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Assessment of airborne bacteria and fungi in an indoor and outdoor environment

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ABSTRACT: Airborne indoor and outdoor bacteria and fungi were assessed during the spring season using conventional methods to investigate the enumeration and identification of airborne micro-organisms. This was determined through air quality sampling using the 'open plate technique'. The air samples were collected during the spring season (March-May) from four different locations. Conventional enumeration of airborne micro-organisms relies on culture-based or microscopic methods. Although a culture-based analysis is most widely used for bio-aerosol, four public places located in urban residential areas were selected for indoor/outdoor air bio-pollutant measurement. The public places included kitchens, classrooms, recreational areas, laboratories. Public parks are an important facility associated with the environmental exposure of children. Cultivation and total microscopic enumeration methods were employed for the sample analysis. 26 groups of bacteria and fungi, either of human or environmental origin were detected. Environmental agents generally predominated while significantly higher counts were detected as the level of hygiene or standard of housing dropped. Seven genera of fungi, mainly members of the genus Aspergillum, were isolated from all residents. Bacteria shows higher growth numbers as opposed to the slow growing fungi. Sample collection and pretreatment, determination techniques and performance results are summarized and discussed.

Keywords: Aspergillum; Bioaerosols; Conventional methods; Microorgansim

INTRODUCTION

Exposure to bio-aerosols, containing airborne microorganisms and their by-products, can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity pneumonitis and toxic reactions (Gorny et al., 2002; Fracchia et al., 2006). Fungi are common in indoor and outdoor environments and nearly 10 % of people worldwide have fungal allergy (Pasanen et al., 1996). In many environments including hospitals, animal sheds, clean-rooms, pharmaceutical facilities and spacecraft environments, the presence of bio-aerosols can compromise normal activities, making efficient monitoring crucial (Venkateswaran et al., 2003; Gorny, 2004; Stetzenbach, 2007; Okafor and Opuene, 2007). Microbial damage in indoor/outdoor areas, is caused most frequently by molds and bacteria. These micro-organisms have a very important role in the biogeochemical cycle, as their task consists of disintegrating organic mass to reusable metabolites. In the environment spores of molds and bacteria may become airborne and are therefore ubiquitous. They can enter indoor areas either by means of passive ventilation or by means of ventilation systems. Many genera are also emitted by indoor sources like animals, flowerpots and wastebaskets. In most cases, normal flora is not harmful. However, growth conditions like excessive humidity and/or a high water content of building materials are encountered on a more frequent basis, which in most cases can be described as the limiting factor for microbial growth. This is caused by shortcomings of the buildings such as the lack of thermal insulation, as well as the incorrect behavior of users of rooms. The relative humidity and/ or the moisture content of the materials determines that to what extent different micro-organisms are able to grow on indoor or outdoor materials (Dhanasekaran et al., 2009). These may cause destruction, adverse health effects and unpleasant odors. Therefore, the task of microbial examinations is to differentiate between

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normal indoor micro-organisms, airborne or adherent to walls and floors and fast growing species, attaching itself to building materials and producing microbial products and ultimately causing adverse health effects (Madukasi et al., 2010). Air sampling of microorganisms is a popular method of conducting microbial examinations, as it allows a direct toxicological evaluation. These results can be related to a concentration expressed in colony forming units per cubic meter. Sometimes information might even be available on a particle which allows for an estimation of how deep those particles may penetrate into the lungs of a human being. Micro-organisms are generally not equally distributed in indoor air. They mostly occur in clouds and are often overlooked in air measurements, especially if the microbial damage is hidden by paneling, walls, etc. Another reason for false-negative results obtained by air measurements is that fungal spores are not released during all the stages of its growth. In this case, other techniques are helpful, for example, the sampling of household dust, the sedimentation method or direct sampling from surfaces. The differentiation of bacteria is performed by a biochemical methods as a rule, whereas in most cases the differentiation of molds is done microscopically, especially when the forms of spores need to be detected. On many occasions, the growth behavior and patterns on different nutrient agars also have to be evaluated. Non-sporulating species have to be triggered to produce spores, otherwise "sterile mycelium" will result, which means they cannot be named by genera or even species. Methods of genetic fingerprinting are still in their early stages and only available for some genera or species. In the meantime enzymatic tests have become available to decide between mold growth and normal quantities on building surfaces. Searching for hidden mold growth can be a very difficult task. An example of this is if adverse health effects like the fungal syndrome is observed (Velmurgan et al., 2008; Cuthbertson et al., 2010). The fungal syndrome is characterized by the occurrence of unspecific symptoms. The analysis of microbial volatile organic compounds or even the use of specially trained sniffer dogs are some of the methods used to detect hidden mold growth. However, these methods have not been scientifically evaluated. The odor alone perceived by human beings is not reliable enough to detect mold damage. As far as the rehabilitation of the indoor environment is concerned, it has to be pointed out very clearly that microbial

damage has to be removed. The extermination of microorganisms is often carried out, but this procedure is not sufficient because non-viable spores for example, keep their allergenic potential. The acuteness of the rehabilitation procedures is normally considered according to the extent of the microbial damage. Adverse health effects are supposed to be linked with microbial growth in indoor areas and is mostly related with mold growth. Allergies is a predominant condition which has to be mentioned, followed by toxic alveolitis and reactions like (allergic) bronchitis, chronic obstructive pulmonary disease, as well as the aggravation of asthma. Infections by molds and bacteria are very rare, but persons with an immunodeficienc are especially susceptible to fungal infections. It has been found that spores of fungi contain fungal toxins (mycotoxins), which are well known from food contaminations. It has however not been confirmed whether these mycotoxins show toxic effects if fungal spores are inhaled. On the whole, the dose relationship between the concentration of microbial particles already mentioned and the adverse health effects described, is not very well established. When sanitary effects are observed, the susceptibility of the individual is very often crucial. The result of this is that guidelines concerning microbial products in indoor areas are sparse and mostly not scientifically sound. In non-industrial indoor environments, the most important source of airborne bacteria is the presence of human (Stetzenbach, 2007). Specific activities like talking, sneezing, coughing, walking, washing and toilet flushing can generate airborne biological particulate matter. In addition food stuffs, house plants and flower pots, house dust, pets and their bedding, textiles, carpets, wood material and furniture stuffing, occasionally release spores of Alternaria, Aspergillus, Botrytis, Cladosporium, Penicillium, Scopulariopsis into the air (Cox and Wathes, 1995; Maeir et al., 2002). Although indoor environments are considered to be protected, they can become contaminated with particles that present different and sometimes more serious risks when their concentrations exceed recommended maximum limits than those related to outdoor exposures (Banerjee, 2008). The recommended maximum limits are: 1000 CFUs/m³ for the total number of bio-aerosol particles set by the National Institute of Occupational Safety and Health (NIOSH); 1000 CFUs/m3 set by the American Conference of Governmental Industrial Hygienists

(ACGIH) with the culturable count for total bacteria not to exceed 500 CFUs/m3 (Cox and Wathes, 1995; Jensen and Schafer, 1998). Human beigns build the home to be protected in the environment. Indoor air pollution can be as much more worses than that of outdoor air, it can cause a wide range of health problems. Mold, mildew, fungi, bacteria, viruses, microorganisms, chemical fumes, organic odors, dust pollen and other floating particles are potential threats in many households. Most people assume that this particular problem is addressed if they filter the air. The truth is that filters will not remove all the particles from the air. Even if a high-efficiency particulate air filter (HEPA) is used, the problem will not be effectively addressed. HEPA filters will only remove particles the size of 3 microns or larger. Consequently, dust particles smaller than 3 microns will pass through unhindered. Unfortunately, filters can also become breeding grounds for mold and bacteria. A filter only collects and does not kill toxic particles. For a filter to work effectively, air has to pass through it. If a person inhales air prior to it passing through a filter, the particles would have already entered the persons lungs. In addition, if a filter collects only mold spores, it does not solve the problem. Effectively, the mold that created the spores is still alive and continues to generate mold spores. A filter is not designed to eliminate the source itself. Ultraviolet lights (UV) are claimed to kill 99.9 % of all organisms. Even though UV has the potential to kill 99.9 % of all organisms, it will only kill that which passes by the light. In addition not all UV rays have the same potential to kill organisms. Some UV rays are designed to produce ozone. The amount of ozone produced is in such small quantities, 0.01 ppm. (parts per million), that it will not have any major effect on indoor air quality. UV lights also present some problems. Certain UV lights have the ability to damage the retina of the eyes especially when a person gaze directly into it. UV rays burn out or stops creating the frequency of UV that produces ozone after 10 to 12 months and it then has to be replaced. Most equipment producing ozone, create oxides of nitrogen (NOx) as a by-product. Oxides of Nitrogen creates nitrous and nitric acids when combined with water vapor or moisture. Nitric acid is used to etch metals. When the upper atmosphere is combined with water vapor, it manifests as acid rain. If NOx is inhaled, it could combine with the moisture in your nose, throat and lungs and create nitrous and nitric acid. This is toxic

and has the potential to destroy healthy tissue (Nkwocha and Egejuru, 2008). The output of machines designed to produce ozone, can also not target the source to stop the toxidity. Abdul Hameed *et al.* (2009) studied airborne bacterial and fungal composition in the industrial town of Helwan in Egypt using a slit impactor sampler during the period from March 2006 to February 2007. Airborne bacterial concentrations were usually higher than fungi. Bacteria and fungi had similar diurnal variation patterns.

The objective of this study was to investigate the airborne fungi and bacteria collected in indoor and outdoor environment. The study was carried out in four areas, using conventional enumeration of airborne micro-organisms and relied on a culture-based method for bio-aerosol sampling, aimed at generating an exposure database and examine the relationship between the in- and outdoor culturability of fungi and bacteria. The primary goal of the bio-aerosol sampling was the quantitative evaluation of the viable airborne bacteria and fungi. Besides the standard enumeration of culturable microbes as CFU/m³, this study attempted to identify and evaluate the colonies through their specific colour, turbidity or other characteristics that appear when grown on selective media. The qualitative assessment was based on the characteristics given in Merck's "Microbiology Manual 2000".

MATERIALS AND METHODS

Description of locations

Kuwait is located in the north-east corner of the Arabian Peninsula and is one of the smallest countries in the world in terms of land area. The flat, sandy Arabian desert covers most of Kuwait. Kuwait is the only country in the world which has no natural lakes or water reservoirs. There is little difference between the country's highest and lowest points, with the highest point in the country being 306 m above sealevel. There are nine islands which are part of Kuwait, all of which with the exception of Failaka Island are uninhabited. Bubiyan is the largest island which is part of Kuwait, covers 860 km² and is connected to the rest of the country by a 2,380 m long bridge. The land area is considered arable and sparse vegetation is found along its 499 km long coastline. Kuwait City is located on the Kuwait Bay, a natural deep-water harbor. Kuwait has some of the world's richest oil fields with the Burgan field which has a total capacity of approximately 70 billion barrels $(1.1 \times 10^{10} \text{ m}^3)$ of proven

oil reserves. During the 1991 Kuwaiti oil fires, more than 500 oil lakes were created covering a combined surface area of about 35.7 km². The resulting soil contamination due to oil and soot accumulation have made the eastern and south-eastern parts of Kuwait uninhabitable. Sand and oil residue have reduced large parts of the Kuwaiti desert to semi-asphalt surfaces. The oil spills during the Persian Gulf War have also drastically affected Kuwait's marine resources.

Kuwait has an arid continental climate. Summer season, which lasts from May to September, is extremely hot and dry with temperatures easily exceeding 45 °C during daytime. Kuwait has a fairly high diurnal temperature range (day-night temperature difference). Winter season, from November through February, is cool with some precipitation and average temperatures around 13 °C with extremes from -2 °C to 27 °C. Annual rainfall averages less than 127 mm and occurs chiefly between October and April. The spring season in March is warm and pleasant with occasional thunderstorms. The frequent winds from the northwest are cool in winter and spring and hot in summer. Southeasterly winds, usually hot and damp, spring up between July and October whilst hot and dry south winds prevail in spring and early summer. The Shamal, a northwesterly wind which is common during June and July, causes dramatic sandstorms. The sampling locations are the Kuwait University (KU) classrooms and garden which are located in the Alkhaldiya area. The latter is the second sampling location (KH), while the third location is at the Kuwait Institute for Scientific Research Laboratory (KISR). The fourth location is the Qurain area (QU) which is located towards the south of the Kuwait city. Outdoor samples were taken from the Kuwait university garden.

Air sampling

Field measurements include air sampling the inand outdoor air. Measurements were taken concurrently or consecutively at each measurement site. As far as recreational facilities and households are concerned, the majority of indoor air measurements were recorded from the middle of the facility or living room at breathing height, while the outdoor air measurements were taken from outside a window of the surveyed facility or room. The air sampling was performed during regular evening hours (between 19:00 and 22:00) on weekdays (Monday thru Thursday). The classroom samples were collected during morning class time and break times and were conducted from the rear of the classroom so as to minimize interrupting classes. All the bio-aerosol samples were taken without controlling any indoor environmental conditions. Petri dish containing a mycological medium was used for fungi test and brain heart infusion agar (BHI) for bacteria incubation. Fungi were incubated for 24 h at 25 °C and bacteria were incubated for 24 h at 37 °C. This is illustrated in Fig. 1. Fungi were identified from microscopic to genus and bacteria were performed in Gram stain.

RESULTS AND DISCUSSION

The experiments are an investigation into the types and numbers of airborne micro-organisms and were carried out in four varying types of areas. 26 groups of bacteria and fungi either of human or environmental origin were detected. Environmental agents generally predominated while significantly higher counts were detected as the level of hygiene or standard of housing dropped. 7 genera of fungi, mainly members of the genus Aspergillus, were isolated from all residents. Microbial occurrence and indoor air quality in the more affluent areas were similar to that reported in a clean hospital environment.

The study showed that fungal spores *Aspergillus* niger and *Penicillium spp*. The indoor and outdoor median viabilities of fungi were 55 % and 25 %,



Fig. 1: Incubation of the samples

respectively, which indicates that an indoor environment provides more favorable conditions for the survival of aerosolized fungi. The highest in- and outdoor culturability of fungi was observed in the spring. Cladosporium had the highest median value of culturability (38 % and 33 % for indoor and outdoor, respectively) followed by Aspergillus/Penicillium (9 % and 2 %) among predominant genera of fungi. Increased culturability of fungi inside the homes may have serious implications because of the potential increase in the release of allergens from viable spores and pathogenicity of viable fungi on immunocompromised individuals. The four parameters surveyed in the present study were all found to influence the indoor and outdoor bio-aerosol levels: sampling time in outdoor and indoor, agar type for measuring the fungal and bacteria species, location, and spring survey periods.

In this experiment, the plate was exposed to air for a distinct period of time starting from 1 h and ending after 3 h to provide the optimum condition for different organisms to grow as illustrated in Tables 1 and 2. In Tables 3 and 4 the CFU/m³ were represented where high CFU/m-3 were found outdoors in periods of 3 h. High CFU/m-3 where found in- and outdoors in the Al-Qurain area.

Brain heart infusion agar is a rich media suitable for cultivation of a wide variety of organism types. Samples were collected using BHI agar for the enumeration of bacterial and fungal colony forming

Out door	1h	2 h	3 h
		Staphylococcus	Aerococcus urinae
	Diphtheroids	haemolyticus	Micrococcus Ietus
		5types diphtheroids	Staphylococcus haemolyticu
KH		(gpb)	Acinetobacter haemolyticus
		Asperigllus niger	3type diphtheroids
			Asprigllus niger
KISR	Staphylococcus	Diphtheroids	Staphylococcus haemolyticu
KISK	haemolyticus	Micrococcus letus	6types diphtheroids
	Diphteroids	Escherichia coli	Sphingomonas paucimobilis
	Leclercia	Staphylococous	Acinetobacter lowffii
QU	adecarboxylata	hominis	Kocuria krsitinae
QU	Staphylococcus	Diphtheroids	Ochrobacterum authropi
	warneri	Staphy lococcus	Diphtheroids (gpb)
		warneri	
	Diphtheroids	Kocuria kristinae	6 typesdiphtheroids
	Stap.haemolyticus	Ochrobactrum Antropi	Acinetobacter lowffii
KU		Diphtheroids	Micrococcus letus
			Staph haemolyticus
			Kocuria kristihae

Table 1: Colony identification for outdoor air open plate technique

	Table 2: Color	y identification	for indoor	air open	plate technique
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Indoor	1h	2 h	3 h
	Diphtheroids	Staphylococcus	Staph. Haemolyticus
KU	-	haemohyticus	Aerococus viridous
			Asprigllus niger
	Staphylococcus	Diphtheroids	Micrococcus leteus
KISR	Haemolyticus	Staphylococcus leutus	Staphylococcus hominis
	-		Staphylococcus xylocsus
	Staphylococcus Lentus	Stahpylococcus Lentus	Staph. Leutus
	Acinetobacter lowffii	Strep. Sanguinis	Paracoccus yeeii
QU		Acinetobacter lowffii	Staphylococcus gallinaium
			3Streptococcus Sanguinis
			Asprigllus niger
	Streptococcus	Kocuria kristinae	Acinetobacter lowffii
VII	Sanguins	Ochrobactrum anthrop	Kocuria kristinoe
KU	-	Dipheroids	Diptheroids
		-	Staphylococcus lentus

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Indoor	1h	2 h	3 h
KH	1 large yellow colony	11arge yellow colony	1 larg yellow colony
		2 fungi colonies	13 small white colonies
		-	1 fungi colony
KISR	2 medium white colonies	1 big yellow colony	3 small yellow colonies
		2 small white colonies	3 big white Colonies
		5 yellow small colonies	
QU	3 medium white colonies	10 white small colonies	13 medium white colonies
			1 fungi colony
KU	1 medium white colony	8 medium white colonies	9 medium white colonies
	Table 4: Colony for	ming unit/ plate for outdoor air sam	nling
Out door	1h	2 h	3h
Outdoor	In	2 П	3 11
KH	2 large yellow colonies	4 large yellow colonies	2 large orange colonies
	3 large white colonies	2 penicillium spp.	4 small orange colonies
		1 large orange colony	1 large yellow colony
		3 small yellow colonies	7 medium white colonies
		3small white colony	3 large white colonies
		12 small white colony	10 small white colonies
KISR	5 small white colonies	3 large orange colonies	1 large yellow colony
		8 small yellow colonies	1 large white colony
		4 small white colonies	1 medium white colony
			1 medium orange colony
			4 small yellow colonies
			7 small white colonies
QU	7 large white colonies	11 large white colonies	10 large white colonies
	10 small yellow colonies	1 large orange colony	1 large yellow colony
		1 medium yellow colony	1 medium white colony
		10 small white colonies	18 small white colonies
KU	1 large yellow colony	10 large white colonies	1 large orange colony
	2 medium white colonies	2 medium white colonies	2 large white colonies
	8 small yellow colonies	1 medium yellow colony	6 medium yellow colonies
		1 small white colony	10 small white colonies

Table. 3: Colony	1 forming	unit/ n	late for	indoor	air	sampling

units (CFU). Figs. 2-4 indicate BHI agar after one, two and three hours. After incubating the plate for 24 h, a variety of organism species are grown. The levels of occurrence of the bacteria and fungi identified in the in- and outdoor air from four different micro-environments; Khaldya, KISR, Kuwait University, AlQurain are represented in Tables 1-3. The numbers of colonies during in- and outdoor air pollution are shown in both Figs. 5 - 6 and Tables 3 -5. In the four areas different and an abundance of bacteria which appeared rapidly in four places were detected. A high diversity of micro-organisms appeared in the AlQurain area. The in- and outdoor bio-aerosol concentration measured during spring were significantly higher compared to the measurement during winter. The reason for this is that temperature and relative humidity are closely associated with microbial growth. It is essential to evaluate the quality of the air we breath whether

indoors or outdoors. The majority of people in Kuwait spend most of their time indoors as a result of extreme weather conditions. All buildings are air-conditioned for most of the year. Ventilation is one of the key factors which affects particle deposition rates indoors (Jamriska, 2000; Howard-Reed et al., 2003; Wallace et al., 2004). The guidelines in both the United Kingdom and the United States and also in Kuwait avoid any discussion of the risks posed by airborne microorganisms. Instead, the focus is on providing a comfortable environment. In this study, the air pollution indoor and outdoors along with its effects on residents from four different residential areas were investigated. 3 BHI agar plates are used for each area with different lengths of recording time starting from 1 h and extending up to 3 h. This is the optimum time that different species need to grow other bacteria like fungi (The more the plate is exposed to the air, the more growth there will be occured).



Fig. 2: BHI agar plate after 1 h



Fig. 3: BHI agar plate after 2 h



Fig. 4: BHI agar plate after 3 h

Bacteria occur in most environments; particularly in dusty, dirty places inhabited by human or other animals. Many of the species of bacteria isolated from the buildings are harmless and frequently include members of the genera bacillus and micrococcus and also diphtheroids bacillus. Species that have been isolated and can cause problem are pseudomonas spp.

Microbial occurrence and indoor air quality in the more affluent areas were similar to that reported in a clean hospital environment. It was concluded that although the numbers and types of microbial population in domestic homes were high, they have little adverse effects on human health. Brain heart infusion agar is a general-purpose medium suitable for the cultivation of a wide variety of organism types, including bacteria, yeasts and molds. With the addition of 5 % or 10 % sheep blood, it is used for the isolation and cultivation of a wide variety of fungal species, including systemic fungi 1 from clinical and non-clinical sources. BHI Agar derives its nutrients from the brain heart infusion, peptone and dextrose components. The peptones and infusion are sources of organic nitrogen, carbon, sulfur, vitamins and trace substances. Dextrose is a carbohydrate source that micro-organisms utilize by fermentative action. The medium is buffered through the use of disodium phosphate. When defibrinated sheep blood is added to the basal medium, it provides essential growth factors for the more fastidious fungal organisms.

Microbial flora of indoor air depend on several factors, including the number and hygienic standard of people present, the quality of the household system and mechanical movement within the enclosed space. In poor quality and crowded domiciles, the higher number of residents confined to a small space result in the build-up of airborne microbes shed by the human body. For both the inand outdoor air samples, the concentration of total bacteria were higher than the concentration of total fungi for all the areas. For individual fungi species the concentration order in both the in- and outdoor air was Cladosporium, Penicillium, Aspergillus and Alternaria in descending order. For both the total bacteria and the total fungi, the outdoor concentration for the four different areas were usually higher compared to the indoor concentrations. The outdoor bacterial concentrations were also significantly higher than the indoor bacterial concentrations. The indoor concentrations of Aspergillus and Penicillium were usually higher than the outdoor concentration in kitchens, the concentration of organism is high and

Indoor and outdoor bacterial contamination

Organism	No.
Diphtheroids(non pathogenic, gram positive bacilli)	31
Staphylococcus haemolyticus (non pathogenic, gram positive coccus)	17
Acinetobaetor(non pathogenic, gram negative bacilli)	13
Kocuria Kristinae (non pathogenic, gram positive coccus)	8
Micrococcus luteus(non pathogenic, gram positive coccus)	8
Ochrobactrum anthropi (non pathogenic, gram negative bacilli)	4
Staphylococcus hominis(non pathogenic, gram positive coccus)	4
Fungi(Asperigillus, penicillum)	15



Fig. 5: Indoor air colony counts for the three hours and four locations



Fig. 6: Outdoor air colony counts for the three hours and four locations

very similar to that found outdoors due to the fact that kitchens naturally have higher temperatures, more humidity, and more nutrients available for bacteria to exist naturally. In comparison to the above, indoor air pollution in areas of 'lesser quality' had higher bacterial counts. These poor quality houses generally had a lower hygienic standard, a higher number of residents. Consequently, less provision is made for effective ventilation systems. The higher the number of residents confined to a small space, the higher the build -up of airborne microbes shed by the human body. In comparison, residences found in more affluent areas were normally better ventilated, enabling a sweeping out effect and also had resident cleaning staff and a higher awareness of hygiene.

CONCLUSION

An assessment of the airborne bacteria and fungi in the indoor and outdoor environment were experimentally investigated. Experiments of the types and numbers of airborne micro-organisms were carried out at four varying types of areas during spring season. The current study clearly indicates that there is significant assessment of the indoor and outdoor airborne bacteria and fungi. The following conclusions can be made:

- (a) Bacteria show higher growth comparing to slow growing fungi
- (b) In- and outdoor median viabilities of fungi were 55 % and 25 %, respectively
- (c) Cladosporium had the highest median value of culturability
- (d) Microbial occurrences and indoor air quality in the houses of the more affluent residents houses and the indoor concentrations of Aspergillus and Penicillium were usually higher than the outdoor concentration.

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