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RESEARCH ARTICLE

MICROBIOLOGICAL INDOOR AND OUTDOOR AIR QUALITY OF SELECTED PLACES IN VISAKHAPATNAM CITY, INDIA

[1]Kirti Bomala, *,[2]Geetha Saramanda, [3]Byragi Reddy. T and [4]Jyothi Kaparapu

[1, 3]Department of Environmental Sciences, Andhra University, India
[2]Department of Microbiology, Andhra University, India
[4]Department of Botany, Andhra University, Visakhspatnam, Andhra Pradesh, India

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ABSTRACT

A study on indoor and outdoor air microbiological contamination in various rooms of public places such as RTC Complex, Government School and College building (Government degree college for women) in Visakhapatnam city, India. Investigations were conducted in the period 2014-2015. Air samples were taken twice a day, in the morning and in the afternoon. In all of the tested places a multiple growth of bacteria and significant increase of mould spores were observed in afternoons. The predominant bacteria and moulds isolated from investigated air samples were: *Staphylococcus sp., Micrococcus sp., Aspergillus sp., Penicillium sp., Rhizopus sp., Cladosporium sp. and Alternaria sp*. Among these microbes the presence of pathogenic and strongly allergenic microorganisms was detected. The results seem to suggest that the concentrations of bioaerosols identified in the studied areas in the afternoon were higher than the values established in the morning for the indoor background – the concentrations of both bacteria and fungal aerosol were higher in the afternoon. The air in the school principal's room and college laboratory were considered the least contaminated with microbial aerosol due to the specific features of the rooms.

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INTRODUCTION

The air inhaled by people is bundantly populated with microorganisms which form so-called bioaerosols (Wojtatowicz *et al.,* 2008). Bioaerosol is a colloidal suspension, formed by liquid droplets and particles of solid matter in the air, whose components contain or have attached to them viruses, fungal spores and conidia, bacterial endospores, plant pollen and fragments of plant tissues (Karwowska, 2005).They account for 5-34% of indoor air pollution. Indoor air plays a special role as a health determinant, and management of indoor air quality requires approaches that differ from those used for outdoor air. For these reasons, the working group preparing the global update of the WHO air quality guidelines (WHO 2006) recommended that WHO also prepare guidelines for indoor air quality. Microbial damage in indoor and outdoor areas is caused most frequently by molds and bacteria. This constitutes a common problem all over the world. The most significant environmental factors influencing the viability of microorganisms are temperature, relative humidity (RH), and wind velocity (Jones 2003).

Corresponding author: Geetha Saramanda
Department of Microbiology, Andhra University, India.

Also, the additional influences are exerted through oxygen, air ions, solar irradiance, and open-air factors. Hence, the monitoring of outdoor airborne microorganisms is necessary to evaluate the risk on human health and to study its evolution, and the interest in bioaerosol characterization has increased over the last few decades (Douwes *et al*., 2003). Most of these studies were carried through airborne fungi (Hurst, 1991). The aim of the present study to investigate airborne bacteria at two different locations in Visakhapatnam, no survey of airborne bacteria has been attempted till now. Moreover, to estimate the influence of meteorological factors on bioaerosol along with bioaerosol pollution through modeling and quality indexing approach respectively.

MATERIALS AND METHODS

Study area

Visakhapatnam is the largest city, both in terms of area and population in the Indian state of Andhra Pradesh. Visakhapatnam is located 363 kilometres (226 mi) north east of the proposed state capital of Amaravati and 587 kilometres (365 mi) of Hyderabad, the common capital of Andhra Pradesh and Telangana. As of 2011, the population of the city was

recorded as 1,897,823, making it 15th largest city in India. Visakhapatnam is the principal commercial hub of the state, and contributes to its economy in many sectors such as heavy industries, tourism, and fishing and information technology. The investigation carried out at Government School, Government Women's College and RTC complex in Visakhapatnam city during the period of 2014 -15.

Collection and Processing of Samples

Air sampling was conducted with the impaction method, with the use of air sampler, based on the principle of the Andersen air sampler (corresponding to its 5th stage (Pascual *et al*., 2003 Karbowska *et al*., 2011) which guarantees that all particles > 1µm were collected). During the sampling, the device was placed at a height of 1.0-1.5m above the floor (one sampling site in the middle of the room) or at the ground level (for outdoor measurements) to simulate aspiration from the human breathing zone. Sterile plates of nutrient agar and saboraud dextrose agar with streptomycin were exposed to air within the class room of the teaching school and college between the hours of 8.00 am and 10.00 am and in the afternoon between 12 noon and 2.00 pm daily. The experiment was carried out in triplicate so also the data generated and sample collection was done within five days. The plates were exposed for about 15 minutes at strategic locations (1m above the floor) in the ward and transported in a clean container to the laboratory for microbiological examination immediately after collection. The nutrient agar plates were incubated at 37°C for 18-24 hours while the saboraud dextrose agar plates were incubated at room temperature, (20°C - 28°C) for 3-5days. The total number of colony forming unit (CFU/m^3) for bacterial isolates and spore forming unit (CFU/m^3) for the fungal isolates were determined after incubation. The biochemical and physiological characteristics of identified bacterial species were performed according to standard procedures (Cheesbrough, 2006). A wet mount preparation of each fungal colony was prepared by using Lactophenol-cotton-blue solution and examined microscopically. Identification of fungi was based mainly on growth of colonial appearance, microscopic examination of the spore and hyphal characteristics of the stained preparations (Barnett and Hunter, 1972).

RESULTS

Table 1. Bacteria and Fungi present in the selected sites

Sampling Place	Name of Bacteria Isolates	Name of Fungal Isolates
School	*Micrococcus, Bacillus, Staphylococcus, Streptococcus*	*Aspergillus, Penicillium, Fusarium, Mucor, Rhizopus*
College	*Micrococcus, Bacillus, Staphylococcus, Pseudomonas, Streptococcus, Legionella,*	*Aspergillus, Penicillium, Fusarium, Mucor, Rhizopus, Legionella*
RTC Complex	*Micrococcus, Bacillus, Staphylococcus, Streptococcus, Mycobacterium, Pseudomonas, Legionella, Klebsiella pneumonia*	*Aspergillus, Penicillium, Fusarium, Mucor, Rhizopus , Alternaria , Cladosporium, Trichoderma*

DISCUSSION

The survival of aerosolized Gram-negative bacteria (including *Pseudomonas*, and *Klebsiella* species) was found to be greatest in high relative humidity, low temperature (Marthi *et al*., 1990; Walter *et al*., 1990). Studies of indoor air have demonstrated that Gram-positive cocci (*Micrococcus, Staphylococcus* species) are the most commonly found bacteria in indoor air environments, though some Gram-negative bacteria (Pseudomonadaceae family, Aeromonas species) are also often present (Gorny *et al*., 1999; Gorny & Dutkiewicz 2002). *Cladosporium, Penicillium, Aspergillus,* and non sporulating fungi were the most common fungi indoors and outdoors in each season and in each region (Brian *et al*., 2002). Different types of bacteria and fungi present in the indoor and outdoor air microbiological examination were presented in the Table 1. The results of total bacteria and fungi present in the indoor and outdoor of selected sites were depicted in Table 3. The results indicate that the total amount of bacteria in the investigated school rooms, which constituted indoor background, ranged from 1.2 x 10^2 to 7.2 x 10^2 CFU/m^3, of air, while the amount of bacteria at different sampling sites of outdoor ranged from 3.6 x 10^2 to 9.2 x 10^2 CFU/m^3. The amount of bacteria found in the Indoor and outdoor air of college ranged from 1.2 x 10^2 to6.8x 10^2 CFU/m^3 and 4.4x 10^2 to 10 x 10^2 CFU/m^3.The count of bacteria in indoor and outdoor air of RTC bus station 2.2 x 10^2 to 8.8 x 10^2 CFU/m^3 and 4.8 x 10^2 to 10.2x 10^2 CFU/m^3 .The concentration of mould fungi in the indoor air of school was ranged from2.2 x 10^2 to 4.2 x 10^2 CFU/m^3 in the morning, but fluctuated in the afternoon (depending on the sampling site). The concentration of mould fungi which constituted outdoor background ranged from 4.2 x 10^2 to 6.8 x 10^2 CFU/m^3. In the College the amount of mould fungi in the indoor air was 2.1 x 10^2 to 6.8 x 10^2 CFU/m^3 and in the outdoor air , the concentration of fungi are 4.2 x 10^2 to 8.2 x 10^2 CFU/m^3. The amount of fungi found in the indoor and outdoor air of RTC bus station ranged from 3.2 x 10^2 to 6.6 x 10^2 CFU/m^3, and 4.8 x 10^2 to 9.2 x 10^2 CFU/m^3. Indoor air flora of selected sites was lower than the concentration of bacteria and fungi of outdoor air flora.

Remarkably, air contamination reached the highest level in areas characterized by a large circulation of people. The study shows that the lowest microbiological air contamination was noted in the school principal's room and college laboratory. This can be explained by the fact that this area is well isolated from the influences of the outdoor environment. The results (Table 2) seem to suggest that the concentrations of bioaerosols identified in the studied areas in the afternoon were higher than the values established in the morning for the indoor background – the concentrations of both bacteria and fungal aerosol were higher in the afternoon. For bacterial aerosol, the most important and continuously active sources of its emission in the environment are people and animals (Cox and Waters, 1995). The most significant sources of fungal aerosol, however, are found in the outdoor environment, and include the soil, water, plants, etc. Regular outside air inflow into interiors is the main process resulting in biological contamination of the indoor environment (Jain, 2000). Relatively high concentrations of bacteria observed in the RTC

Table 2. Bacterial and fungal concentration in the indoor outdoor air of selected sites

	Sampling Place	Time of taking samples	Total Number of Bacteria [CFU/m^3]		Total Number of Fungi[CFU/m^3]	
			Indoor	Outdoor	Indoor	Outdoor
SCHOOL	Class Room (Ground Floor)	morning	3.9×10^2	7.2×10^2	2.4×10^2	5.2×10^2
		afternoon	6.2×10^2	9.2×10^2	2.2×10^2	4.8×10^2
	Class Room (First Floor)	morning	5.2×10^2	8.8×10^2	3.2×10^2	5.0×10^2
		afternoon	7.2×10^2	9.2×10^2	4.2×10^2	6.8×10^2
	Principal Room	morning	1.2×10^2	3.6×10^2	2.4×10^2	4.2×10^2
		afternoon	2.2×10^2	5.2×10^2	3.2×10^2	5.6×10^2
COLLEGE	Lecture Room	morning	$3.2x\ 10^2$	$7.8x\ 10^2$	2.2×10^2	4.2×10^2
		afternoon	$5.6x\ 10^2$	10×10^2	2.1×10^2	5.2×10^2
	Library	morning	$4.2x\ 10^2$	$6.5x\ 10^2$	4.2×10^2	7.8×10^2
		afternoon	$6.8x\ 10^2$	$9.8x\ 10^2$	6.8×10^2	8.2×10^2
	Laboratory	morning	1.2×10^2	$4.4x\ 10^2$	3.2×10^2	5.2×10^2
		afternoon	$3\ .2 \times 10^2$	7.2×10^2	3.6×10^2	5.0×10^2
RTC	Ticket Counter	morning	4.8×10^2	6.6×10^2	3.2×10^2	4.8×10^2
		afternoon	7.9×10^2	$10.2x\ 10^2$	$4.8x\ 10^2$	7.2×10^2
	Waiting Room	morning	2.2×10^2	4.8×10^2	5.6×10^2	8.2×10^2
Bus Station		afternoon	4.6×10^2	9.2×10^2	6.6×10^2	9.2×10^2
	Canteen	morning	5.6×10^2	8.2×10^2	4.8×10^2	7.2×10^2
		afternoon	8.8×10^2	10×10^2	5.2×10^2	8.2×10^2

Table 3. Evaluation of air quality according to the sanitary standards for non-industrial premises (CEC, 1993)

Group of microbes	Range of values (CFU/m3)	Pollution degree
Bacteria	< 50	Very small
	50-100	Small
	100-500	Medium
	500-2000	High
	>2000	Very High
Fungi	<25	Very small
	25-100	Small
	100-500	Medium
	500-2000	High
	>2000	Very High

ticket counter, (where a large cloakroom is located), canteen and toilet are entirely understandable and confirm the observations of other researchers (Muszynski *et al*., 1992). Remarkably, air contamination reached the highest level in areas characterized by a large circulation of people. In the toilet, these were the toilet bowl, washbasin and humidifier, apart from the people who produce large amounts of microorganisms in the air. In the canteen, directly connected to the kitchen, tiny particles which may form a suspension in bioaerosols are released into the air during different stages of food preparation. Stairs located nearby leading to the first floor of class room constitute an additional source of air contamination since they are responsible for large amounts of dust entering into the room. Similar results were observed by Agnieszka *et al*., 2012.

Evaluation of the air quality in the designated areas (School, College, RTC bus station) was based on the sanitary standards for non-industrial premises formulated by the European Commission in 1993 (Table 2). According to this classification, the air in the school class rooms of first and ground floor, outdoor air flora of college lecture room, college library and RTC bus Station were highly contaminated with bacteria. The study shows that the lowest microbiological air contamination was noted in the indoor flora of school principal room and college laboratory. This can be explained by the fact that this area is well isolated from the influences of the outdoor environment and are efficiently ventilated.

Numerous studies emphasise the fact that rooms with efficient ventilation or air conditioning systems and guaranteed air tightness are less contaminated than rooms where air-conditioning was not installed (Gorny, 2004, Wlazlo *et al*., 2008; Lugauskas *et al*., 2004). The results of the research into the concentration of mould fungi on the premises of the selected areas showed that a high level of fungal contamination was determined in air samples collected in the waiting hall and canteen of RTC bus station and in college library. At both sampling sites, half of the air tests indicated moderate air contamination. The same level of contamination was also observed in more than half of the air tests conducted in the college library and in school class room of first floor. The lowest level of air contamination with fungal aerosol was noted in the college lecture room and ground floor class room indicated a low level of contamination.

Conclusion

According to the criteria for microbiological cleanliness in the interiors submitted by the European Commission in 1993, the air was considered heavily or moderately contaminated with bacteria, while the air contamination with mould fungi was described as moderate. The air in the school principal's room and college laboratory were considered the least contaminated with microbial aerosol due to the specific features of the rooms.

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