m3) significantly higher than the outdoor total fungal count (4121 *cfu*/m3) from an outdoor environment in Upper Silesia. One possible cause is that the soil surface would be a significant source of bacteria, since higher concentrations of bacteria were present when dust was raised (Jones and Harrison, 2004).

The mean bacterial counts in Urban center (UC) were 1.386×10^3 CFU/m^3 (wet) and 2.018×10^3 CFU/m^3 (dry) as compared to mean fungal counts which were lower in number 9.94×10^3 CFU/m^3 (wet) and 1.777×10^3

CFU/m^3. Mean bacteria counts were found to be lower in both seasons in Housing Estate and Local Residence than the mean fungal counts as shown in table 1 and figures 2 and 3. Again, mean fungal counts outnumbered the mean bacterial counts in the Secretariat Complex only in the dry season sampling. However, bacteria and fungi were isolated from the outdoor air.

Five bacterial genera were constantly found in the outdoor air including *Staphylococcus*, *Proteus*, *Bacillus*, *Escherischia*, *Micrococcus*; others were *Pediococcus*, *Pseudomonas*, *Serratia*, *Salmonella*, and *Shigella*. Six fungal genera were consistently found in the outdoor air and comprised: *Aspergillus, Fusarium, Geotricum, Verticillium, Cladosporium and Penicillium*. Other fungi: *Absidia, Cephalosporium, Epicoccum, Botrytis, Moniliella, Eurotium,Diplodia, Rhizopus,Phoma, Candida, Alternaria, Scopulanopsis, Pichia, Trichoderma, Sacchromyces, Monilia*, and *Humicola* were present in very low numbers and varied according to location and season.

The results in the present study agree with Malecka – Adamowicz *et al*., (2007) who found that, the air at landfill site in Warsow - Poland, was highly contaminated by both heterotrophic bacteria and actinomycetes. Actually, the high bacterial concentration at the landfill site is attributed to the composting processes, waste handling and pile shredding.

It was found that bio-aerosol concentrations were more prevalent in dry season than wet season as shown in Table 1. Di-Giorgio, Krempff, Guiraud, Binder, Tiret and Dumenil (1996) reported that seasonal patterns in bioaerosol concentrations are caused by temperature, moisture availability and hours of daylight. They further affirmed that culturable bacteria are more prevalent in dry season than wet season in some regions due to dry, dusty conditions and associated agricultural or human activities in dry season in contrast to wet conditions with snow cover in 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.

REFERENCES

Ariya PA, Amyot M (2004). New Directions: The Role of Bio-aerosols in Atmospheric Chemistry and Physics. Atmos. Environ. 38: 1231–1232.

Burrell R (1991). Microbiological agents as health risks in indoor air. Environmental Health Perspectives, 95: 29–34.

Den Boer JW, Yzerman EPF, Schellekens J, Lettinga KD, Boshuizen HC, Van Steenbergen JE, Bosman A, Van den Hof S, Van Vliet HA, Peeters MF (2002). A Large Outbreak of Legionnaires' Disease at a Flower Show, the Netherlands, 1999. Emerg. Infect. Dis. 8: 37– 43.

Di-Giorgio C, Krempff A, Guiraud H, Binder P, Tiret C, Dumenil G (1996). *Atmospheric pollution by airborne microorganisms in the City of Marseilles,* Atmos. Environ. 30: 155-160

Douwes J, Wouters I, Dubbeld H, Zwieten LV, Steerenberg P, Doekes G, Heederik D (2000). Upper airway inflammation assessed by nasal lavage in compost workers: A relation with bioaerosol exposure. Am. J. Ind. Med. 37(5): 459-468.

Friberg B, Friberg S, Burman LG (1999). Correlation between surface and air counts of Particles carrying aerobic bacteria in operating rooms with turbulent ventilation: An experimental study, J. Hosp. Infect. 42: 61 - 68

Handley BA, Webster AJF (1995) Some factors affecting the airborne survival of bacteria outdoors. J. Appl. Bacteriol. 79: 368-378

Jones AM, Harrison RM (2004). The effects of meteorological factors on atmospheric Bio-aerosol concentrations — A review. Sci. Total Environ. 326: 151–180.

Karra S, Katsivella E (2007). Microorganisms in bio-aerosol emissions from wastewater treatment plants during summer at a Mediterranean site. Water Res. 41 (6): 1355–1365.

Lee BU (2011). Life comes from air: A short review on bio-aerosol control. Aer. Air Qty. Res. 11: 921 – 927

Levetin E, Dorsey K (2006). Contribution of leaf surface fungi to the air spora. *Aerobiologia; 22: 3-1.*

Maier R, Pepper MIL, Gerba CP (2000). Environmental Microbiology. Academic Press, San Diego, CA.

Pasquarella C, Pitzura O, Savino A (2000). The index of microbial air contamination. J. Hosp. Infect. 46: 241 – 256

Pastuszka JS, Tha-Paw UK, Lis DO, Wlazlo A, Ulfig K (2000). Bacterial and fungal aerosol in indoor environment in Upper Silesia, Poland. Atmos. Environ. 34: 3833-3842.

Paya-Vicent M, Suarez-Fernandez G (1984): *A contribution towards the study of Madrid air microflora II. Genus cladosporium.* Allergol. Immunopathol. Madr. 12: 397-402.

Ren P, Jankun TM, Belanger K, Bracken MB, Leaderer BP (2001). The relation between fungal propagules in indoor air and home characteristics. Allerg. 56: 419-424.

Savery S (1986). Relative humidity and wind velocity associated with diurnal rhythmicity of aerial dispersal of Puccinia arachidis urediniospores. − Netherlands J. Plant Sci. 92: 115-125.

Shaughnessey RI, Morey PR, Cole EC (1999). *Prevention and control of microbial contamination*. In J. Macher (ed.), *Bioaerosols: Assessment and control. American Conference of Governmental Industrial Hygienists*, Cincinnati, OH. 10 (1): 10 - 13

Sinha R, Klisch P, Groniger M, Hader ADP (2000). Mycosporine-like amino acids in the marine red alga Gracilaria cornea, effects of UV and heat. Environ. Exp. Bot. 43: 33 - 43.

Udosen CE (2006). Land Use and Erosion Risk in Ikpa River Basin. Niger. J Agric. Food Environ. Faculty of Agriculture University of Uyo. 2: 51-57.