AEROMYCOFLORA OF JIZAN, SAUDI ARABIA

Syeda Fatima Manzelat

Department of Botany, College of Science and Arts, Ad Darb,
Jizan University, Jizan, Saudi Arabia.

Corresponding author, Email: drsyedafatima@hotmail.com

Abstract

This is the first study to isolate and identify the aeromycoflora of Jizan province. The study was carried out to estimate quantitatively and qualitatively the mycoflora present in the air of different sampling sites of the province. Open petri plate method with Potato Dextrose agar media was used with an exposure time of 2 mins. at each of the seven sampling sites. Total number of eight genera were isolated from the seven indoor and outdoor sampling sites of the province represented by *Aspergillus niger, Aspergillus sp., Chaetomium, Cladosporium, Nigrospora, Penicillium, Rhodotorula, Scedosporium and Ulocladium.* Seventy two fungal isolates were obtained from the eight represented genera. *Aspergillus* was the predominant genera. The Colony forming unit $(CFU)^3$ was calculated for all of the sampling sites under study to know the level of contamination. The high wind speed and the busy hours of business were the major factors responsible for large number of fungal counts during this study from the province.

Key words: *Aeromycoflora, Jizan , Saudi Arabia*.

Introduction

Air is a composite mixture of microbes in which can be found the diverse groups of fungal spores responsible for the decomposition of wastes and also responsible for the diseases of plants , animals and human beings. The more the number of these spores the more is the pollution of the environment , hence they are the bioindicators of pollution in the indoor and outdoor environments of the place. The present study evaluates the qualitative and quantitative analysis of the aeromycoflora which can help in building the pollution index of that place so that effective monitoring and control measures can be undertaken. There are only few reports of aeromycoflora of Saudi Arabia . Some studies have been done in the Arabian Gulf countries such as Qatar and United Arab Emirates (Ababutain, 2013).

There are reports of aeromycoflora of Abu Arish, Ad Darb and Shuqaiq regions of Jizan but this is the first comprehensive study from Jizan province. *Aspergillus niger, Aspergillus flavus and Penicillium* were reported in the indoor air of Abu Arish (Mostafa et al., 2012). The outdoor aeromycoflora was represented by *Aspergillus niger, Aspergillus flavus, Aureobasidium, Curvularia, Dresclera* ,

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Epicoccum, Fusarium, Penicillium and Yeast. The indoor aeromycoflora was represented by *Aspergillus, Colletotrichum, Penicillium, Chrysosporium and Ulocladium.* The most predominant genus was *Aspergillus* (Manzelat, 2017).

Fungi inhabiting household environments in the West, East and Central localities of Riyadh city were screened. The screened area included bedrooms, drawing rooms, living rooms, kitchens and bathrooms.

The common genera of fungi isolated were *Alternaria, Aspergillus, Cercospora, Chaetomium, Cladosporium, Curvularia, Drechslera, Embellisia, Fusarium, Mucor, Penicillium, Rhizopus, Scytalidium, Trichoderma, Torula* and *Ulocladium* (Bokhary and Parvez, 1995).

Household dust samples from Riyadh, Saudi Arabia showed *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp., *Acremonium* spp., *Botryodiplodia* spp., *Circenella* spp., *Myrothecium* spp., *Syncepalastrum* sp. (Khan, 2012).

The dominant species at the two sites (Al-Batha, a more developed area in the south and Al-Ulia, a less developed area in the north) in Riyadh, were members of the genera *Alternaria, Aspergillus, Cladosporium, Penicillium* and *Ulocladium* (Mahmoud and Alghmadi, 2015).

Though air is not a medium in which microorganisms can grow but has a carrier particulate matter, dust and droplet which can be laiden with microbes. The number and type of microbes in the air is determined by the source of contamination in the environment. For example: microorganisms can spread by coughing, sneezing from the human respiratory tract. Circulation of dust particles from the surface of the earth by air current can also disperse microorganisms in the atmosphere (Aneja, 2003). The result revealed that the temperature and humidity factors were more effective than most weather condition and wind speed on the fungi presence in air (Ababutain, 2013). *Rhodotorula* spp, though considered a common saprophyte, recently has been reported as causative agent of opportunistic mycoses. We present a case of meningitis in an immunocompromised human immunodeficiency virus infec-ted patient who presented with longstanding fever (Scinde et al., 2008).

It commonly leads to skin infections, but in immunocompromised patients it can present with disseminated organ involvement, commonly in brain, lung and bones (Sharma and Singh, 2007). The underlying pulmonary architectural distortion and mucosal defects predispose these patients to colonization and infection with *Scedosporium*, Zygomycetes, and dematiaceous moulds (Kubak and Huprikar, 2009). The most prevalent species were *Aspergillus niger, A. flavus, Penicillium chrysogenum, Stachybotrys chartarum, Ulocladium atrum, Mucor racemosus* and *Fusarium solani* and *A. niger, A. flavus, Trichoderma viride, P. Chrysogenum, Ulocladium atrum, Chaetomium globosum, C. spirale, Stachybotrys chartarum* and *Mucor racemosus* on the two media, respectively (Bagy and Gohar, 1988).

Rhodotorula species have been reported as a causative agent of opportunistic mycoses in immunocompromised hosts. We report a case of sepsis and meningoencephalitis caused by *Rhodotorula glutinis* in a 20-year-old female patient with systemic lupus erythematosus (SLE), which was diagnosed at autopsy

(Pamidimukkala et al., 2007).

S*cedosporium apiospermum* is the asexual form of a rare fungus *Pseudallescheria boydii*. It is usually present in soil, sewage and dirty water in ditches, ponds, etc. It commonly leads to skin infections, but in immunocompromised patients it can present with disseminated organ involvement, commonly in brain, lung and bones (Shinde et al., 2008). *Nigrospora sphaerica* was isolated from date palm leaves which showed severe symptoms of leaf and stem spot (Abass et al., 2013).

Objectives of this research were to know the occurrence, distribution of the fungi in the busy places of Jizan and to isolate and identify them. This will help in monitoring the environmental status and will also help in knowing the pathogenic genera. Hence the fungal genera in the air can cause asthma, rhinitis, aspergillosis, mycoses, sepsis and other deadly diseases in humans and animals apart from different types of leaf spot and fruit rot diseases etc. in plants

Materials and methods

Sampling site

Aeromycoflora was isolated from seven different indoor and outdoor sites during the year 2017 from the busy locations of Jizan province (Fig. 1).

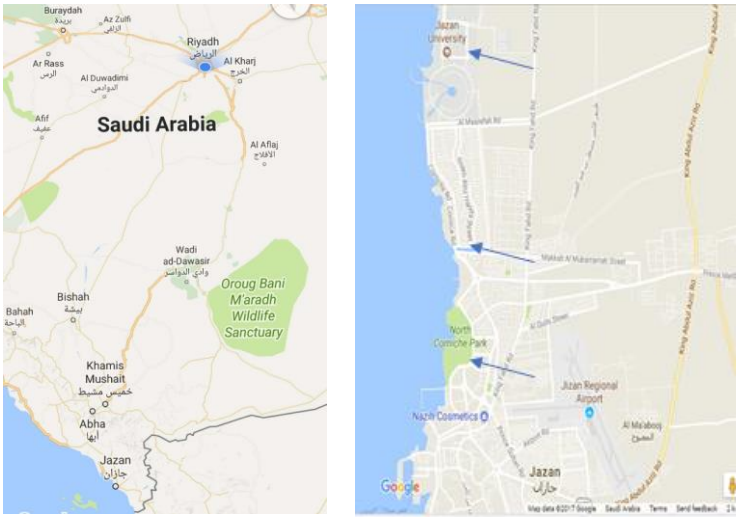

Figure 1
Location map of Jizan with sampling sites.

Indicates the sampling sites

Outdoor environments selected for the study were the sea shore of the red sea at Jizan , fast food restaurant in the center of the city ,public office and the Jizan University. The indoor places were also the fast food restaurant in the center of the city, public office and the Jizan University (Fig. 2).

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Figure 2. *Pictures of the sampling site from Jizan*

Meteorological data of the sampling site

The samples were collected on 1 st of May 2017 with 31°C mean maximum temperature and 29°C mean minimum temperature . The humidity recorded was 71% and there was no rainfall . It was a very windy day near the seashore with a wind speed record of 27 km/hr. The high wind speed was a major factor responsible for large number of fungal counts during this study from the province (Table 1).

Table 1. *Temperature, humidity and rainfall of Jizan on the sampling date.*

Name of the sampling site	Date of sampling	Temperature of the Place (°C)		Humidity (%)	Wind speed (km/hr)	Rainfall
		Maximum	Minimum			
Jizan	1 May 2017	37	29	71	27	Nil

The high wind speed and the busy hours of business were the major factors responsible for large number of fungal counts during this study from the province. Environmental factors such as relative humidity and wind speed shows positive correlation with culturable aeromycoflora.

Weather affects the pollen and mold counts. Air temperature, wind speed, and humidity all affect how much pollen and mold is airborne at a particular moment. Hot, dry, windy days generally mean more pollen and mold is in the air. Pollen levels tend to be lower on rainy, cloudy or windless days (http://wsav.com/2013). Local and regional pollen and mold counts - which measure the amount of airborne allergens are often broadcast along with local weather.

Isolation and identification

Potato Dextrose agar media and Czapek Dox agar media were employed during the study. Petri plates with 10 ml of the media were opened and exposed at the sampling site for a period of two minutes for each of the samples. The fungal cultures on the petri plates with Potato Dextrose Agar media were incubated at room temperature for five days. Lactophenol and cotton blue in lactophenol were used as mounting and staining media for preparing semi permanent slides which were sealed with DPX mountant. The fungal isolates were counted and identified by macroscopic and

microscopic characters using standard procedures. Research microscope with adequate high power has been used through out the study. Identification was carried out by using standard manuals and keys to the identification. Macroscopic and microscopic were used in the identification of the isolated fungal genera. Macroscopic morphological characters like the size and colour of the fungal colony on the surface and reverse of the culture on the petri plate were used in the identification. Microscopic characters include like nature, size and colour of mycelium, sexual, asexual structures and spores. Photographs were taken of all the infected post harvest fruit samples and the fungal cultures on the petriplates. Photomicrographs of the slides were also taken under the microscope with a camera (Fig. 3 a-o, 4a-v).

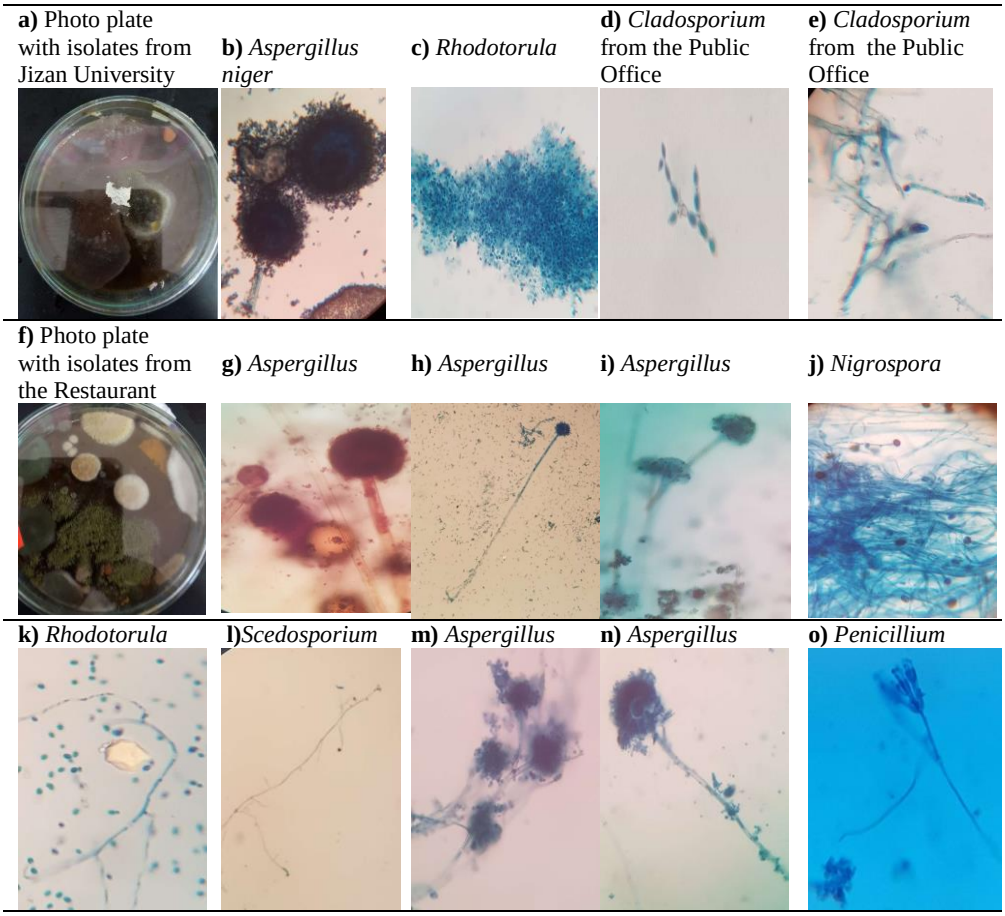

Figure 3. *Photomicrographs of the Indoor Mycoflora*

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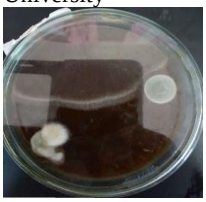
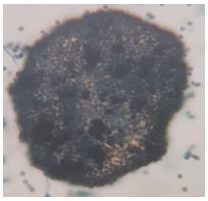
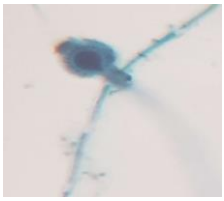
a) Photo plate with isolates from Jizan University



b) *Aspergillus*

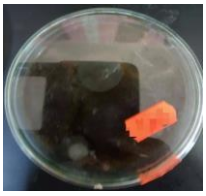


c) *Aspergillus*

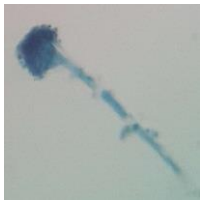


d) *Penicillium*

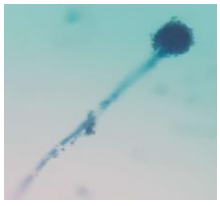
e) Photo plate with isolates from the Restaurant



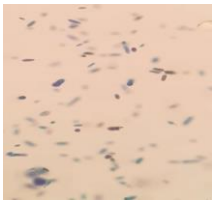
f) *Aspergillus*



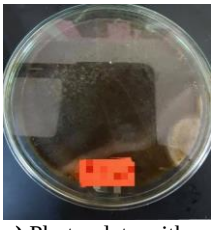
g) *Aspergillus*



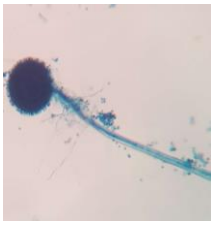
h) *Ulocladium*



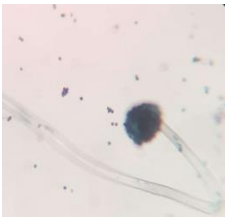
i) Photo plate with isolates from the Public Office



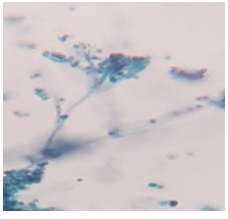
l) *Aspergillus*



m) *Aspergillus*

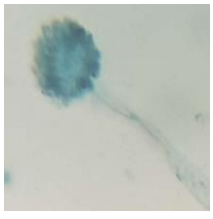


n) *Ulocladium*

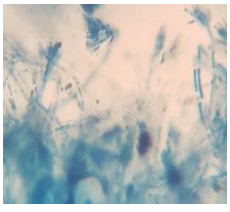


o) Photo plate with isolates from the Jizan Seashore

p) *Aspergillus*

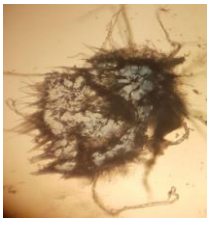


q) *Penicillium*

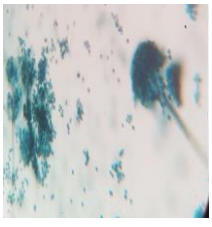


r) *Penicillium*

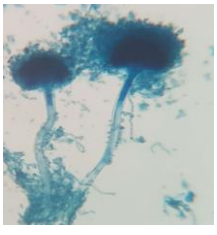
s) *Chaetomium*



t) *Aspergillus s.p*



u) *Aspergillus sp.*



v) *Rhodotorula*

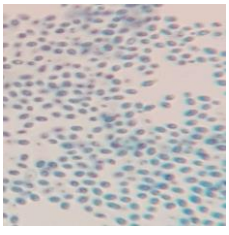


Figure 4. *Photomicrographs of the Outdoor Mycoflora.*

Results and Discussion

Total number of eight genera were isolated from the seven indoor and outdoor sampling sites of the province represented by *Aspergillus, Chaetomium, Cladosporium, Nigrospora, Penicillium, Rhodotorula, Scedosporium and Ulocladium.* Seventy two fungal isolates were obtained from the eight represented genera (Fig. 5).

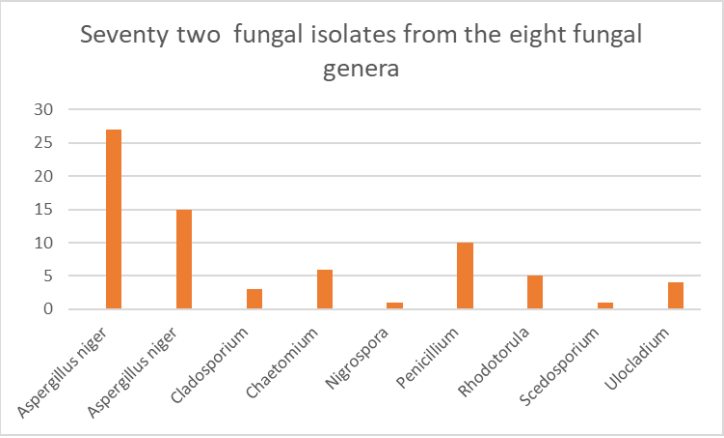


Figure 5
Seventy two fungal isolates from the eight fungal genera.

Total number of six genera were isolated from the three indoor sampling sites of the province represented by *Aspergillus niger* (Fig.3b, 3g, 3h) *Aspergillus sp.(Fig. 3n), Cladosporium* (Fig. 3d, 3e), *Nigrospora* (Fig.3j), *Penicillium* (Fig.3o), *Rhodotorula* (Fig.3c, 3k) *and Scedosporium* (Fig. 3l). Thirty fungal isolates were obtained from the six represented genera (Table 2).*Scedosporium* is the uncommon genera reported in this study

	Name of the sampling site			
	1. Jizan University	2. Public Office	3. Fast food Restaurant	Total fungi isolated
Aspergillus niger	+(3)	+(2)	+(7)	12
Aspergillus sp.	-	-	+(6)	6
Cladosporium	-	+(3)	-	3
Nigrospora	-	-	+(1)	1
Penicillium	-	-	+(4)	4
Rhodotorula	+(2)	-	+(1)	3
Shedosporium	-	-	+(1)	1
Total Number of Colonies (Type)	5	5	20	30
Colony Forming Unit (CFU/m^3)	125	125	500	750

(+) = Present and the number of isolates of the fungi from the site.

Table 2.
Total fungal colony count from the Indoor Environment

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Total number of five genera were isolated from the four outdoor sampling sites of the province represented by *Aspergillus niger* (Fig.4b, 4g, 4l), *Aspergillus sp.* (Fig. 4c, 4f, 4m, 4p, 4t, 4u), *Chaetomium* (Fig. 4s), *Penicillium* (Fig. 4e, 4q, 4r), *Rhodotorula* (Fig. 4v) *and Ulocladium* (Fig. 4h, 4n). Forty two fungal isolates were obtained from the six represented genera (Table 3). The outdoor aeromycoflora was represented by *Aspergillus niger, Aspergillus flavus, Aureobasidium, Curvularia, Dresclera, Epicoccum, Fusarium, Penicillium* and *Yeast*. The indoor aeromycoflora was represented by *Aspergillus, Colletotrichum, Penicillium, Chrysosporium* and *Ulocladium* (Manzelat, 2017). The most common genera in floor dust of Riyadh were *Aspergillus, Penicillium* and *Cladosporium* (Saad and el-Gindy, 1990). The samples from the restaurant were taken during the busy hours of lunch time from a very highly crowded queue. This place showed the largest fungal count both qualitatively and quantitatively thereby proving that the fungal count of a place is directly proportional to crowd and human activity of the place. This is a significant finding, as it is widely recognized that fungal count could decrease in less crowded houses. This finding is supported by several studies which reported that higher fungal spore concentration were associated with crowded places (Ababutain, 2013).

Table 2. *Total fungal colony count from the outdoor environment*

	Name of the sampling site				
	1. Jizan University	2. Public office	3. Fast food restaurant	4. Jizan seashore	Total fungi isolated
Aspergillus niger	+(3)	+(3)	+(5)	+(4)	15
Aspergillus sp.	+(2)	+(2)	+(2)	+(3)	9
Chaetomium	-	-	-	+(6)	6
Penicillina	+(1)	-	+(3)	+(2)	6
Rhodotorula	-	-	-	+(2)	2
Ulocladium	-	+(2)	+(2)	-	4
Total number of colonies (Type)	6	7	12	17	42
Colony Forming Unit (CFU/m^3)	150	175	300	425	1050
(+) = Present and the number of isolates of the fungi from the site.					

Statistical analysis of Colony Forming Unit $(CFU)^3$

The total number of isolates of each genera in the air samples collected from different sites was determined and the colony forming unit $(CFU)^3$ was calculated by using the standard equation given below:

$$(CFU)^3 = \frac{\text{(no. of colonies on the petriplate) x 10000 / (petriplate surface)}}{\text{(petriplate surface)x (petriplate exposure time) x 0.2}} \qquad [1]$$

where Petriplate surface = 10 cm

The colony forming unit $(CFU)^3$ of each of the represented mycoflora was calculated to find out the level of contamination of different sites. The normal standard range of CFU/m^3 is 61-460 set up according to the guidelines of World Health Organization. The values of ranged from 125-500 in the present study.

The $(CFU)^3$ values for the outdoor sampling sites ranged from 150-425 which are all within the normal range. The highest value 425 being from the Jizan Sea Shore. The factor responsible for this high value was the wind speed of 27 km/hr.
The $(CFU)^3$ values for the indoor sampling sites ranged from 125-500. The highest value of 500 was recorded for the fast foor restaurant under study.

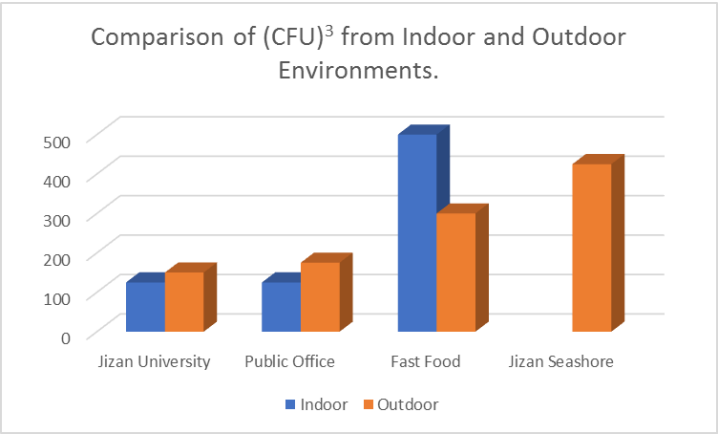

Figure 6
Comparison of $(CFU)^3$ from indoor and outdoor environments.

The major factor responsible for this high value is the crowd in the restaurant during the busy peak hours of afternoon. Hence the we must be careful in crowded places and the restaurants and other public places which maybe potential sources of pathogens. These crowded and busy places must take extra efforts in order to keep these very important places germ free even during the peak business hours in order to maintain cleanliness and hygiene.

Conclusions

The qualitative and quantitative study of aeromycoflora is very important as it can check the harmful mycoflora of the area of study. The area recorded seventy two fungal isolates from the eight represented genera which are potential hazard to the health plants , animals and humans around it. *Aspergillus* was the predominant genera. The Colony forming unit $(CFU)^3$ was calculated for all of the sampling sites under study to know the level of contamination. The study also establishes that high wind speed and the busy hours of business could be hazardous to health as these were the major factors responsible for large number of fungal counts during this study from the province. The study can help in the effective monitoring of air quality thereby helping in the management and minimization of health hazards due to the mycoflora.

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Conflict of interest

The author declares that there is no conflict of interest.

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