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The fungal flora at various historical locations in Izmir, Turkey

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Abstract

In our study, surface and environmental air samples from the historical buildings (houses, kiosks, Turkish bath, khans, train station) in seven different locations were investigated for their fungal flora. Sampling was performed in seven locations in Izmir (Bornova, Buca, Cesme, Foca, Karsiyaka, Konak and Tire) in autumn and summer of 2009. Totally 192 isolates were obtained from the air and surface samples of these places. Air samples were collected using a MAS-100 Microbial Air Sampler (Merck) and fungal load were detected by using Rose Bengal Chloramphenicol Agar (RBCA, Merck). Biofilm samples were obtained from different surfaces (stone, wood, plaster, marble, limestone, brick and paint). As a result of our study, 25 genera related to *Zygomycota*, anamorph *Ascomycota* and teleomorph *Ascomycota* were identified. Besides, *Aspergillus sp., Penicillium sp., Phoma sp., Alternaria sp., Chaetomium sp.* and *Cladosporium sp.* were appeared the most frequently fungi genera. However, 6 isolates were not identified. It was observed that *Aspergillus sp.* was at the first stage with frequency of 21.88% while *Penicillium* sp. was at the second stage with 17.70%. In our study it was also shown that fungal load was much more in autumn than in summer due to its high moisture content and optimum temperature.

Keywords: Airborne fungi, Ascomycota, Biofilm, Biomass, Microfungi.
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İzmir'de çeşitli tarihi bölgelerin mikrofungal florası

Özet

Bu çalışmada İzmir'deki bazı önemli tarihi binalarda bulunan fungal flora incelenmiştir. Örnekler, 2009 yılının sonbahar ve yaz dönemlerinde İzmir'deki yedi farklı lokasyondan (Bornova, Buca, Çeşme, Foça, Karşıyaka, Konak and Tire) toplanmıştır. Bu lokasyonların hava ve yüzey örneklerinden toplam 192 izolat elde edilmiştir. Hava örnekleri MAS-100 Microbial Air Sampler (Merck) kullanılarak toplanmıştır ve fungal yük Rose Bengal Chloramphenicol Agar (RBCA, Merck) kullanılarak belirlenmiştir. Biyofilm örnekleri çeşitli yüzeylerden (tahta, taş, plastik, mermer, kireçtaşı, tuğla ve boya) alınmıştır. Bu çalışmanın sonucunda *Zygomycota*, anamorf *Ascomycota* ve teleomorf *Ascomycota'*ya ait 25 genus bulunmuştur. Bunun yanında, en sık rastlanılan fungus genusları *Aspergillus sp., Penicillium sp., Phoma sp., Alternaria sp., Chaetomium sp.* ve *Cladosporium sp.* olarak bulunmuştur ancak 6 izolat tanımlanamamıştır. *Aspergillus sp.* %21,88 oranla en sık görülen genus olmuştur. Bunu %17,70 oranla *Penicillium sp.* izlemektedir. Aynı zamanda bu çalışmada, yüksek nem oranı ve optimum sıcaklığa bağlı olarak fungal yükün sonbahar döneminde, yaz dönemine göre çok daha fazla olduğu gösterilmiştir.

Anahtar kelimeler: Havalı kaynaklı funguslar, Ascomycota, Biyofilm, Biyokütle, Mikrofunguslar

Introduction

Cultural heritage provides positive supplement to economy and tourism of a country besides its high civilization expression. Historical artifacts of our country vary widely depending on past cultural wealth of our country. The transfer of these well-preserved historical monuments is an indispensable requirement for continuation of cultural evaluation.

The three important factors influencing the deterioration of monuments and buildings belonging to the cultural heritage are biodeterioration processes, atmospheric deterioration and natural and anthropogenic pollution (Herrera and Videla 2004).

Biodeterioration problems in historic buildings are well recognized and during the past decades the role of the microbes in biodeterioration processes has been acknowledged. Biodeterioration cannot usually be related to single microbial groups; rather, they are a result of complex microbial interactions. After the microbiota contaminates a historical site, they can initiate damaging biodeterioration processes. Typically, although microbes can colonize permanently on various materials in historical sites, their damaging activity is not constant, but rather periodical (Saarela et al. 2004).

In the previous studies, fungi and bacteria were determined from historical wood (Bjordal et al. 1999; Blanchette et al. 1990; Blanchette 2000; Erickson et al. 1990.), mortar (Saarela et al. 2004; Shirakawa et al. 2003), stone (Crispim et al. 2004; Gaylarde and Gaylarde 2000; Krumbein 1988; Lamenti et al. 2000; Lewis et al. 1988; Resende 1996), limestone (Videla et al. 2000), glass (Gurtner et al. 2001), frescos and wall paintings (Berner et al. 1997; Gorbushina et al. 2004; Guglielminetti et al. 1994; Jeffries 1986; Karpovich-Tate and Rebrikova 1990; Krumbein 1988; Saiz-Jimenez and Samson 1981; Sampo and Luppi 1989; Savulescu and Ionata 1971; Sorlini et al. 1987).

The purpose of our study was to characterize fungal flora of some historical buildings belong to 18th and 19th centuries in Izmir in this study. Besides, the interaction between air and biofilm samples was also investigated.

Material and methods

Sampling, isolation and counting

Sampling was performed in seven locations (Fig. 1) such as Bornova, Buca, Cesme, Foca, Karsiyaka, Konak and Tire in Izmir in autumn and summer of 2009. Both airborne and biofilm associated fungi were determined on the historical monuments in these sites.

Air was sampled using a MAS-100 Microbial Air Sampler (Merck) for standard 90 mm petri dishes which contain Rose Bengal Chloramphenicol Agar (RBCA, Merck) to collect a 100-l air sample. Visible biofilms were collected from different surfaces (Stone, wood, plaster, marble, limestone, brick and paint) in the historical buildings. Sterile cotton swabs moistened with sterile saline solution were used to collect sample of the surfaces. These samples were directly spread over the RBCA agar surfaces for isolation.

The number of colony forming units (CFU) in samples was calculated referring to the table for the MAS-100 as per manufacturer's instructions. Numbers of fungi growing on RBCA media were enumerated.

Identification of Fungi

Isolates with different colony morphologies were selected from RBCA and inoculated onto suitable growth media. The growth media and incubation conditions were shown in Table 1.

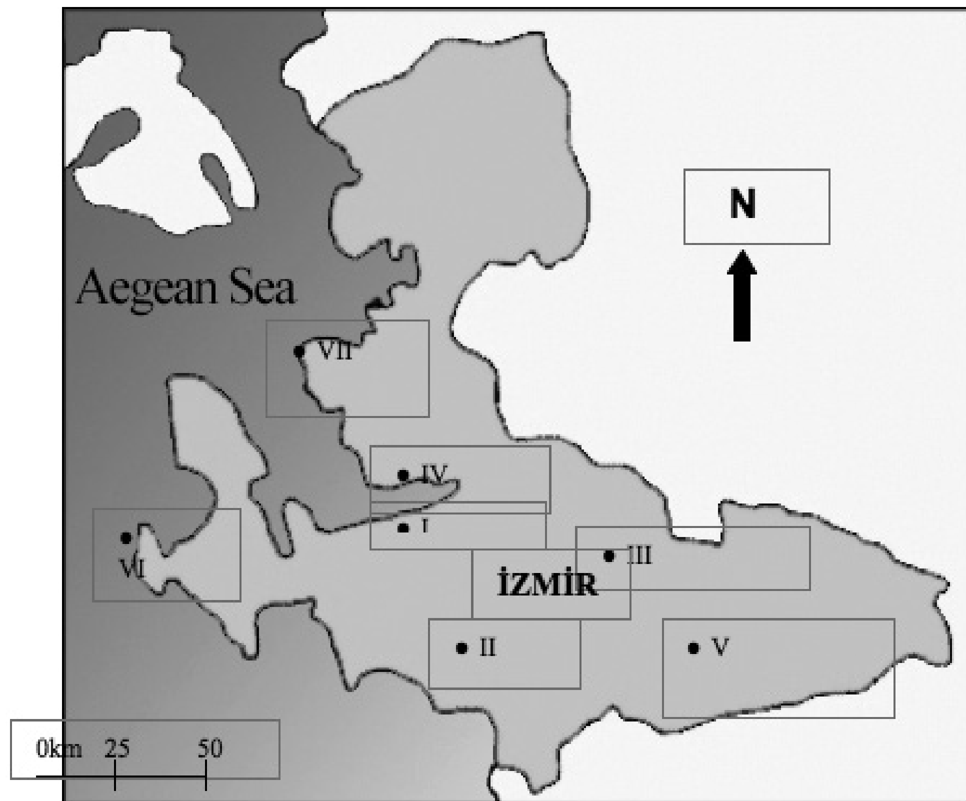

Figure 1. Sampling sites; N: North. I: Konak, II: Buca, III: Bornova, IV: Karsiyaka, V: Tire, VI: Cesme, VII: Foca.

Table 1. Culture media used in the cultural identification and incubation conditions

Genera	Medium	Incubation condition
Aspergillus sp.	Malt Extract Agar (MEA, Oxoid)	25°C, 7-10 days
	Czapek Dox Agar (CDA, Oxoid)	25°C, 7-10 days
	Czapek Yeast Agar (CYA, Oxoid)	25°C and 37°C, 7-10 days
	Czapek Yeast Agar with 20% Sucrose (CY20S)	25°C, 7-10 days
Penicillium sp.	Malt Extract Agar (MEA, Oxoid)	25°C, 7 days
	Czapek Yeast Agar (CYA, Oxoid)	5°C, 25°C and 37°C, 7 days
	25% Glycerol Nitrate Agar (G25N)	25°C, 7 days
Other genera	Malt Extract Agar (MEA, Oxoid)	27°C, 7-10 days
	Potato Dextrose Agar (PDA, Merck)	27°C, 7-10 days

A stereoscopic light microscope with a magnification of 400x and 1000x was used for identification of the fungi. The fungi were identified due to their typical colony and conidia morphology characters according to Pitt and Hocking (1985), Barnett and Hunter (1998), Pitt (2000), Klich (2002), Watanabe (2002).

Results

Quantitative analysis

The number of airborne fungi detected in the historical buildings in summer and autumn were presented in Fig 2.

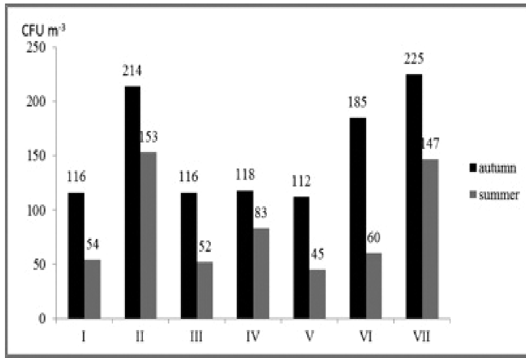

Figure 2. Numbers of airborne fungi; I: Konak, II: Buca, III: Bornova, IV: Karsiyaka, V: Tire, VI: Cesme, VII: Foca.

In our study, it was shown that average numbers of fungi in air were higher in autumn than in summer. The highest number of airborne fungi was detected in the sampling site VII (225 CFU m^{-3}), whereas the lowest number was detected in the sampling site V (45 CFU m^{-3}) (Fig. 2). Totally 192 different fungi and 25 genera were isolated both from air and biofilms. The most dominant fungi were identified as *Aspergillus sp.* (21.88%), *Penicillium sp.* (17.71%), *Phoma sp.* (7.81%) and *Alternaria sp.* (6.25%). 6 isolates obtained from various samples were unidentified (Fig. 3).

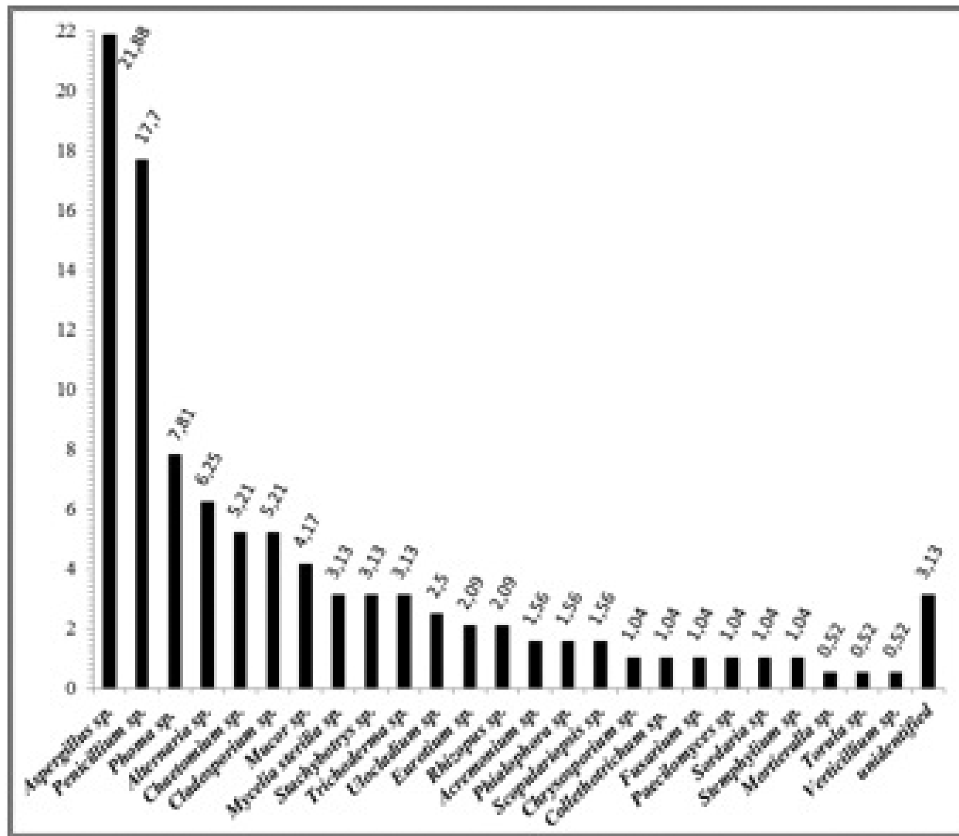

Figure 3. The range of isolated fungi genera.

Qualitative analysis

The results of qualitative analyses for air and biofilm samples were shown in Table 4. Fungi that were identified in our study belong to *Zygomycota*, anamorphic *Ascomycota* and teleomorphic *Ascomycota*. *Aspergillus sp., Penicillium sp., Phoma sp., Alternaria sp., Chaetomium sp.* and *Cladosporium sp.* were the most dominant genera isolated from both air and biofilm samples. The most dominant species were identified as *Aspergillus foetidus, A. niger*, *Penicillium chrysogenum* and *P. janthinellum*.

Table 2. Fungi present in the air and biofilm samples of sampling sites.

Fungi	I		II		III		IV		V		VI		VII	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Acremonium sp.									x			x	x	
Alternaria sp.	x	x		x				x		x	x			
A. alternata	x					x	x							
Aspergillus sp.	x											x		
A. awamori		x		x										
A. candidus	x			x										
A. carbonarius				x										
A. flavipes	x													
A. flavus		x												
A. foetidus		x		x					x					
A. fumigatus											x			
A. niger		x			x		x	x		x	x			
A. ochraceus												x		
A. parasiticus	x	x	x					x						
A. pseudoglaucus	x													
A. sclerotiorum								x			x			
A.terreus						x								x
A. versicolor										x				x
A. wentii	x													
Chaetomium sp.								x		x				x
C. cochliodes		x												
C. gelasinosporum		x												
C. indicum								x						
C. spinosum		x												x
Chrysosporium sp.											x		x	
Cladosporium sp.	x		x		x		x		x	x			x	x
Collethotrichum sp			x											
E. amstelodami					x			x						
E. chaevalieri								x						
Fusarium sp.									x				x	
Mortierella sp.											x			
Mucor sp.			x				x			x	x			
M. circinelloides								x						
M. hiemalis												x		
Mycelia sterilia			x			x				x	x			
Paecilomyces sp.												x		
P. variabilis								x						
Penicillium sp.	x	x	x							x				
P. chrysogenum	x			x		x		x				x		
P. commune								x						
P. expansum	x												x	
P. glabrum	x						x							
P. italicum				x					x				x	
P. janthinellum		x				x				x	x		x	
P. miczynskii										x				
P. oxalicum										x				
P. roqueforti			x											
P. waksmanii					x					x				
Phialophora sp.						x							x	x
Phoma sp.		x	x			x			x			x	x	x
Rhizopus sp.	x						x							
R. stolonifer	x											x		
Scopulariopsis sp.								x				x		
S. brevicaulis						x								
Sordaria sp.										x				
S. fimicola										x				
Stachybotrys sp.		x				x					x			x
Stemphylum sp.											x			
Trichoderma sp.		x				x		x		x				x
T. viride										x				
Torula sp.				x										
Ulocladium sp.			x		x						x	x		
Verticillium sp.												x		

I: Konak, II: Buca, III: Bornova, IV: Karsiyaka, V: Tire, VI: Cesme, VII: Foca; A: isolated from air; B: isolated from biofilm

The results showed that the airborne and biofilm samples had both similarities and differences (Table 2). The fungal flora of biofilm was more diverse than air samples. Many fungal genera were isolated from both air and biofilm (*Acremonium* sp., *Alternaria* sp., *A. alternate, Aspergillus* sp., *A. candidus, A. foetidus, A. niger, A. parasiticus, A. sclerotiorum, Cladosporium* sp., *Eurotium amstelodami, Mucor* sp., *Mycelia sterilia, Penicillium* sp., *P. chrysogenum, P. italicum, P. janthinellum, P. waksmanii, Phoma* sp., *Rhizopus stolonifer, Stachybotrys* sp., *Ulocladium* sp.) while some of them were present in only air (*Aspergillus flavipes, A. fumigatus, A. pseudoglaucus, A. wentii, Chrysosporium* sp., *Collethotrichum* sp., *Fusarium* sp., *Mortierella* sp., *Penicillium expansum, P. glabrum, P. roqueforti, Rhizopus* sp., *Scopulariopsis brevicaulis* and *Stemphylum* sp.) or biofilm (*Aspergillus flavus, A. awamori, A. ochraceus, A. terreus, A. versicolor, Chaetomium* sp. and the other species of this genus, *Eurotium chaevalieri, Mucor circinelloides, M. hiemalis, Paecilomyces* sp., *P. variabilis, Penicillium commune, P. miczynskii, P. oxalicum, Scopulariopsis* sp., *Sordaria* sp., *S. fimicola, Trichoderma* sp., *T. viride, Torula* sp. and *Verticillium* sp.)

Discussion

Besides their beneficial effects, fungi have some harmful impacts such as mycotoxins, pathogenicity, allergens, food spoilage and biodeterioration of materials (Sterflinger 2010). In recent years, people in charge of the conservation of cultural heritage objects have become more aware of the problems relating to microbial colonization and biodeterioration. Physical and chemical weathering, environmental pollutants and presence of micro- and macro-organisms represent the main agents responsible for the deterioration of cultural heritage. The impact of varying combination of physical, chemical and biological effects leads to an extent of damage caused by biodeterioration at different locations in a historical site ranging from inconspicuous to obvious aesthetic and physical and/or chemical damage (Lamenti 2000). In our study, fungal flora of some historical buildings (mosque, house, hostel, hammam) belonging to 14th-19th centuries in Izmir which are important historical centers were investigated. Air and surface samples were collected from seven sampling sites such as Bornova, Buca, Cesme, Foca, Karsiyaka, Konak and Tire in autumn and summer of 2009.

Guiamet et al. (2012) found that *Aspergillus* sp. and *Penicillium* sp. were the most abundant microorganisms since they can be easily cultivated. They also detected *Fusarium* sp., *Candida* sp. and *Rhodotorula* sp. in their samples. Irbe et al. (2012) revealed that they identified commonly *Antrodia, Gloeophyllum, Athelia, Hyphoderma, Hyphodontia, Pharenochaete, Postia* and *Botryobasidium* from their samples. In this study, 192 isolates were characterized from both air and biofilm samples. 25 of them belong to *Zygomycota*, anamorphic and teleomorphic *Ascomycota*. Among these fungi *Aspergillus sp., Penicillium sp., Phoma sp., Alternaria sp., Chaetomium sp.* and *Cladosporium sp.* were the most frequent genera (Fig. 3). 6 isolates obtained from various samples were not identified. Duncan et al. (2010) expected that they would obtain the highest level of airborne fungi in the end of the summer. However, their results were variable for summer and winter. Oliveira et al. (2010) studied the effects of meteorological conditions of Amares and Porto on fungal growth. *Cladosporium, Agaricus, Aspergillus/ Penincillium, Alternaria, Coprinus* and rusts were the most abundant fungi in their study. They found the lowest values in winter while the highest values were in Autumn. This was compatible with our results as the highest value was found in autumn (13.3%) while the lowest

value was found in summer (2.7%) in our study (Fig. 2). These results may be obtained due to the weather condition in Izmir in Autumn and Summer.

Saarela et al. (2004) studied air and biofilm samples obtained from catacombs in some locations in Italy. They reported that fungi were more diverse in air than biofilm. In our study, we also observed that fungi were more abundant in air than biofilm as genera rates were given in Fig. 3 and the species that were found in air were given in results section.

In conclusion, characterization of the microbiota including fungi colonized on historical monuments and understanding of complex biological interactions will provide more information about biodegredation processes. Besides, it will be useful for the determination of biodegradative and bioactive metabolites for further studies. This investigation is essential to restrict damaging microbial growth in terms of protecting the historical artifacts in order to transfer our historical wealth to next generations.

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