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Fungal Bioaerosols in Indoor Air Environments of Health Services Vocational School in Marmaris, Turkey

Vedat Kadir ÖZKAN
Muğla Sıtkı Koçman University, Health Services Vocational School, 48700, Marmaris, Muğla, Turkey
Orcid ID: 0000-0002-1049-8282/ vedatkozkan@mu.edu.tr

Abstract: In this study, it was aimed to determine the fungal bioaerosols in the indoor air environments of Health Services Vocational School and a total of 50 samples were taken from offices, classrooms, dining hall and canteen indoor air. The samples were taken by using Petri dishes containing Rosebengal Chloramphenicol Agar and they were kept open for 30 minutes at a height of 1.5 meters from the ground. Fungi obtained for diagnostic purposes were incubated in Czapek Dox Agar and Malt Extract Agar media at 27^0C for two weeks. After incubation, morphological and microscopic examinations were carried out and fungi were diagnosed with the help of related references. As a result, 27 microfungi species belonging to the 12 genera were identified. The dominant microfungi strains are *Cladosporium, Alternaria, Acremonium* and *Aspergillus*, respectively, and microfungus species is *Cladosporium herbarum*. Fungal bioaerosols levels did not exceed acceptable values in all sampling areas. This research has revealed that fungi affecting human health have been found, the indoor air quality has to be checked for a certain period of time, and more work has to be done on the subject.

Key words: Fungal bioaerosols, Microfungi, Microfungal contamination, Indoor air quality

Marmaris (Türkiye) Sağlık Hizmetleri Meslek Yüksekokulu Kapalı Alanlarında Fungal Biyoaerosoller

Öz: Sağlık Hizmetleri Meslek Yüksekokulu kapalı alanlarında fungal biyoaerosolleri belirlemeyi amaçlayan bu çalışmada ofis, sınıf, yemekhane ve kantin iç ortam havalarından toplam 50 örnekleme yapılmıştır. Örnekler, yerden 1.5 metre yükseklikte Rosebengal Chloramphenicol Agar içeren petrilerin kapakları 30 dakika süreyle açık bırakılarak alınmıştır. Elde edilen funguslar teşhis amacıyla Czapek Dox Agar ve Malt Extract Agar besiyerlerine ekilerek 27^0C'de iki hafta inkübe edilmiştir. İnkübasyondan sonra morfolojik ve mikroskobik incelemeler yapılarak ilgili referanslar yardımıyla funguslar teşhis edilmişlerdir. Sonuçta 12 cinse ait 27 mikrofungus türü belirlenmiştir. Dominant olarak elde edilen mikrofungus cinsleri sırasıyla *Cladosporium, Alternaria, Acremonium* ve *Aspergillus*, mikrofungus türü ise *Cladosporium herbarum*'dur. Fungal biyoaerosol düzeylerinin, örnekleme yapılan bütün alanlarda kabul edilebilir değerleri aşmadığı görülmüştür. Bu araştırmada insan sağlığını etkileyen fungusların bulunmuş olması, iç ortam hava kalitesinin belirli sürelerle kontrol edilmesi ve konu ile ilgili çalışmaların daha çok yapılması gerekliliğini ortaya çıkarmıştır.

Anahtar kelimeler: Fungal biyoaerosoller, Mikrofunguslar, Mikrofungal kontaminasyon, Kapalı alan hava kalitesi

Introduction

Indoor air quality affects human health because of people spending most of their time indoor. Due to poor microbiological quality of indoor air quality, various infections, respiratory diseases, allergic reactions, toxicities and infiammations can ocur in humans and their performance can be affected and work efficiency can be decreased (Gorny et al. 2002; Fischer and Dott 2003; Fung and Hughson 2003; Hardin et al. 2003; Curtis et al. 2004; Singh 2005; İnal et al. 2007; Ökten and Asan 2009; Arshad 2010; Çeltik et al. 2011; Meheust et al. 2014; Ghosh et al. 2015; Heo et al. 2017). Microbiological factors that worsen indoor air quality are biologically derived particles called bioaerosols. These include pathogenic or nonpathogenic, live or dead fungi, bacteria, bacterial endotoxins, mycotoxins,peptidoglycans, viruses, high molecular weight allergens, polen, etc. (Güllü and Menteşe 2007; Ökten and Asan 2009; Heo et al. 2014; Ghosh et al. 2015; Heo et al. 2017).

Fungi affecting indoor air quality constitute a signicifant part of bioaerosols. Consentrations of fungi in indoor environments vary depending on such factors as temperature, humudity, materials, number and circulation of people, building characteristics, geographical and climatological conditions, heating-cooling and ventilation systems (Güllü and Menteşe 2007; Kalyoncu 2008; Saylam et al. 2011; Alçay and Yalçın 2015; Ghosh et al. 2015; Heo et al. 2017; Yılmaz et al. 2017). In indoor environments where environmental conditions are appropriate, concentrations of fungi are increasing and adversely affect human health. The effects of fungi are allergy, infection and toxicity (Gorny et al. 2002; Fischer and Dott 2003; Fung and Hughson 2003; Hardin et al. 2003; Curtis et al. 2004; Nunes et al. 2005; Singh 2005; Güllü and Menteşe 2007; İnal et al. 2007; Kalyoncu 2008; Ökten and Asan 2009; Arshad 2010; Çeltik et al. 2011; Saylam et al. 2011; Övet et al. 2012; Heo et al. 2014; Meheust et al. 2014; Sharifzadeh et al. 2014; Alçay and Yalçın 2015; Ghosh et al. 2015; Heo et al. 2017; Yılmaz et al. 2017). Because of their impact on human health, many researches have taken an interest, and therefore complation of fungal bioaerosols in indoor air has been prepared (Fischer and Dott 2003; Hardin et al. 2003; Curtis et al. 2004; Singh 2005; Arshad 2010; Meheust et al. 2014; Alçay and Yalçın 2015; Ghosh et al. 2015), and also the determination of bioaerosol levels (Gorny et al. 2002; Güllü and Menteşe 2007; Heo et al. 2014), the effects human health (Fung and Hughson 2003; İnal et al. 2007; Heo et al. 2017), hospital environments (Nunes et al. 2005; Ökten and Asan 2009; Sharifzadeh et al. 2014), school environments (Çeltik et al. 2011; Övet et al. 2012), office environments (Nunes et al. 2005; Saylam, et al. 2011), shopping centers (Nunes et al. 2005), nursing homes (Yılmaz et al. 2017) and residental buildings (Kalyoncu 2008) and fungi.

The identification of fungal bioaerosols in indoor air is very important because they adversely affect human health, performance and work efficiency. Thus, by taking precautions against fungal aerocontaminants, it will be easier and more conscious to be protected from their harmful effects, ensure indoor hygiene and increase air quality. The aim of this study is to determine the fungal bioaerosols in indoor air environments of Health Services Vocational School.

Material and Method

In the Health Services Vocational School, 50 samples were taken from 22 indoor environments (13 offices, 7 classes, 1 dining hall and 1 canteen). The sampling was done in triplicate in September 2017 and the windows were kept closed. The samples, petri dishes containing Rosebengal Chloramphenicol Agar (RCA), are 1.5 meters high from the ground and the lids were left open for 30 minutes. The petri dishes were immediately brought to the laboratory and incubated at 27^0C for two weeks. After incubation, the microfungi colonies were one by one counted and transferred to the tubes containing the Potato Dextrose Agar (PDA) and isolated. After two weeks of incubation, the fungi developed in each tube were planted in the petri dishes containing Malt Extract Agar (MEA) and Czapek Dox Agar (CDA) for diagnostic purposes. The microfungi colonies that developed after two weeks of incubation at 27^0C were examined for their morphological and microscopic characteristics and were identified according to the relevant references (Domsch et al. 1980; Hasenekoğlu 1991; Ellis et al. 2007; Refai and El-Yazid 2014).

Results and Discussion

In this study, 27 microfungal species belonging to 12 genera were obtained. The distributions of the obtained microfungi species and genera in the sampled areas are given in table 1 and 2. *Cladosporium* was isolated from all indoor environments and it was determined to be the most dominant (43.88%) fungus genus qualitatively and quantitatively (Table 1, 2). *Alternaria* (14.44%), *Acremonium* (10.55%) and *Aspergillus* (10.00%) were observed respectively. Among microfungi species, *Cladosporium herbarum* was the highest frequency (22.22%) and the most common

species (Table 2). Previous studies (Fischer and Dott 2003; Fung and Hughson 2003; Curtis et al. 2004; Güllü and Menteşe 2007; İnal et al. 2007; Kalyoncu 2008; Ökten and Asan 2009; Arshad 2010; Çeltik et al. 2011; Saylam et al. 2011; Övet et al. 2012; Meheust et al. 2014; Sharifzadeh et al. 2014; Ghosh et al. 2015; Yılmaz et al. 2017) have indicated that the high frequency and commonly found fungi in this study are common fungi frequently isolated from the indoor air environments. This study findings are paralel to the relevant references.

In present study, the highest fungal concentration was found in offices (46.66%), followed by classrooms (35%), dining hall (12.22%) and canteen (6.11%) (Table 1,2). It is natural that fungal concentration is high because of the higher number of samples in offices. It can also be said that living plants and soils in the pots in offices may have increased fungal concentration. The high concentration of mold (35%) in the classrooms can be related to the circulation of students and the transfer of fungal fragments to the classroom environments with their clothes and things, especially the shoes.

Table 1. Distributions of microfungal genera in indoor air environments

Microfungal genera	Classroom	Offices	Dining hall	Canteen	Colony numbers	Ratio to total colony number (%)
Acremonium	4	12	3	-	19	10.55
Alternaria	12	4	6	4	26	14.44
Aspergillus	4	13	-	1	18	10.00
Cladosporium	31	40	5	3	79	43.88
Curvularia	-	2	-	-	2	1.11
Drechslera	3	-	2	-	5	2.77
Fusarium	4	2	-	-	6	3.33
Penicillium	1	5	-	-	6	3.33
Phoma	1	2	2	-	5	2.77
Pithomyces	1	4	1	1	7	3.88
Rhizopus	2	-	1	1	4	2.22
Ulocladium	-	-	2	1	3	1.66
Total colony numbers and present rates	**63 (%35)**	**84 (%46.66)**	**22 (%12.22)**	**11 (%6.11)**	**180 (%100)**	

Table 2. Distributions of microfungal species in indoor air environments

Microfungal species	Classroom	Offices	Dining hall	Canteen	Colony numbers	Ratio to total colony number (%)
Acremonium kiliense Grütz	4	12	3	-	19	10.55
Alternaria alternata (Fr.) Keissler	5	4	-	1	10	5.55
Alternaria brassicicola (Schwein.) Wiltshire	5	-	4	1	10	5.55
Alternaria tenuissima (Kunze) Wiltshire	2	-	2	2	6	3.33
Aspergillus fumigatus Fresen.	-	2	-	-	2	1.11
Aspergillus niger Tiegh.	4	1	-	1	6	3.33
Aspergillus sclerotiorum Huber	-	1	-	-	1	0.55
Aspergillus versicolor (Vuill.) Tirab.	-	9	-	-	9	5.00
Cladosporium cladosporioides (Fr.) Vries	5	8	-	-	13	7.22
Cladosporium herbarum (Pers.) Link	14	18	5	3	40	22.22
Cladosporium macrocarpum Preuss	5	5	-	-	10	5.55
Cladosporium oxysporum Berk.	7	2	-	-	9	5.00
Cladosporium sphaerospermum Penz.	-	7	-	-	7	3.88
Curvularia lunata (Wakker) Boedijn	-	2	-	-	2	1.11
Drechslera biseptata (Sacc.&Roum.) Richardson&Fraser	3	-	2	-	5	2.77
Fusarium oxysporium Schltdl.	1	-	-	-	1	0.55
Fusarium Link *sp.1*	1	2	-	-	3	1.66
Fusarium Link *sp.2*	2	-	-	-	2	1.11
Penicillium canescens Sopp.	-	3	-	-	3	1.66
Penicillium citrinum Thom	-	1	-	-	1	0.55
Penicillium glabrum (Wehmer) Westling	-	1	-	-	1	0.55
Penicillium lanosum Westling	1	-	-	-	1	0.55
Phoma Sacc. *sp.1*	-	2	2	-	4	2.22
Phoma Sacc. *sp.2*	1	-	-	-	1	0.55
Pithomyces chartarum (Berk.&Curtis) Ellis	1	4	1	1	7	3.88
Rhizopus stolonifer var. *stolonifer* (Ehrenb.) Vuill.	2	-	1	1	4	2.22
Ulocladium botrytis Preuss	-	-	2	1	3	1.66
Total colony numbers and present rates	**63 (%35)**	**84 (%46.66)**	**22 (%12.22)**	**11 (%6.11)**	**180 (%100)**	

Acceptable fungal concentration levels in indoor environments should not exceed 1.000 cfu/m^3 according to the Turkish Standards Institute (Yılmaz et al. 2017). This value 500 cfu/m^3 according to World Health Organization (WHO), 200-500 cfu/m^3 according to American Industrial Hygiene Association (AIHA) and there is no standardization and it varies between countries (Gorny et al. 2002; Curtis et al. 2004; Nunes et al. 2005; Güllü and Menteşe 2007; İnal et al. 2007; Çeltik et al. 2011; Meheust et al. 2014; Ghosh et al. 2015; Heo et al. 2017; Yılmaz et al. 2017). The fungal concentration levels in this study were below the acceptable limits in all areas sampled. In addition to the building properties and climatological factors, cleaning of the offices and the classrooms daily, cleaning of the flor and iner walls of the dining hall and canteen by using detergent twice a day, frequent cleaning of tables, less student circulation and ventilation are among the factors that reduce fungal concentration. The fact that the fungal concentration is below the acceptable limits should not mean that there will be no fungal diseases and that it will not affect people. Because, a single fungus spore or piece that can grow under appropriate conditions can increase vegetative volume and produce a large number of spores. As a result of this, allergies, infections, toxicities and inflammations may occur.

Acremonium, Alternaria, Aspergillus, Cladosporium, Curvularia, Drechslera and *Penicillium* obtained in present study are indicated as they lead to various allergic disorders in different sources (Fischer and Dott 2003; Fung and Hughson 2003; Hardin et al. 2003; Curtis et al. 2004; Singh 2005; İnal et al. 2007; Kalyoncu 2008; Ökten and Asan 2009; Arshad 2010; Çeltik et al. 2011; Saylam et al. 2011; Meheust et al. 2014; Ghosh et al. 2015; Yılmaz et al. 2017). In some studies (Fischer and Dott 2003; Curtis et al. 2004; Singh 2005; Ellis et al. 2007; Güllü and Menteşe 2007; Saylam et al. 2011; Meheust et al. 2014; Refai and El-Yazid 2014; Ghosh et al. 2015), it is suggested that *Acremonium, Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium* and *Rhizopus* constitute various infections. In some works (Fischer and Dott 2003; Fung and Hughson 2003; Hardin et al. 2003; Curtis et al. 2004; Ellis et al. 2007; Ghosh et al. 2015; Yılmaz et al. 2017) fungi belonging to *Aspergillus, Fusarium* and *Penicillium* are reported to adversely affect human health by producing mycotoxin.

Phoma isolated in this research but not mentioned above is widely found in soil and plants in nature and rarely shows pathogenicity in humans (Ellis et al. 2007; Refai and El-Yazid 2014). However, it has been reported that it may cause cutaneous, subcutaneous, corneal and systemic infections (Refai and El-Yazid 2014). *Pithomyces* is commonly found in plant materials, in the air, in soil, in straw and dry grass, in lumberand in the ceiling plaster (Ellis et al. 2007). *Pithomyces chartarum* causes facial eczema in some animals, usually in sheep (Ellis et al. 2007; Refai and El-Yazid 2014). *Ulocladium* is a fungus which inhabits the soil and decaying herbaceous plants. It is widely distributed in nature and may be isolated from paper, textiles and wood as well. *Ulocladium* is commonly considered as a contaminant and it may very rarely cause human disease. *Ulocladium* species may lead to phaeohyphomycosis and particularly subcutaneous infections (Refai and El-Yazid 2014).

Increase in the concentration of fungal bioaerosols in indoor environments can lead to respiratory diseases, allergies, hypersensitivity, irritations, cutaneous, subcutaneous and systemic infections, mycotoxicoses and can cause major problems in human health (especially in atopic, elderly, immunocompromised, organ transplanted individuals). For this reason, improving the indoor air quality also means protecting human and environmental health.

The presence of fungi affecting human health in this study suggests that indoor air in common areas of humans should be clean. This is also important in terms of productivity in work places, which depends on human performance. Based on all these, it shows that necessary to carry out such researches in order to make education-training activities more effective in indoor environments in schools. Because the identification of fungi, which can create a risk for human health in indoor environments, will facilitate the fight against them and ensure that protective precautions to be taken.

To improve air quality and protect human health in indoor, the following may be recommended:

a. Central heating-cooling systems and air purifying systems should be used,

b. Temperature and humidity of enclosed spaces must be controlled,

c. Clean all materials, surfaces and flooring in the enclosed area with appropriate disinfectant,

d. Environment and building characteristics should be improved,

e. Bioaerosols affecting indoor air quality should be measured and controlled at certain intervals,

f. Compliance with individual and environmental hygiene regulations,

g. Environmental and health education should be given importance.

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