Indoor air studies of fungi contamination at the Tabacco factory in Kavala, Greece

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ABSTRACT

Purpose: The aim of the study was to assess the presence of airborne fungi at the tabacco Factory in Kavala, Greece.
Material and methods: Materials for the tests were: the air samples (in front of the building and the selected rooms) of the Museum tabacco factory-old, Museum tabacco factory-new, and old tabacco factory. The air pollution was determined using SAS SUPER 100 (Pbi International). The microbial flora from walls was assessed using the Count-Tact applicator and the plate Count-Tact (BioMerieux). Humidity and temperature were evaluated by a termohygrometr.
Results: The following fungal pathogens isolated from air were *Aspergillus, Candida albicans, Candida spp*., and *Penicillium species.* The dominated fungal pathogens isolated from the air samples were *Aspergillus* and *Candida albicans.* We found a comparable number of fungi colonies in these three museums. No significant correlation between CFU of fungi in air and temperature in the tested museums was noted. Similarly, no relationship between CFU of fungi in air and humidity was found.
Conclusions: The main fungal pathogens isolated from the air samples were *Aspergillus* and *Candida albicans* in the tabacco factory in Kavala.
Key words: indoor air fungi, SAS SUPER 100, Greece

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INTRODUCTION

Fungi and moulds are biological hazards that are ubiquitous both in the communal and occupational environments. Human exposure to airborne fungi in residential, occupational, and industrial settings has been shown to cause a variety of negative health effects.

Numerous studies have demonstrated that onset of Sick Building Syndrome (SBS) could at least be partially owing to the exposure to the biological agents [1,2,3]. Common moulds such as *Alternaria, Aspergillus, Penicillium, Mucor, Drechslera, Cladosporium, Fusarium* and *Ulocladium*, which occur frequently in almost all the environments, are reported to cause various diseases like rhinitis, dermatitis and allergic asthma [3,4].

Moulds can pose a health hazard to library, archive and museum workers [4].

In the literature [2,5,6], it is emphasized that all construction buildings create excellent conditions for the settlement, growth and reproduction of numerous and varied organisms. It is estimated that several dozen species of bacteria can live in buildings (mainly Gram negative), more than 400 species of fungi (mainly *Aspergillus, Cladosporium, Penicillium, Fusarium* genus), several species of fungi causing decay processes of wood and wood-based materials, many species of algae, bryophytes, lichens, plant seeds, including decorative (e.g. benjamin ficus, abutilon), and over 30 species of mites (mainly in house dust), over 300 species of insects (posing parasitological and sanitation threats and destroying the structural wood of houses), several species of rodents, several species of birds (living on the roofs and external walls of buildings) and several species of bats [6-9].

Generally, museum workers are at no particular health risk if the collections are stored under appropriate hygienic and climatic conditions and the construction standards for this type of facilities are complied with [10,11]. If the storage conditions change, e.g. the indoor temperature or humidity increases, the collections may become colonized with microscopical fungi presenting a health hazard to the workers.

To our knowledge, no study on environmental contamination by airborne fungi in the museum of old Tabacco factory in Kavala (Greece) was performed.

The aim of the study was to assess the presence of airborne fungi at the tabacco Factory in Kavala, Greece.

MATERIAL AND METHODS

The fungal air pollution was done using a SAS SUPER 100 (pbi international) with international measure standards (EN 50081-1, EN 500 50082-1). Air sampling were carried out in the tabacco Factory in Kavala (Greece) in May 2008. Material into the mycological studies was air sampled at the entrance of factory building, hall and the selected brooms of factory and museum of the tabacco Factory.

Sample has a flow rate of 100 liters air/min. At each site, a 100 liters sample was taken with the sampler placed at a height of 150 cm above floor level in the middle of the room, with all windows and doors closed. Plates from SAS SUPER 100 were incubated. After incubation number of fungal colonies and number of fungi in air volume was counted. In according to producer, at the first part of investigation number of fungal colonies at plates (real number of colonies-RNC) was corrected on statistical probability multiple passage of particle through the same hole (number of colonies corrected). In according to formula, it was estimated CFU (number of colonies at 1000 L of air): X = (P x 1000) : V, where : V- volume of air sample , r – number of counted colonies at contact plate, P - corrected number of colonies (in according to producer instrument), X – number of colonies (CFU) at 1000 L (1 m3) of air. The microbial flora from the walls was detected using the Count-Tact applicator and the plate Count-Tact (BioMerieux).

Classification of isolated fungi was made with an accordance to the current procedures. After incubation at 27°C for three days quantitative analysis and morphological evaluation of fungal colonies were carried out, and depending on the nature of the fungi cultures the plates were incubated for up to 14 days to allow identification. The raw counts of colonies on the agar plates were adjusted by reference to statistical scaling tables applying to the particular sampler. The cultured fungi were identified from macroscopic and microscopic characteristics, and biochemical tests were appropriate.

Yeast-like fungi were identified by means of original Candida ID (bioMerieux) medium, API 20C AUX (bioMerieux) identification sequence as well as CandiSelect (Bio-Rad) medium. For moulds, microscopical evaluation of morphological elements in the preparations was performed. Temperature and humidity were measured by thermo-hygrometer PWT-401 (Elmetron). Laboratory studies were performed in Poland.

Wilocoxon's paired test and Spearman rank test were used. Significance was defined as a p value of 0.05. This analysis was performed on a personal computer with a commercially available statistics program (Statistica 7.0 PL).

RESULTS

Table 1 presents fungal occurrence in the air of rooms of the New Museum of Tabacco factory in Kavala, Greece. The following fungal pathogens were isolated from air were: *Aspergillus species, Penicillium species Candida albicans, Candida sp.., Botyris sp.,* and *Cladosporium sp..* Numbers of the airborne culturable fungi were the highest in the archive room, the I room of tabacco processing and tabacco bath. Mean number of fungi colonies isolated from air was 224.93 ± 261.66, mean temperature 25.66 ±1.48°C, and humidity 60.96 ± 4.64. No significant correlation (R= - 0.339; p=0.257) between CFU of fungi in the air

Table 1. Fungal occurrence in the air of rooms of the New Museum of tabacco Factory in Kavala, Greece.

Room	Number of colony	Type/ Species	Temperature	Humidity	Air flow
Archive room I	560	Botrytis sp., Candida sp.	24.0	50.7	0.01
Archive room II	18	Penicillium sp., Aspergillus sp.	24.5	55.4	0.001
Tabacco bath	600	Aspergillus sp.	25.1	63	0.11
Band after starting	450	Botrytis sp., Penicillium sp. Aspergillus sp.	25.8	65	0.01
Room III 1 st floor	9	4 Cladosporium sp., 5 C.albicans	26.2	65	0.01
Room corner	500	Aspergillus sp.	23.5	65.3	0.01
Room of tabacco processing 4 th floor	580	Aspergillus sp.	25.6	62.1	0.01
Old cellar	21	6 Penicillium sp., 15 C. albicans	24.3	60.1	0.01
Room	30	23 Penicillium sp., 7 C. albicans	27.5	56.3	0.01
Room III 4 th floor	5	2 Penicillium sp. 3 C. albicans	24.6	67.5	0.03
Corridor between floors	29	1 Penicillium sp.,21 C. albicans, 7 Candida sp.	26.5	63.7	0.01
Office	119	94 Penicllium sp., 20 C. albicans, 5 Candida sp.	27.2	61.3	0.02
Outside of building	3	3 C. albicans	28.8	57.2	0.86

and temperature was noted. Similarly, no relationship (R=0.02; P=0.941) between CFU of fungi in air and humidity was found. Table 2 presents fungal occurrence *Candida albicans sp., Aspergillus sp., Acremonium strictum* and *Penicillium*. Mean number of fungi colonies isolated from air was 74.25±180.14, mean in the air of rooms of the Old Museum of Tabacco factory in Kavala, Greece. The following fungal pathogens isolated from air were: *Candida albicans*, temperature 26.25 ±0.88°C, and humidity 56.87 ±21.39. No significant correlation (R=-0.411;p=0.695) between the CFU of fungi in air and temperature was noted.

Table 2. Fungal occurrence in the air of rooms of the Old Museum of tabacco Factory in Kavala, Greece.

Room	Number of colony	Type/Species	Temperature	Humidity	Air flow
Outside of building	3	1 Aspergillus sp., 2 C. albicans	27	63.9	0.03
Store room 2nd floor	520	Aspergillus sp.	26.6	56.7	0.02
2 nd floor	11	3 Acremonium strictum 1 Penicillium, 7 C. albicans	26.5	64.5	0.02
3 rd floor	10	1 Aspergillus 3 Acremonium strictum	25	77.9	1.02
4 th floor	15	1 Penicillium sp. 3 Acremonium strictum, 11 C. albicans	26.6	61.9	0.09
Room II near main entrance	15	2 Acremonium strictum 10 C. albicans, 3 Candida sp.	26.2	65	0.08
Corridor near entrance	11	9 C. albicans, 2 Candida sp.	28.2	6.18	0.13
5th floor	9	1 Acremonium strictum 8 C. albicans	26.2	63.8	0.09

Table 3 presents of fungal occurrence in the air of rooms of the Old Tabacco factory in Kavala, Greece. The following fungal pathogens isolated from air were: *Candida albicans, Candida albicans sp., Penicillium sp., Cladosporium sp., and Aspergillus sp.*. Mean number of fungi colonies isolated from air was 215.40 ± 283.18, mean temperature 26.00 ± 2.82°C, and humidity 66.00 ± 2.91. Significant ($p<0.05$) difference in air fungal contamination between the museums of Old Factory and the Old Factory was found.

Table 3. Fungal occurrence in the air of rooms of the Old tabacco factory in Kavala, Greece.

Room	Number of colony	Type/ Species	Temperature	Humidity	Air flow
Outside of building	12	2 Pencillium sp., 2 Aspergillus sp., 5 C. albicans., 3 Candida sp.	31.4	64	0.03
Room II 1 st floor	10	1 Cladosporium sp., 3 Penicllium sp., 6 C. albicans	25	67.5	0.04
Room I 1 st floor	500	Cladosporium sp.	25.7	69.5	0.03
Room 2 nd floor	550	Cladosporium sp.	24.3	68.7	0.02
Ground floor	5	1 Penicillium sp., 4 C. albicans	25.6	62.8	0.01

DISCUSSION

In the present study, we demonstrated considerable numbers of fungi in the air of the Museums and Old Tabacco factory in Kavala in Greece. The following fungal pathogens isolated from air were: *Aspergillus species, Penicillium species Candida albicans, Candida sp., Botyris sp., and Cladosporium sp.*. We performed the study in May. In the literature there is clear evidence of seasonal differences in the numbers of fungi in indoor air [12,13]. Mean temperature was about 26^{o}C and low humidity. We did not assess symptoms of allergy in museum workers.

Museum employees are exposed to fungi and storage mites in the workplace. Wiśniewska et al. [10] evaluated the prevalence and risk factors of sensitization to moulds, as well as clinical symptoms associated with allergy in museum workers of the Polish National Museum in Warsaw. A total of 103 employees, potentially exposed to fungi during their work, were assessed using a questionnaire and skin prick tests to common allergens and fungal extracts.

The level of total and serum-specific IgE to moulds was evaluated, and spirometry was performed in all subjects. Mycological analysis of the workplace was also performed. *Penicillium, Aspergillus, Cladosporium, Alternaria, Trichoderma, Acremonium and Paecilomyces* were the most frequent species isolated from investigated exhibits of the museum. Furthermore, 30% of museum employees were sensitized to at least one of the fungal allergens. Logistic regression analysis revealed that duration of occupational exposure lasting > 5 years, family history of atopy, presence of a cat at home, sinusitis, allergic rhinitis and a history of frequent respiratory infections were risk factors for the development of sensitization to fungi in this working group. These findings are partially in agreement with our results on incidence of air borne fungi.

Similar findings also described Krysińska-Traczyk [5]. A study on mycological contamination at an archive facility conducted by Krysińska-Traczyk [5] revealed a large number of mould species: *Peniciullium* genus, *Cladosporium herbarum*, *Geotrichum candidum*, *Cephalosporium glutineum*, *Mucor racemosus*, *Trichoderma viride*, and *Aspergillus niger* in dust samples collected from the contaminated books.

Zielinska-Jankiewicz et al. [11] analyzed, both in quantitative and qualitative terms, workplace air samples collected in a library and archive storage facilities. Occupational exposure and the related health hazard from microbiological contamination with moulds were assessed in three archive storage buildings and one library. Air samples (total 60) were collected via impact method before work and at hourly intervals during work performance. The air sample and surface sample analyses yielded 36 different mould species, classified into 19 genera, of which *Cladosporium* and *Penicillium* were the most prevalent. Twelve species were regarded as potentially pathogenic for humans: 8 had allergic and

11 toxic properties, the latter including *Aspergillus fumigatus*. Quantitative analysis revealed air microbiological contamination with moulds at the level ranging from 1.8 x 10(2)-2.3 x 10(3) cfu/m(3). In surface samples from library and archive artifacts, 11 fungal species were distinguished; the number of species per artifact varying from 1-6 and colony count ranging from 4 x 10(1) to 8-10(1) cfu/100 cm(2). Higher contamination levels were found only for *Cladosporium cladosporioides* (1.48 x 10(3) cfu/100 $cm^{(2)}$) and *Paecillomyces varioti* (1.2 x 10(2) cfu/100 $cm^{(2)}$). At the workposts examined, although no clearly visible signs of mould contamination could be found, the study revealed abundant micromycetes, with the predominant species of *Cladosporium* and *Penicillium*. The detected species included also potentially pathogenic microorganisms which can cause allergic and toxic effects, such as *Aspergillus fumigatus*, that could be hazardous to workers' health. Moreover, for some species, the concentration levels exceeded the values considered the proposed hygienic standards for total microscopical fungi in occupational settings. These results are in accordance with our findings.

The season variation and time of day have an impact on incidence of fungi. We examined the air in the morning and during a spring (May).

Chen et al. [14] collected airborne microbes by means of gravitational sedimentation on open Petri dishes at the Emperor Qin's Terra-Cotta Museum. Three parallel samples were collected at the same time each day, and samples were subsequently incubated in the laboratory. They found 13 bacterial genera and eight genera of fungi were identified from indoor and outdoor air at the museum. As for the comparison of indoor and outdoor samples, the average concentrations of fungi were higher during the afternoon (13:00) than for the morning (09:00). The average concentrations of bacteria in indoor air were higher during the afternoon (13:00) than for the morning (9:00), and in outdoor air, they were lower during the afternoon (13:00) than for the morning (9:00). The average concentrations of five dominant groups of bacteria and three dominant groups of fungi were higher during the afternoon (13:00) than for the morning (9:00) in the indoor air, but the average concentrations of fungi were higher and those of bacteria were lower during the afternoon than for the morning, for outdoor air. As for the comparison of indoor samples, the bacterial daily concentrations and fungal daily concentrations were higher during the afternoon (13:00) than those for the mornings (9:00) over the 10 days. For the comparison of outdoor samples, the bacterial concentration was lower, and the fungal concentrations were higher during the afternoon (13:00) than those for the morning (9:00) over the 10 days. They concluded that museum air was affected by human activity; therefore, it is imperative that the number of visitors be strictly limited and that windows be opened regularly to avoid air pollution.

CONCLUSIONS

In conclusion, the main fungal pathogens isolated from the air samples of the tested tobacco museums were *Aspergillus* and *Candida albicans*. We found a comparable number of fungi colonies in these three museums. No significant differences between the number of fungal colonies, temperature and humidity of air were found.

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