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Detection of fungi and yeast in the air dust outdoors of the city of Morelia, Mich, Mexico

Abstract

The air we breathe contains suspended dust particles which can host filamentous fungi that can cause respiratory problems and allergies in humans. Generally, the propagules can survive depending upon the environmental conditions, such as humidity, temperature and cleanness. The aim of this research work was to detect the fungi in dust of outdoor air at the city of Morelia, Mich, México. So far similar study is not reported in this city. For this purpose, the samples were collected at fixed places from October to December 2017 at two times during the day. Results demonstrate the existence of the various fungi genera, from high to low concentration levels: *Aspergillus fumigatus, Penicillium* sp, *A. niger*, *Monilia* sp, *Alternaria* sp, *Mucor* sp, *Acremonium* sp *Cladosporium* sp, and *Chrysosporium* sp including more than one genus of yeasts such as *Candida tropicalis* and *Rhodotorula rubra* were isolated from the dust precipitated from the outside air, regardless of the intensity of solar radiation The existence of *A. fumigatus* and *C. tropicalis* represents a risk for human population who lives in this city in that sense a sanitary measures should be apply.

Keywords: dust, public health, open environments, filamentous fungi, allergies

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Introduction

The content of fungal spores in the air varies according to the location. For example, in the air of tropical forests it constitutes up to 45% by mass of the fine particles greater than 1 µm, while in the air of rural or urban regions it is around 4-11% by mass of particles less than or equal to 2.5 µm. Methods to determine the biological composition of particulate matter collected from outdoor air. It has been reported that on average, the content of fungal spores in the air of the continents is around 103-104 m-3 and the mass concentration of approximately 1 µg / m3, which makes a global emission of 50 Tg / year of organic aerosol, which represents one of the major emission sources.[1] Contribution of fungi to primary biogenic aerosols in the atmosphere: wet and dry discharged spores, carbohydrates, and inorganic ions. Air is the primary means of transport for the dispersal of spores and also, some fungi are major pathogens or allergens for plants, animals and humans.[2,3]

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They found the highest concentration of fungi in summer and the lowest in winter and spring, geographically the highest levels were in the West, Southwest and Southeast, the most abundant fungi in the outdoor air dust were: *Cladosporium*, *Penicillium*, mycelia sterilia and *Aspergillus*. This provides industrial hygienists, allergy specialists, and public health physicians with data on the most common fungi in airborne dust in the United States. While, Sitkowska et al.,[6] conducted an investigation on fungi carried in airborne dust from outside a furniture factory in Poland, central Europe. In order to evaluate the dominance and variation of fungi in air dust and on factory surfaces. By the methods of impact and surface contact. Air dust samples were inoculated onto malt extract agar. The results indicated a high density of: *Aspergillus* sp, *Absidia* sp, *Penicillium* sp, in the dust in the air outside the factory. In this work it is concluded that continuous exposure to dust can cause respiratory infections and allergies in susceptible workers. Cabrales et al.,[7] reported the distribution of allergens in open environments in the city of Bucaramanga, Colombia. They used the Durham harvester, which was slipped with glycerinated gelatin for a period of seven days. The results indicated

85.34% fungi among which 45.06% were deuteromycete conidia, 35.35% ascospores and 7.46% basidiospores. 13.42% corresponded to pollen grains and 1.23% to fern spores. It is concluded that studies are required to determine the clinical importance of these fungi in airborne dust from open environments. Lugauskas et al., 2004. Evaluated the density of fungi in the dust of open environments from the city of Vilnius, Lithuania. Air dust samples were taken from 20 sites in the city, using the plate exposure method with malt extract agar and Czapek. The fungi isolated from outdoor air dust were *Alternaria, Aspergillus fumigatus, A. niger, Cladosporium, Aureobasidium, and Geotrichum*. Which were isolated in higher concentration during spring and in lower concentration during winter. They concluded that this can have a negative impact on the health of people, plants and animals. These results provided information to further assess the risks related to public health. The aim of this research was to detect of filamentous fungi at dust of the air at open places in the city of Morelia, Michoacan, Mexico.

Material and methods

The dust collection in open environments used in this study was carried out in five specific sites in the city, at 9:00 a.m. and 2:00 p.m Table 1. Dust collection from air suspended outside the city of Morelia, Mich, Mexico.

Table 1 Selected sites for the analysis of filamentous fungi and yeast on dust suspended and deposited on the surfaces outdoors of Morelia City, Michoacán, México

Place / population density	Collection sites
Mall/High	Independence Market (IM)
Downtown/High	Valladolid Square (VS)
Mall/High	City Hall (CH)
Residental/ Low	Ocolusen Garden (OG)
Green area and school zone/High	University City, Michoacana University of San Nicolás de Hidalgo (UC)

Technique for the detection of filamentous fungi from outdoor air dust in the city of Morelia, Mich, México (Table 1). The collection of dust from open environments was carried out with an Evar ® brand vacuum pump,[8] 40 liters of air were bubbled for 3 minutes, at a height of 1.35 to 1.50 m from the ground, in a Kitasato flask with 1L of sterile 0.1 N NaCl saline solution with Tween 20 at a concentration of 1 mL / L, to separate the fungi from the dust, the solution was centrifuged at 3000 rpm / 20 min, the precipítate dust was resuspended in 50 mL of saline solution and it was Vortex for 30 sec. The saline solution was filtered on a membrane of 0.2 µm (Milipore [MR]) and the membrane was transferred to a 100 mL Erlenmeyer flask with 25 mL of saline solution, it was shaken and 0.1mL was inoculated on Petri dishes with PDA to pH 5 and chloramphenicol at a concentration of 2IU / L, the samples from air were inoculated with glass beads on PDA which were incubated at 28 °C / 3 to 5 days.[9,5]

Collection of dust from the air and deposited on the surfaces of open sites in the city of Morelia, Mich, Mexico. This technique consisted of spreading a swab over an area of approximately 25 cm2, to collect the dust deposited from the air outside the collection sites, the swab was placed in a tube with 4.5mL Sabouraud broth with 2IU / L of chloramphenicol, which was incubated at 28 oC / 3 days, after Sabouraud broth 0.1 mL was sown in petri dishes with APD for 28 oC / 5 days. The fungi were identified based on the macroscopic and microscopic characteristics and were conserved in soil.[9] Identification of yeasts from the dust precipitated from the air outside the city of Morelia, Mich.

The yeast isolated from the dust on the air and precipítate from the air outside the city of Morelia, their macro and microscopic morphology were determined, and their biochemical identification was carried out by an auxonogram with the assimilation system of carbon and nitrogen sources called API-20 from BioMérieux.[10] For this, a representative group of yeasts from each area of the city was selected, which was suspended in saline solution and inoculated in the microtubes of the API-20C gallery to determine the assimilation response to carbon and nitrogen sources: Nitrate (NO2-N2), Tryptophan (TRP), Glucose (GLU), Arginine-dehydrolase (ADH), Urea (URE), Equalin (ESC), Gelatin (GEL), p-nitrophenyl-ß-D-galactopyranoside (PNG), Glutamine (Glu), L-arabinose (ARA), Mannose (MNE), D- mannitol (MAN) (MAN), N-acetiglucosamine (NAG), Maltose (MAL), Gluconate (GNT), Caprate (CAP), Adipate (ADI), Malate (MLT), Citrate (CIT) and Phenyl-acetate (PAC). The gallery inoculated with each yeast was incubated at 37 °C / 24-48h. The yeast response to the assimilation of carbohydrates and nitrogen was read by comparing each microtube, with the corresponding gallery used as a control inoculated with the yeast but without a carbon source.[10]

Results

The temperature was recorded on each sampling day during the study. The average minimum and maximum temperatures on those days were 12.6 and 25.2, 8.6 and 26.6, 7.8 and 23.2 °C, in October, November and December of that year (2017) respectively. However, with these variations in temperature, did not observe a clear relationship with the fungal and yeasts analyzed on dust suspended and precipitated of air outdoor of Morelia, City.

The results showed in Table 2 of the analysis of outdoor air dust suspended and precipitated on the surfaces of the 5 sites in the city of Morelia, Mich, indicated that were 29% *Aspergillus fumigatus*, *A. niger*, 20%, 18% *Penicillium* sp, 8%. *Monilia* sp, 7% *Alternaria* sp, 7% *Mucor* sp, 5% *Acremonium* sp, 3% *Cladosporium* sp, and 3% *Chrysosporium* sp, while in the genera of *A. fumigatus* were found with 45%, *Penicillium* sp 22% and *A. niger*, *Alternaria* sp and *Rhizopus* sp with 11% at dust precipited in all sites analyzed of the city of Morelia.

On Table 3 shows the main macroscopic and microscopic characteristics of yeasts in the dust precipitated from the outside air of each selected area of the city of Morelia Mich; one with white pigment was detected in the Plaza Valladolid, which is equivalent to 80.0% of the total analysis of the dust precipitated from the outside air. The same in Table 3 showed a 20.0% of *C. rubra* RMS-1 that synthesized an orange pigment. In the City Hall, in the precipitated dust from the outside atmosphere, a white one was found 75.0% in *C. tropicales* RMS-2 and 25.0% with an orange pigment.

On Table 4 is showed the main biochemical proprieties of the yeast recovered, the dust precipitated from the outdoor air in the city of Morelia, Mich, the biochemical profile belong to *Candida tropicales* (close associated to *C. albicans* human pathogen yeast).

Table 2 Frequency and distribution of filamentous fungi on the dust of suspended of the open places at Morelia, Mich, México

Fungy genus	Analysis sites				
	Independence Marquet	**Valladolid Square**	**City Hall**	**Ocolusen Garden**	**University City**
Aspegillus fumigatus	3•/8*	3•/8*	2•/8*	5•/8*	4•/8*
A. niger	3•/8*	2•/8*	3•/8*	3•/8*	0•/8*
Penicillium sp.	2•/8*	2•/8*	2•/8*	2•/8*	4•/8*
Alternaria sp.	0•/8*	1•/8*	1•/8*	0•/8*	2•/8*
Monilia sp.	0•/8*	1•/8*	1•/8*	1•/8*	2•/8*
Mucor sp.	0•/8*	0•/8*	0•/8*	3•/8*	1•/8*
Cladosporium sp.	0•/8*	0•/8*	2•/8*	0•/8*	0•/8*
Acremonium sp.	0•/8*	0•/8*	0•/8*	0•/8*	3•/8*
Chrysosporium sp.	0•/8*	0•/8*	2•/8*	0•/8*	0•/8*

• Number of times the filamentous fungus was found during the samplings. *Number of analyzes performed on the site

Table 3 Detection of yeasts in airborne dust suspended and precipitated outdoor of Morelia, city Mich, México

Hour	Specific sites of the analysis in the city of Morelia, Mich, Mexico	[a]Macroscopic /[b]microscopic morphology / [c]isolation percentage (%)
9:00am	Downtown City Hall (CH)	[a]Colony 2-3 mm, white, round, smooth edge, creamy, opaque, characteristic odor: fruity
		[b]spheroidal and blastoconidia
		[C]75.0
14:00pm	Valladolid Square (VS)	[a]Colony 3-4 mm, white, round, creamy, characteristic odor: fruity
		[b]spheroidal and blastoconidia
		[C]80.0
14:00pm	Independence Market (IM)	[a]Colony 2-3 mm, orange pigment, round, characteristic odor: fruity
		[b]spheroidal and blastoconidia
		[c]20.0
9:00 AM	University City, Michoacana University of San Nicolás de Hidalgo (UC)	[a]Colony 3-4 mm, orange pigment, round characteristic odor: fruity
		[b]spheroidal and blastoconidia
		[c]20.0
9:00 AM	Ocolusen Garden (OG)	[a]Colony 2-3 mm, white, round, smooth, creamy, characteristic odor: fruity
		[b]spheroidal and blastoconidia
		[c]66.66

Table 4 Identification of yeasts in the dust suspended and precipitated from the air outside of the city of Morelia, Mich., México, by assimilation of carbon sources and nitrogen according to API-20C system

*Carbono and nitrogen source assimilation	Genus and species of yeasts	
	Rhodotorula rubra*	**Candida tropicalis***
NO2 - N2	-	-
TRP	+	+
GLU	+	+
ADH	+	+
URE	-	+
ESC	+	+

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Table Continued...

*Carbono and nitrogen source assimilation	Genus and species of yeasts	
	Rhodotorula rubra*	**Candida tropicalis***
GEL	+	+
PNG	+	+
GLU	+	+
ARA	+	+
MNE	+	+
MAN	+	+
NAG	+	+
MAL	+	+
GNT	+	+
CAP	+	+
ADI	+	+
MLT	+	+
CIT	+	+
PAC	+	+

Nitrate., = NO_2-N_2, Tryptophan., = TRP, Glucose., = L-Arginine-dehydrolase.,= ADH, Urease = URE, Escalin, = ESC, Gelatinose, =GEL, P-nitrophenyl-ß-D-galactopyranoside., = PGN, Glutamine, = Glu, Arabinose, = ARA, Mannose., = MNE, D-Mannitol., = MAN, N-acetiglucosamine, = NAG, Maltose, = MAL, Gluconate., = GNT, Caprate., = CAP, Adipate, = ADI, Malto., = MLT, Citrate., = CIT and Phenyl-acetate., = PAC. # Most frequent yeast genera detected in the precipitated dust from the air outside of Morelia, Mich. (+) = Positive assimilation, (-) = No assimilation

Disscusion

The data presented on Table 2 showed that differences in the percentage of fungal propagules showed variation among the five sites at the city of Morelia, specifically at site UC, which had the highest concentration and fungal diversity. This site is located close cemetery the oldest and largest of the city, and it is near to housing and busy avenue that was measured outdoor propagule forming units (PFU) represented as a percentage. In environmental outdoor those genus fungy have been reported related to many allergic humans' problems.[11,12]

The resistance of the propagules of the fungy at dust from the suspended and precipitated air of the city of Morelia, Mich, to radiation depends in part on the melanin of the hyphae of those fungy.[5,13–16]

Related to the most species of *A. fumigatus* is important due is a potential capacity for producing mycotoxins besides its allergenic proprieties, regarding that except at Ocolusen Graden all the places have a high density of the human population which means a health risk for those who are living or working at those sites.[17,12]

While diversity of fungy and yest detected in the city of Morelia, have a negative impact on the public health mainly in the post pandemic condition caused by COVID-19 since fungal diseases have recently been reported to kill 1.5 million people worldwide per year and about half are caused by 3 three opportunistic pathogens: *A. fumigatus* and *Candida albicans*.[12] In this sense, the health authority must establish health programs to prevent respiratory diseases associated with fungi and yeasts in the diverse environment of the city of Morelia.[18]

While on the Table 3 is showed that the fact that yeasts with pigment have been isolated in both sites indicates that they were necessary for them to survive in the air dust in open environments, given the changes in the intensity of solar radiation from 0.68-1.73 KJ and in temperature of 5.7-28.8 °C[7] they reported that these physical factors could decrease the probability of survival in the dust of the atmosphere of open environments[1] even that those yeasts are on the dust of air outdoor of the city.

Finally on the Table 4 is showed that *C. tropicales* and *Rhodotorula rubra* got a pigment to protect them from any stress due to meteorological conditions that prevailed before, during and after the collection of dust air samples. At first it was evident the survival of various genus of yeasts in the dust precipitated from the outdoor air, underline in places with the highest population density and human activity, on the contrary, in the Ocolusen Garden, only one type or none was detected, which is probably justified by the scarce number of inhabitants of that residential area, with low human activity, compared to what happens in the City Hall, University City-UMSNH and Valladolid Square.[19–24]

Conclusion

The diversity genus of filamentous fungi and yest detected in airborne dust and deposited in open spaces and surfaces in the city of Morelia Mich, are indicated that is important to educate to the people to choose a health living style. The genus and species of *A, fumigatus,* as well as *C. tropicalis* detected in higher density represents what has a potential risk of opportunistic fungal diseases especially at certain times of the year due to environmental conditions, which implies establishing sanitary measures in now underline post pandemic conditions caused by COVID-19 to prevent them in the human population of the city of Morelia, Mich, Mexico an important responsibility that the city health authority would have to attend.

Ethical considerations

The approval of the research by the Ethics Committee of the Universidad Michoacana de San Nicolas de Hidalgo followed the guidelines established by that committee.

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Conflicts of interest

The authors declare that there is no conflict of interest for the publication of this article. We are grateful to the project 2.7 (2022) of the CIC-UMSNH Morelia, Mich, Mexico and BIONUTRA, S.A. from CV Maravatio, Mich, Mexico for the support.

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