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Fungal Characterization of the Air Outdoor and Indoor in Five Areas of the City of Puebla-Mexico

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The presence of filamentous fungi as aerosols in the atmosphere outside and inside is a risk to human health and determines air quality. This study aimed to identify the genres of the population of filamentous fungi in indoor and outdoor air of the north, northeast, central, south and southeast of the city of Puebla-Mexico, including buildings of two universities located within these areas during the four seasons of a year. It has been made air monitoring with a volumetric meter "Microflow" and Rodac plates, containing selective media for filamentous fungi. In each selected point they filtered 0.25 m^3 of air by 1.48 minutes. It isolated and identified eleven genera of filamentous fungi in indoor and outdoor air of the la city of Puebla-Mexico, being the predominant genre *Aspergillus* sp with 40.3%, followed by *Alternaria* 11.25%, *Stachibotrys* 9.64% and *Penicillium* with 7.66%. Most colony forming units (CFU) of filamentous fungi were detected in summer with an average temperature of 23 °C and a humidity on 59.33%. The southern region recorded the highest load of filamentous fungi with respect to other areas, due to the emission of aerosols of the Atoyac River, an open and adjacent tributary to the urban area.

Key words: Filamentous fungi, colony forming units (CFU), Environment and air quality.

Exposure to airborne pathogens is a major risk factor for human health[1]. It has been shown that filamentous fungi and bacteria from environmental sources can be dispersed over long distances by air currents and ultimately be inhaled or ingested, constituting a risk factor to human health[2]. The estimate of the density and diversity of these microorganisms in the environment is an indicator of air quality[3].

Aerosols are formed by particles of biological origin or biological activity that can affect living things through processes infectious, allergic, irritating and toxic[3, 4].

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There are reports that some genera of filamentous fungi can be allergens or opportunistic pathogens[5, 6, 7]. Among the known species of fungi, approximately 100,000 have medical interest in outdoor and indoor environments and belong to the Deuteromycetes class or imperfect fungi, with some exceptions[8].

In indoor environments they have shown that fungal diseases are invasive (EFI) and may be an important cause of morbidity and mortality[9, 10]. This study aimed to identify the genres of the population of filamentous fungi in indoor and outdoor air in the north, northeast, central, south and southeast of the city of Puebla-Mexico for four weather stations in the period 2013-2014.

MATERIALS AND METHODS

Sampling areas

The Puebla-Mexico City was divided into five areas, which are described below:

1) North Zone: Is taken outdoor air sample 200 m from Puebla 2000m Industrial Park and 150 m from Atoyac river, for the indoor air, a sample of the Technological University of Puebla was taken, specifically in the Environmental Engineering building, which had a modern building with two levels. This building presented currents on the gateway and the presence of sunlight was limited. 2) Zone Northeast: air sample taken in the exterior and interior of the bus station of Puebla (CAPU), a building which was inaugurated in 1980, this building has a large number of units, approximately 50, 000 passengers a day, is one of the largest bus terminals in Latin America and the country's largest[11] The southern region: Is taken outdoor air sample a 300 meters away from the Atoyac river and for the indoor air, is taken the building sample of high school October 2 (BUAP), specifically in the library "Ernesto Che Guevara", which occupies an area of 28 x 15.83 m, the interior is made up of a coordinating library for 1 person, television room for 40 people, 2 cubicles advisory, presents a collection of 4,192 books, distributed in 4,129 titles and 8,906 volumes located on 7 shelves of metal. 4) The southeastern area: air sample taken in the exterior and interior of the campus of the Benemerita Universidad Autonoma of Puebla (BUAP) and 5) The downtown area: Is taken outdoor air sample and interior the central building of the Faculty of Philosophy and Letters (BUAP), specifically the observatory Alfonso Reyes was monitored.

Environmental Monitoring

Monthly sampling of indoor and outdoor air each of the point described above, starting in September 2013 until in August 2014 was performed. Air samples were collected with an air sampler (M Air T Millipore Co., MA). The air sampler was placed in a position of 1.20 m with airflow of 250 L (0.25 m^3) at a time of 1.48 min. The samples were collected in triplicate in plates Rodac and in Sabouraud dextrose agar and V8 agar. The plates Rodac were incubated at 25 °C by 72 h, subsequently counting colony forming units (CFU) of fungi in each plate[12].

Isolation and Identification

Each fungal colony developed in the culture medium is selected; subsequently it has been made the strain collection of pure colonies where microcultures were performed in blocks of 1 cm agar Sabouraud Dextrose. The identification was made through taxonomic keys based on the colony color, morphology, production method and arrangement of the spores and is also important to know the size and disposition of the hyphae[13, 14, and 15].

Statistic analysis

The obtained data were processed in the statistical package SPSS Statistics version 17 (Statistical Package for Social Sciences) to perform the analysis of variance (ANOVA) and then the multiple comparison test of Tukey ($p < 0.05$), was applied to determine differences between genres of the population of filamentous fungi in indoor and outdoor air of the north, northeast, central, south and southeast of the city of Puebla-Mexico.

RESULTS AND DISCUSSION

Frequency of fungi in the city of Puebla-Mexico

They were isolated and identified a total of 248 fungi corresponding to eleven genera from the outdoor and indoor air. The overall frequency of fungi isolated for a year of the 5 areas studied is shown in table 1.

The gender *Aspergillus* has a frequency of isolation of 40.30%, followed by *Alternaria* with 11.25% and of the *Stachybotrys* gender with 9.64%. The genders *Pythium*, *Ulocladium*, *Rhizopus* and *Cladosporium*, they were less abundant in this study, but nine of the eleven isolated genres dominated the outdoor air. The genus *Aspergillus* is considered a major cause of respiratory diseases in humans caused by bioaerosols, in this study the genre with greater frequency of isolation was *Aspergillus* in the environment outer 30% and 11.2% in indoor. These results are consistent with a study conducted in India; where *Aspergillus* was identified in a percentage higher than 40%. Where 5 species isolated showed greater relevance in cases of allergy[12].

The genera of fungi isolated and identified from the outside air and inside the five zones of the city of Puebla-Mexico, have been associated with allergic and infectious diseases[16] and they coincided with genera of fungi isolated from indoor and outdoor environments of studies of Taekhee and Rashmi Sharma[12, 13].

Work of Kasprzyk and Cordeiro[17, 18] they reported that the main genera of fungi found in indoor air are *Cladosporium*, *Mucor*, *Alternaria alternata*, *Penicillium glaucum*, *Penicillium notatum*, *Aspergillus glaucus*, *A. niger*, *A. versicolor*, *Rhizopus*, *Paecilomyces Scopulariopsis*, among others. These results agree with some of the genera isolated in this work, where gender *Cladosporium* sp had the lowest percentage of isolation.

The gender Ulocladium was found in the same frequency in both exterior and interior air, only gender Stachybotrys had a greater frequency of isolation in indoor air. These results are shown in the table 2. The figure 1 shows some micrographs optical of the morphology of the fungi most commonly isolated.

The highest frequency of isolation of fungi from indoor and outdoor air by area of study (table 1), it was observed that in the south with eleven genders identified (38.26%), followed by the north (25%), the Northeast (20.12%), the center (9%) and Southeast (7.64%). The importance of the genera *Aspergillus* sp, *Stachybotrys* sp and *Fusarium* sp is that they are producers of mycotoxins and are potentially pathogenic to humans, as there are reports that cause allergies and respiratory infections[16].

Table 1. Categories of fungi identified in the northern, south, northeast, southeast and center of the city of Puebla-Mexico

Gender	Study area											
	North		South		Northeast		Southeast		Center		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Aspergillus sp	27	10.88	30	12.09	19	7.66	14	5.64	10	4.03	100 a	40.30
Alternaria sp	6	2.41	9	3.62	4	1.61	3	1.20	6	2.41	28 b	11.25
Stachybotrys sp	5	2.01	8	3.22	6	2.41	2	0.80	3	1.20	24 c	9.64
Penicillium sp	5	2.01	10	4.03	4	1.61	0	0	3	1.20	22 d	9.00
Mucor sp	9	3.62	5	2.01	0	0	0	0	0	0	14 e	5.63
Curvularia sp	5	2.01	4	1.61	5	2.01	0	0	0	0	14 e	5.63
Fusarium sp	0	0	8	3.22	5	2.01	0	0	0	0	13 e	5.23
Phytium sp	5	2.01	4	1.61	2	0.80	0	0	0	0	11 f	4.42
Ulocladium sp	0	0	10	4.03	0	0	0	0	0	0	10 f	4.03
Rhizopus sp	0	0	7	2.82	0	0	0	0	0	0	7 g	3.00
Cladosporium sp	0	0	0	0	5	5	0	0	0	0	5 g	2.01
Total	62	25	95	38.26	50	20.12	19	7.64	22	9.00	248	100

Table 2. Frequency of fungal genera isolated from the outside air and inside of five zones of the city

Gender	Outside		Inside	
	No.	%	No.	%
Aspergillus sp	74	30	26	10.30
Alternaria sp	22	8.87	6	2.38
Stachybotrys sp	9	3.62	15	5.02
Penicillium sp	12	4.83	10	4.17
Mucor sp	10	4.03	4	1.60
Curvularia sp	10	4.03	4	1.60
Fusarium sp	9	3.62	4	1.61
Phytium sp	9	3.62	2	1.00
Ulocladium sp	5	2.02	5	2.01
Rhizopus sp	4	1.61	3	1.39
Cladosporium sp	3	1.20	2	1.00
Total	167	67.45	81	32.08

In Spain, Tormo[19] showed that the largest number of fungal spores found in the environment belong to the genus *Cladosporium* (176.8 spores / m^3), also could compare the air inside was at least 4 times fewer spores than outdoor air. Studies in different countries offer varying results in the total concentration of fungi and their distribution because it basically depends on the season, geographic location and living conditions as well as substrates for fungal growth in different countries[17, 20 and 21]. The Levine test used in the statistical analysis showed both indoor and outdoor air, there was a normal distribution with a

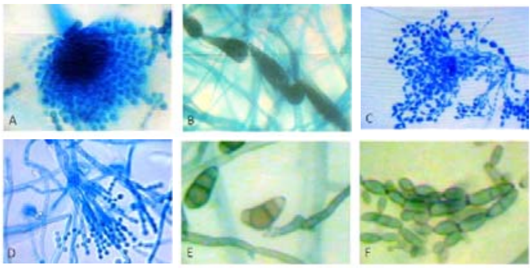

Fig. 1. Optical micrographs (40x) of microcultures of fungi most isolated (AE) and lower isolation frequency (F), blue-stained cotton. A) *Aspergillus* sp., B) *Alternaria* sp., C) *Stachybotrys* sp., D) *Penicillium* sp., E) *Curvularia* sp., and F) *Cladosporium* sp

value of (p <0.001). Comparing the frequency of isolation of indoor and outdoor air with respect to the four weather stations, it significant difference was found of (p <0.005).

To the outside air in summer, it found a significant difference between the numbers of isolates of the south with the southeast, north, northeast and center. In spring, the South and the Northeast presented significant difference from with respect to other areas of study (fig-2). In indoor air, the higher frequency of isolation also corresponded to summer. In summer, spring and autumn the south present a higher frequency of

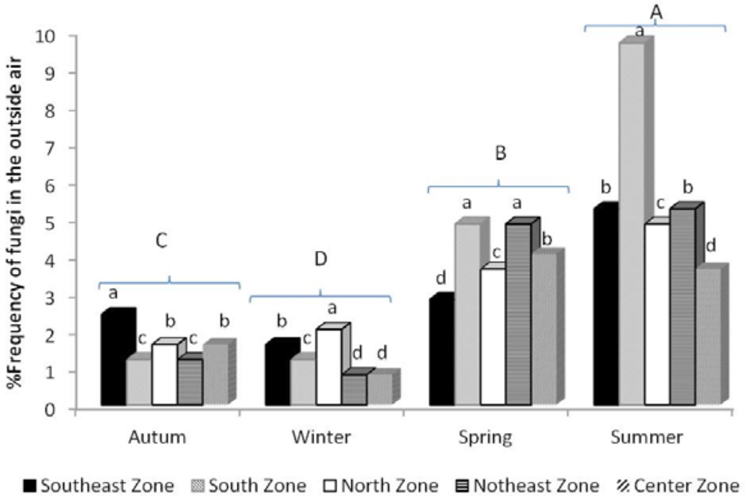

Fig. 2. Percentage of fungi isolated from the outside air in the city of Puebla, during different weather seasons. *Means with different letters in the column indicate significant differences with the Tukey test (α = 0.05), for every season and sampled area.

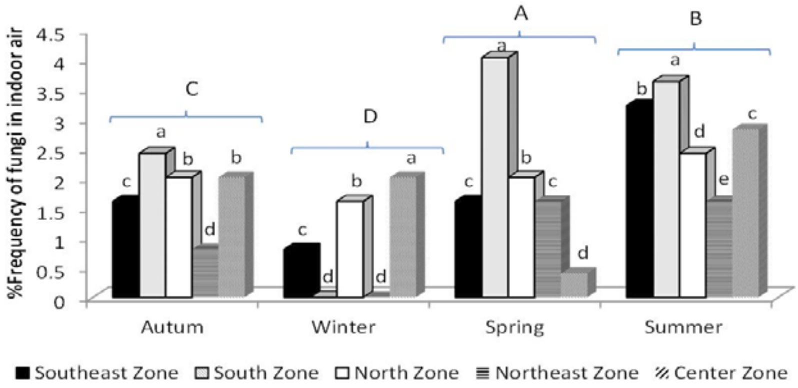

Fig. 3. Percentage of fungi isolated from the indoor air in the city of Puebla, during different weather seasons. *Means with different letters in the column indicate significant differences with the Tukey test (α = 0.05), for every season and sampled area

isolation, without significant differences with other areas of study. In autumn, the downtown area presented a slightly higher frequency of isolation without presenting significant difference from the southern, southeast, north and northeast (fig-3).

It has been reported that fungal spores are present throughout the year; however, there are significant seasonal variations affecting sporulation, dispersion and deposition. These seasonal variations depend on the type of climate, in temperate climates, the maximum concentration of spores occurs in the summer and early fall; while in tropical and sub-tropical regions of the greater abundance of spores it occurs in the coldest months [16]. On the other hand Kasprzyk[17] reported that the hot, dry weather favors the development of conidia, with the largest concentration daily at noon or in the afternoon, when higher temperatures and lower humidity values are recorded, data that are very similar in this investigation.

Climate change may increase the incidence of respiratory diseases, increasing a greater willingness of allergens in the environment[21]. It reported that normal levels of ozone and biofilms could increase the adverse effects due to a synergist at high temperatures[23, 24].

CONCLUTION

In based on the literature and the results obtained in this study, we observed that the frequency of isolated of fungi was higher in the air outside of the five studied areas, the south, was the most contaminated area and has the greatest diversity of species isolated due to the proximity of the Atoyac River and the bioaerosols it emits the river. The genus *Aspergillus* has the highest frequency of isolation in both indoor air and outdoors. The lowest frequency of isolation was for the genus *Cladosporium* (1.2% and 1%) respectively. By season, the most frequently isolated fungi in outdoor air it was in summer (28.6%) and in the case of indoor air, spring won the largest number of fungi isolated.

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