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Full Length Research Paper

A survey of the microflora of the outdoor air environment of Keffi Metropolis, Nasarawa State, Nigeria

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The microbiological quality of the air environment of Keffi metropolis was assessed by determining the concentrations and composition of bacteria and fungi present in the outdoor air. Air samples were collected from ten different locations of the metropolis by the plate sedimentation methods which involved exposing media-filled Petri plates to the air for 30 min. Trypticase soy agar (TSA) was used for the enumeration of total bacterial concentrations while malt extract agar (MEA) was used for the enumeration of total fungal concentrations. Standard microbiological methods were employed for the identification of the bacterial and fungal isolates. The results obtained show that the concentrations of bacteria in the different locations of Keffi ranged from 2.8 x 10^3 to 6.4 x 10^3 CFU/m^3, while the concentrations of fungi ranged from 4.71 x $10^2$to 4.60 x 10^3 CFU/m^3. Six bacterial species belonging to six genera and nine fungal species belonging to seven genera were isolated at varying frequencies of distribution. The quantitative and qualitative analysis of the microbial flora of the outdoor air of Keffi metropolis has provided information on the airborne microorganisms. The fact that some of the bacterial and fungal species isolated are known to be pathogenic to humans has demonstrated that the microflora of Keffi metropolis has public health implication.

Key words: Bacteria, fungi, microflora, air quality, Keffi, Nigeria.

INTRODUCTION

Air quality is one of the most significant factor affecting the health and well-being of people. It has been reported that a single person inhale's an average of approximately 10 m^3 of air every day (Dacarro et al., 2003). However, the air inhaled by people is abundantly loaded with microorganisms which form part of the bioaerosol (Górny, 2004). Bioaerosol is a colloidal suspension, formed by droplets and particles of solid matter in the air, whose components can contain or have attached to them viruses, fungal spores and conidia, bacterial endospores, plant pollen and fragments of plant tissues (Karwowska, 2005). Biological contamination of air is mostly caused by

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bacteria, moulds and yeasts (Flannigan, 2001; Daisey et al., 2003; Pieckova and Kunova, 2002). They can be dangerous as pathogenic living cells but they also secrete some substances harmful to human health (Gutarowska and Jakubowska, 2001).

Airborne microorganisms are usually derived from various natural sources such as soil, animals, and humans (Posfai et al., 2003; Mouli et al., 2005; Fang et al., 2007). Human activities such as sewage treatment, plants and animal rendering, fermentation processes and agricultural activities do emit microorganisms into the air (Recer et al., 2001; Adhikari et al., 2004; Gillum and Leventin, 2008). Several studies have identified human activities like talking, sneezing and coughing (Kalogeraskis et al., 2005), while other human activities such as vehicular transportation and human movements, washing in homes and business centres, flushing of toilets and sewages, sweeping of floors and roadsides can generate bioaerosols indirectly (Kalogeraskis et al., 2005; Chen and Hildermann, 2009). Since micro-organisms can lodge in/on dust particles, dust therefore is a potential source of bioaerosols.

In recent years, monitoring of the number of airborne microorganisms has gained interest due to increasing concerns on public health, the threat of bioterrorism, surface biodeterioration and spread of plant diseases (Douwes et al., 2003; Pieckova and Jesenska, 1999; Stetzenbach et al., 2004). Exposure to bio aerosols, containing airborne microorganisms and their by-products, can result in respiratory disorders and other adverse health effects such as infections, hypersen-sitivity, pneumonitis and toxic reactions (Frachia et al., 2006; Górny et al., 2002).

Exposure to outdoor air microorganisms has been associated with allergic respiratory symptoms, asthma exacerbation, asthma related death and infections (Dales et al., 2004; Peternel et al., 2004). Several findings of epidemiological research indicate that exposure to high concentration of microorganisms frequently leads to allergies, asthma, hay fever (Björnsson et al., 1995; Newson et al., 2000), pneumonia (Siersted and Gravesen, 1993), and many other health side-effects, including infections (Renn et al., 2001). In recent years, dramatic increase in the number of allergic reactions to fungal spores has been reported, and young people do constitute a large group of allergy sufferers, whose symptoms persist throughout the year (Jain, 2000). For this reason, there is need for regular monitoring of the air in order to determine its quality as it affects the health of humans in the public as they go about their daily activities. Presently in Nigeria, attention is yet to be given to the monitoring of airborne microorganisms, whether outdoor or indoor. This study is therefore an attempt to provide some empirical data that could stimulate both outdoor and indoor bioaerosol research in Nigeria.

Keffi, a cosmopolitan settlement, is a typical example of a Nigeria town experiencing very rapid population growth and urbanization. A large proportion of the human inhabitants of Keffi are traders, small-scale entrepreneurs and menial workers, who spend most hours of their day in direct exposure to the outdoor air environment. Thus, the microbiological quality of the outdoor air in this locality is of public health significance. This study therefore aimed at evaluating the microbiological quality of the outdoor air environment of the Keffi metropolis.

MATERIALS AND METHODS

Study area

The study was carried out in Keffi, a fast growing cosmopolitan town geographically located on longitude 7° 50 E and latitude 8° 3 N, north-west of Lafia (the Capital of Nasarawa State, Nigeria), and is situated on an altitude of 850 m above sea level. Keffi, though in Nasarawa State, is about 68 km from Abuja, the Federal Capital of Nigeria (Akwa et al., 2007).

Air sampling and microbiological examination

Air samples were collected from ten different locations of Keffi metropolis by plate sedimentation methods as employed by Stryjakowska-Sekulska et al. (2007) and Ekhaise et al. (2008). The ten locations were Main Campus, Angwan Lambu, Federal Medical Centre (FMC), Emir's Palace, Pyanku Campus, Government Residential Area (GRA), Main Market, Angwan NEPA, Dadin kowa and High Court. Petri plates containing culture media suitable for bacteria and fungi were used as sampling surfaces. Trypticase Soy Agar (TSA) supplemented with cyclohexamide (which inhibits growth of fungi) was used for the determination of total number of bacteria, while Malt Extract Agar (MEA) supplemented with chloramphenicol (which inhibits growth of bacteria) was used for the determination of the total number of fungi (Kalwasińska et al., 2012). Plates in triplicates for each type of culture medium were exposed to air in each of the ten locations for 30 min in order to allow air microorganisms to settle gravitationally directly on the media surfaces of the plates. Plates with TSA were incubated for 48 h at 37°C, while the plates with MEA were incubated at 30°C for 7 days (even though colonies were counted on the 3rd day). The total number of colony forming units were enumerated and expressed as colony forming units per cubic meter of air (CFU/m^3) (Stryjakowska-Sekulska et al., 2007).

Identification of bacterial and fungal isolates

The identification of bacterial colonies was carried out according to the standard microbiological methods as described by Holt (1994), Cheesbrough (2000) and Aneja (2003), in which the colonies were characterized using macroscopic (cultural) and microscopic (morphological) features as well as biochemical tests. The API system (bio-Mérieux, Marcy-l'Etoile, France) was also used to further confirm the identity of the bacterial species.

Identification of all fungal isolates was also carried out using standard methods based on macroscopic and microscopic features as described by Ellis (1971), Domsch et al. (1980), Singh et al. (1991), Barnett et al. (2007) and Barnett and Hunter (1999).

Statistical analysis

Turkey test as recommended by Zar (1999) for analysis of data for

Table 1. Bacterial concentration in the outdoor air environment of different locations of Keffi metropolis.

Location	Mean (cfu/m^3)	Standard deviation
Main Campus	4.2x10^1	± 1.13
Angwan Lambu	4.5x10^1	± 0.83
Federal Medical Centre	4.2x10^1	± 1.13
Emir's Palace	7.6x10^1	± 2.27
Pyanku Campus	5.4x10^1	± 0.07
GRA*	3.0x10^1	± 2.33
Main Market	1.0x10^2	± 4.67
Angwan NEPA	6.0x10^1	± 0.67
Dadin Kowa	4.2x10^1	± 1.13
High Court Area	4.2x10^1	± 1.13

*GRA, Government Reserved Area.

Table 2. Fungal concentration of the outdoor air environment of the different locations in Keffi metropolis.

Location	Mean (CFU/m^3)	Standard deviaton
Main Campus	1.2x10^1	±2.06
Angwan Lambu	1.2x10^1	±2.06
FederalMedical Centre	1.7 x10^1	±1.56
Emir's Palace	3.9 x10^1	±0.64
Pyanku Campus	1.5 x10^1	±1.76
GRA*	4.8 x10^1	±1.54
Main Market	7.0 x10^1	±3.74
Angwan NEPA	5.0 x10^1	±1.74
Dadin Kowa	2.6 x10^1	±0.66
High Court Area	3.7 x10^1	±0.44

*GRA, Government Reserved Area.

multiple-comparison was used to determine the statistical significance of the concentrations of bacteria and fungi in the air sampled from the different locations of Keffi. Statistical Package for Social Sciences (SPSS) version 20.0 software was employed for this analysis. Percentage frequencies of occurrence of species of bacteria and fungi at the different locations were also computed according to the methods of Sampo et al. (1997).

RESULTS AND DISCUSSION

The results of the bacterial concentrations in the outdoor air environment of different locations of Keffi metropolis are shown in Table 1. The bacterial concentrations in the different locations ranged from 2.8 x 10^3 to 6.4 x 10^3 CFU/m^3. The highest bacterial concentration of 6.4 x 10^3 CFU/m^3 was recorded at the Main Market, followed by 6.2 x 10^3 CFU/m^3 and 5.7 x 10^3 CFU/m^3 recorded for Emir's Palace and Angwan NEPA, respectively. The lowest concentration of 2.8 x 10^3 CFU/m^3 was recorded at Government Reserved Area (GRA).

The results of the fungal concentrations in the outdoor air environment of different locations of Keffi metropolis are shown in Table 2. The fungal concentrations in the different locations ranged from 4.71 x 10^2 to 4.60 x 10^3 CFU/m^3. The highest fungal concentration of 4.60 x 10^3 CFU/m^3 was also recorded at the Main Market. The concentrations recorded at High Court Area, Government Reserved Area (GRA) and Dadin Kowa were 3.49 x 10^3 CFU/m^3, 2.52 x 10^3 CFU/m^3 and 2.45 x 10^3 CFU/m^3 respectively. The lowest concentration of 4.71 x 10^2 CFU/m^3 was recorded at Agwan NEPA.

The results of the distribution of the different species of bacteria and fungi isolated are presented in Tables 3 and 4, respectively. Six bacterial species were isolated at varying frequencies of occurrence. The bacterial species with their respective frequencies of occurrence were *Staphylococcus aureus* (100%), *Streptococcus pyogenes* (100%), *Escherichia coli* (90%), *Bacillus* spp. (100%), *Enterobacter aerogenes* (40%) and *Shigella* spp. (50%).

Nine species of fungi belonging to seven genera were isolated with respective percentage frequencies of 100% (*Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer*), 80% (*Penicillium* spp.), 60% (*Aspergillus fumigatus, Candida albicans*) and 30% (*Mucor* spp., *Absida corymbifera, Alternaria alternata*), respectively.

Epidemiological studies have shown that a large number of people around the world are exposed to biological agents (Daisey et al., 2003; Dales et al., 2004; Golofit-Szymczak and Gorny, 2010). Unfortunately, there is no official reference limit for the microbiological quality of air in human environments, whether indoor or outdoor. The lack of quantitative health-based guideline values or thresholds for the acceptable levels of microbial contamination in the air may be due to a lack of dose-response relationship for most of the air microbiological agents (Golofit-Szymezak and Górny, 2010). Several investigators in this area had highlighted that source data on concentrations of biological agents in the air environments

Table 3. Distribution of bacterial species isolated from the outdoor air environment of the different locations in Keffi metropolis.

Bacterial isolate	Location*										Occurrence
	A	B	C	D	E	F	G	H	I	J	(%)
Staphylococcus aureus	+	+	+	+	+	+	+	+	+	+	100
Streptococcus pyogenes	+	+	+	+	+	+	+	+	+	+	100
Escherichia coli	+	+	+	+	+	-	+	+	+	+	90
Bacillus sp.	+	+	+	+	+	+	+	+	+	+	100
Enterobacter aerogenes	-	-	-	-	-	-	+	+	+	+	40
Shigella sp.	-	-	-	-	-	+	+	+	+	+	50

*Locations: A = Main Campus; B = Angwan Lambu; C = Federal Medical Center; D = Emir's Palace; E = Pyanku Campus; F = Government Reserve Area; G = Main Market; H = Angwan NEPA; I = Dadin Kowa; J = High Court Area.

Table 4. Distribution of fungal species isolated from the outdoor air environment of the different locations of Keffi metropolis.

Isolate	Location*										Occurrence
	A	B	C	D	E	F	G	H	I	J	(%)
Aspergillus flavus	-	+	-	+	+	-	+	+	+	+	70
Aspergillus niger	+	+	+	+	-	+	+	+	+	+	100
Aspergillus fumigatus	-	+	+	-	+	-	+	+	-	+	60
Penicillium sp.	-	+	+	+	-	+	+	+	+	+	80
Rhizopus stolonifer	+	+	+	+	+	+	+	+	+	+	100
Absidia corymbifera	-	-	-	+	-	-	+	+	-	-	30
Alternaria alternata	-	-	-	+	-	-	+	+	-	-	30
Mucor sp.	-	+	-	+	-	-	+	-	+	+	50
Candida albicans	+	+	+	+	-	-	+	+	-	-	60

*Locations: A = Main Campus; B = Angwan Lambu; C = Federal Medical Center; D = Emir's Palace; E = Pyanku Campus; F = Government Reserve Area; G = Main Market; H = Angwan NEPA; I = Dadin Kowa; J = High Court Area

are still insufficient (Adhikar et al., 2004; Mouli et al., 2005; Golofit-Szymczak and Gorny, 2010; Kalwasińska et al., 2012). This notwithstanding, the qualitative and quantitative information on the composition and concentrations of microorganisms in the air environment of human habitations at any point in time would help in alerting the public of possible health risk that may be encountered by vulnerable individuals.

Several researchers in this area had earlier reported that exposure to high concentrations of microorganisms in the air frequently lead to allergies, asthma (Björnsson et al., 1995; Newson et al., 2000), pueumonia (Siersted and Gravesen, 1993), and other health side-effects. In addition to public health advantage, routine monitoring of air quality can serve as a means of military surveillance for the detection of any possible biological threat of bioterrorism (Douwes et al., 2003).

Data resulting from this study revealed that the concentrations of bacteria in Keffi ranged from2.8×10^3 - 6.4×10^3 CFU/$m^3$and that of fungi ranged from4.71×10^2 - 4.60×10^3CFU/m^3. However, the concentrations for both bacteria and fungi have been shown to vary ($P<0.05$) in the different locations of the Keffi metropolis. The concentrations of bacteria at all the locations exceeded the recommended limit (10^3CFU/m^3) suggested by National Institute of Occupational Safety and Health (NIOSH). The American Conference of Governmental Industrial Hygienists (ACGIH) had suggested 500 CFU/m^3 for culturable bacteria (Kalogerakis et al., 2005). Górny and Dutkiewiez (2002) earlier presented to WHO Expert Meeting in Berlin, a proposed Residential Limit Values of 250 CFU/m^3 for bacterial concentrations.

Considering all available threshold limits for bacterial concentrations in the air environments, it is clear that the outdoor air of Keffi metropolis is heavily loaded with bacteria. The Main Market that had the highest bacterial concentration followed by the Emir's Palace is among the busiest locations in the metropolis in terms of human and vehicular movements. The high bacterial concentration recorded in these two locations is not surprising, and this

agrees with reports by several researchers (Kalogeraskis et al., 2005; Chen and Hildermann, 2009). Similarly, the concentrations of fungi in most of the locations exceeded the recommended proposal of 10^3 CFU/m3 as threshold limits for fungal concentrations in the air (Górny and Dutkiewiez, 2002).

The qualitative analysis of the microbial flora provides additional information on airborne microorganisms in the outdoor air of Keffi metropolis. In this study, six species of bacteria, *S. aureus*, *S. pyogenes, E. coli, Bacillus* spp., *E. aerogenes* and *Shigella* spp., and nine species fungi belonging to six genera, which included *A. flavus, A. niger, A. fumigatus, Penicillium* spp., *R. stolonifer, A. corymbifera, A.alternata, Mucor* spp. and *C. albicans* were isolated from the outdoor air environment of Keffi. Some of these bacteria and fungi have been shown to be amongst the most common bacterial and fungal species isolated from the air (Burge and Hoyer, 1990).

From this study, *S. aureus, S. pyogenes, Bacillus* spp. and *E. coli* are the most prevalent bacterial species isolated. *S. aureus* is known to be carried in the naso-pharynx, throat, skin, cuts, boils, nails, and such can easily contribute to the microflora in the Keffi metropolis which is always busy with activities involving in most cases human and vehicular movements. *Bacillus* spp. is spore-forming soil bacteria and the most persistent in the atmosphere (Shaffer and Lighthart, 1994). *S. pyogenes* are often found as commensals in the upper respiratory tract of human (Cheesbrough, 2000). If host defenses are weakened or a new highly virulent strain is introduced it can lead to acute suppuration infections (Brooks et al., 2001). *E. coli* is an enteric coliform which is a normal resident flora of the large intestines of mammals including humans and are used as indicators of pollution of feacal origin (Willey et al., 2008). The high prevalence of *E. coli* in the air of Keffi suggests a very low personal and environmental hygiene practice in this town.

A. niger and *Penicillium* spp. are the most predominant species isolated. Maktkovic et al. (2007) in their study reported *Aspergillus* spp. and *Penicillium* spp. as the predominant genera of organisms isolated from the air, while Ekhaise et al. (2008) reported *Aspergillus* species as the most common genus of fungi in the air environment. *Aspergillus* and other species of fungi have been implicated as pathogenic in causing several mycotic infections. The relatively high concentrations of fungi in the air environment of Keffi may pose only little health hazard to healthy individuals, but would pose serious danger and special risk to immunosuppressed persons and other severely immunocompromised individuals (Flannigan et al., 1994). Fungal spores from species of *Penicillium* have been implicated with allergies and elicit asthma in vulnerable individuals (Flannigan et al., 1991).

Conclusion

The importance of evaluating the quality of the air humans breathe whether indoor or outdoor, especially in the urban areas where there is high vehicular traffic and human activities involving rapid movements cannot be over-emphasized. The number and type of airborne microorganisms can also be used to determine the degree of cleanliness as a means of determining the source of human discomfort and certain airborne microbial infections.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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