A Cross Sectional Study on the 24 – Hour Variation of Fungal Pollution in the Atmosphere of Erbil City, Iraq

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Abstracts: Airborne fungi are an important part of aerobiological particles; this group includes several etiologic fungi for humans, animals, and plants. Meteorological parameters as well as plantations are actively influencing the density of outdoor airborne fungi. The current study followed the plate exposure method to collect bihourly air samples for 3 days between April 22 and July 7, 2023. The site of sample collection is 15 meters high in the centre of Erbil city. Sabouraud's dextrose agar was used. Plates were exposed for 15 minutes from 7 a.m. to 5 a.m. the following day. Plates were kept at lab temperature for 3 weeks and examined daily for visible fungal growth. Fungi were morphologically identified at the genus level. A total of 322 colonies belonging to 10 identified genera, as well as sterile mycelia SM, sclerotia bodies, chlamydospore colonies, and creamy yeast colonies, were Hyphomycetes, which represented 6 genera, Ascomycetes 2, Zygomycetes, and Basidiomycetes, one for each.SM group showed the highest frequency (F% =48.2%) and occurrence (O% =100%). Aspergillus spp. has the second incidence level (F=12.7% and O%=91.6%) followed by Alternaria sp. (F=9.3% and O= 25%).

Keywords: Airborne Fungi, Aeromycoflora, Meteorological Parameters, CFUs/Hour, Iraq.

1. INTRODUCTION

Above the land surface, almost 25% of the total aerosol particles are made up of biological materials [1]. The bioaerosols include fungal structures, bacteria, viruses, and biological particles. Studies indicate that outdoor airborne fungi OAF fungi are the main source of indoor airborne fungi IAF [2]. These two groups include etiologic diseases, and the variation of fungal communities according to indoor environments and their relationship with temporal and spatial changes were demonstrated [3]. Studies also focused on ventilation systems as an additional factor related to indoor aeromycoflora [4, 5]. The relationship between asthma patients and IAF was validated [6, 7]. Fungal populations vary as a result of the interaction between atmospheric and interior environmental factors [3].

The effects of atmospheric factors AF have received a lot of attention; these factors have an effect on fungal distribution as well as daily and seasonal fluctuations, and several works deal with one or more meteorological factors having an effect on a specific fungus [8]. Nowadays, the relationship between total aeromycoflora and AF attracts more public awareness, and their relationship with respiratory disorders is clear enough. The predominant genera, such as Cladosporium, Aspergillus, and Alternaria, were discussed thoroughly in several studies [9, 10]. Their daily and seasonal fluctuations showed different patterns, as did the concentration (CFUs/m3) [11, 12].

The present study aims to highlight the 24-hour fluctuation of atmospheric fungi and explain the relationship between meteorological parameters, viz., temperature, humidity, and wind speed, and total fungal CFUs. The study also analyzes the characteristics of the fungal community. It is worth mentioning that the current study is the first to report the OAF fluctuations for 24 hours in Iraq.

2. MATERIEL AND METHODS

2.1. The Site of Sample Collection

The air samples were collected on the roof of a building 15 meters high in a residential quarter, which is 3 km far from

the trading center of Erbil city. There are no high buildings around the location, and the circulation of the air currents was unobstructed in all directions. Ornamental shrubs 2-3m high are the common plantation including Dodonea sp. and Melia sp.

2.2. Meteorological Data

The meteorological data covering 3 times of sampling were provided by the website https://www.weather25.com/ which is a specialized site for providing hourly data.

2.3. Sample Collection

The plate exposure method was followed to collect atmospheric fungi, Sabouraud's dextrose agar was used. 12 samples in 2 hours intervals during 3 days were collected, they were (S1=18-19/6/2023), (S2=22-23/6/2023), and (S3=3-4/7/2023). Petri plates were exposed for 15 minutes, then were covered, and sealed, after that they were transferred to the lab and incubated at 25 ^{o}C±2. The colonies were counted after 4 days, and the developing fungi were morphologically identified to the genera level (except Aspergillus) depending on [13, 14].

2.4. Meteorological Data Quantitative Characteristics of Isolated Genera

The absolute number CFU for each plate was modified to CFU/m^3 by the equation followed by [15].

$$N = 5a \times 104\ (bt)^{-1} \quad (1)$$

When: N: microbial CFU/m^3 of air, a: number of colonies/ plate, b: area of dish surface, (cm^2), t: exposure time (minutes).

The occurrence% and the frequency of occurrence% were calculated for each genus by the following equations followed by [16].

Occurrence% (O%) = (no. of samples in which the genus occurred)/ (no. of total samples) X 100. Frequency% (F%) = (no. of genus colonies / no. of total genera colonies X100.

3. RESULTS

3.1. Qualitative and Quantitative Analysis

From the 36 samples of (S1+S2+S3), a total of 322 fungal colonies belonging to 10 identified genera were counted. viz *Aspergillus spp., Alternaria sp., Penicillium sp., Ulocladium sp., Actinomucor sp., Stachybotrys sp., Scytaledium sp., Eurotium sp., Eupenicilium sp.* and *Rhodotorula sp*. in addition to 4 unidentified isolates viz sterilized mycelia SM, sclerotia bodies, chlamydospores colonies, and creamy yeast colonies (Table-1). Dematiacious Hyphomycetes display 151 isolates (50.6%) viz black and brown SM., *Alternaria sp., Ulocladium sp*., Chlamydospore colonies, *Scytaledium sp., and Stachybotrys sp*. The remaining 141 Hyphomycetes isolates are hyaline, including hyaline, yellow, and gray SM, *Aspergillus spp*., and *Penicillium sp*. Table 1 SM group showed the highest frequency (F% =48.2%) and occurrence (O% =100%). *Aspergillus spp*. has the second incidence level (F=12.7% and O%=91.6%) followed by Alternaria sp. (F=9.3% and O= 25%).

Table 1. The total no. of fungal isolates in S1+S2+S3.

	Fungi	No of isolates	F%	O%	T.G.	Color
1	Sterile mycelia	178	55.2%	100%	H	Hy + D
2	*Aspergillus spp.*	41	12.7%	91.6%	H	Hy
3	*Alternaria sp.*	30	9.3%	25%	H	D
4	*Penicillium sp.*	24	7.4%	27%	H	Hy
5	Yeasts(unidentified)	17	5.2	13.8%	-	-
6	*Ulocladium sp.*	10	3.1	8.3%	H	D
7	Sclerotium bodies	8	2.4	5.5%	-	D
8	Chlamydospore colonies	3	0.009%	5.5%	H	D
9	*Eurotium sp*	3	0.009%	2.7%	A	Hy
10	*Stachybotrys sp.*	3	0.009%	2.7%	H	D
11	*Actinomucor sp.*	2	0.006%	2.7%	Z	-
12	*Eupenicillium sp.*	1	0.003%	2.7%	A	Hy
13	*Rodotorula sp.*	1	0.003%	2.7%	B	-
14	*Scytaledium sp.*	1	0.003%	5.%	H	D
		322	100%			

F%: frequency, O%: occurrence, T.G.: taxonomic group, H: Hyphomycetes, A: Ascomycetes, Z: Zygomycetes, B: Basidiomycetes. Hy: hyaline, D: dematiaceous.

Among the fungal genera, 6 are Hyphomycetes, 2 are Ascomycetes, and one belongs to each Zygomycetes, and Basidiomycetes (figure1).

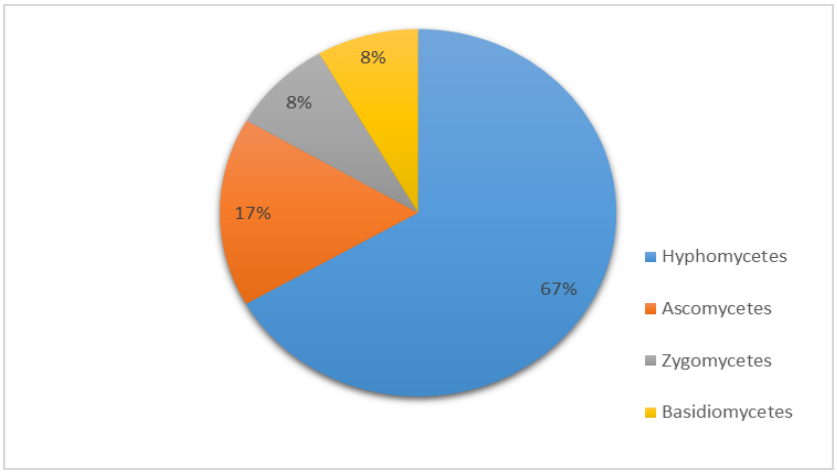

Figure 1. The taxonomic groups of isolated genera.

The number of isolates fluctuated bihourly during sample time since (7am) to (5am) of the next day (12 samples). The mean of (S1+S2+S3) showed that the CFUs/M3 ranging from 67 in 3am to 780 in 1pm and 7pm (figure 2).

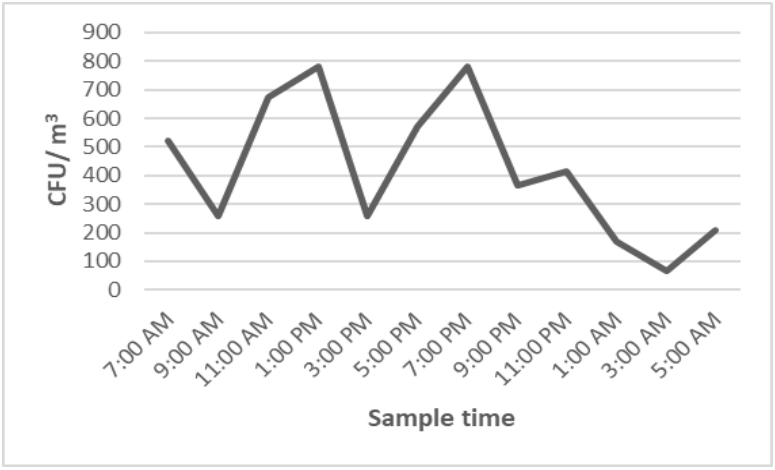

Figure 2. Bihourly fluctuation of CFU/m3 (mean of 3 samples).

The 24 hours were divided into four sampling periods, P1: morning (7,9,11am), P2: noon (1,3,5), P3: evening (7,9,11pm), and P4: midnight and early morning (1,3,5am).

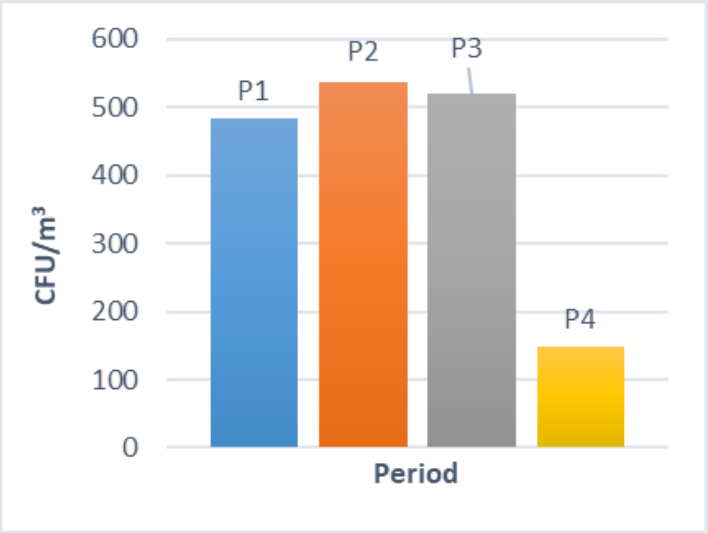

Figure 3. The midday hour (P2) had the highest mean CFUs/m3 value of (S1+S2+S3), followed by the evening. (CFUs/m3 in P1: morning period, P2: midday, P3: evening, and P4: midnight and early morning).

At each sampling period, the total number of genera varied from seventeen at 7 p.m. (5%) to just two at 3 a.m. (1.8%). Table 2 displays the prevalence of fungal genera in the 12 samples throughout a 24-hour period. SM is present in 12 sample occasions (100%), Aspergillus spp. in 9 instances (75%), Alternaria sp. in 8 instances (66.6%), Penicillium sp. in 7 instances (58.3%), and other genera in 3 instances (≤ 25%).

Table 2. Incidence of fungal genera for 24 hours.

	Time / Fungi	7 am	9 am	11 am	1 pm	3 pm	5 pm	7 pm	9 pm	11 pm	1 am	3 am	5 am	no. of incidence
1	Sterile mycelia	4	5	4	6	4	5	7	5	3	3	1	4	12
2	*Aspergillus spp.*	3	-	2	1	1	2	2	1	-	3	1	-	9
3	*Alternaria sp.*	1	2	1	1	-	1	2	1	-	1	-	-	8
4	*Penicillium sp.*	1	1	-	-	-	1	2	2	-	2	-	1	7
5	Yeasts(unidentified)	1	-	-	-	1	-	-	-	1	-	-	-	3
6	*Ulocladium sp.*	1	-	-	-	-	1	-	-	-	1	-	-	3
7	Sclerotium bodies	-	1	1	-	-	-	-	-	-	-	-	-	2
8	Chlamydo colonies	-	-	-	1	1	-	-	-	-	-	-	-	2
9	*Eurotium sp*	-	-	-	1	-	-	-	-	-	-	-	-	1
10	*Stachybotrys sp.*	-	-	-	-	-	-	-	-	1	-	-	-	1
11	*Actinomucor sp.*	-	1	-	-	-	-	-	-	-	2	-	-	3
12	*Eupenicillium sp.*	-	-	-	1	-	-	-	-	-	-	-	-	1
13	*Rodotorula sp.*	-	-	-	-	-	-	1	-	-	-	-	-	1
14	*Scytaledium sp.*	-	-	1	-	-	-	-	-	-	-	-	-	1
	Total no.	11	10	9	11	7	10	17	9	5	12	2	5	108

From the other side of view, the total number of isolates incidence in (S1+S2+S3) is 108 including 75 time at diurnal period (7pam-5pm), and 33 at nocturnal period (7pm-5am). The mean of colonies/sample time is 12.5 and 6.6 respectively, and 7pm samples are the highest diversity as shown in Figure 4.

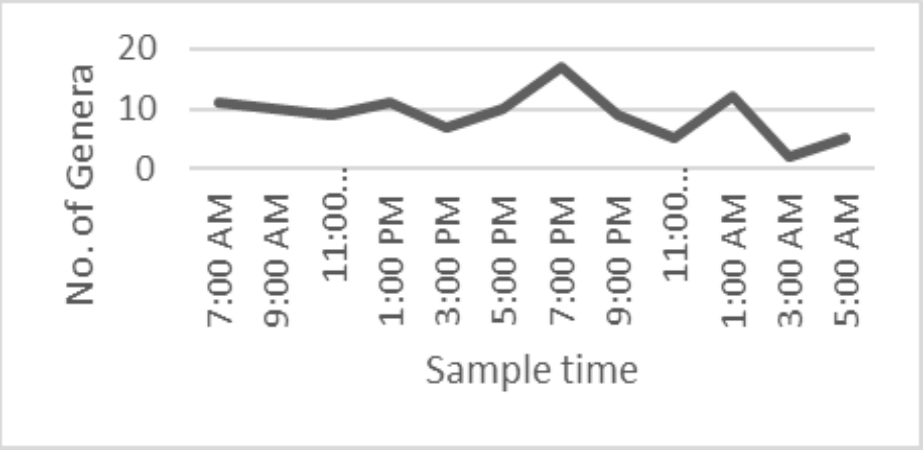

Figure 4. The relationship between CFUs and Meteorological factors.

3.2. Statistical Analysis

While the association between CFUs and humidity is non-significant, there is a significant relationship between CFUs and wind speed and temperature. According to the person correlation index wind speed and temperature show a significant positive effect on total airborne CFUs, (p=0.05 and 0.001, respectively).

Chi-squares test showed significant differences (P<0.05) between different isolates and different genera within 24 hours.

4. DISCUSSIONS

4.1. General Discussion

Spores require dry, warm, and windy weather to become airborne and spread, so airborne spore counts (depending on geographic location) reach maximum levels during sunny afternoons of late summer and early autumn and drop to zero during the winter [17]. The effect of meteorological factors on aeromycoflora resulted in daily and seasonal fluctuations cycle [18]. These fluctuations of airborne fungi were widely investigated around the world. The studies focused on the relationship between aeromycoflora and human and plant diseases, as well as the relationship with meteorological factors [19, 20].

4. 2. Aeromycoflora Characteristics

Many of the fungal genera listed here have been found previously in the aeromycoflora of Erbil City, and the predominant isolates in the present study, Aspergillus spp., Alternaria sp., and Penicillium sp. were also recorded among the predominant airborne genera in this region. [21, 22, 5]. However, the absence of Cladosporium sp. and the predominance of SM recorded here disagree with previous studies conducted in Iraq and surrounding countries [23, 24, 25, 26, 27]. Cladosporium sp. is naturally found on plant materials, and the lack of dead and moist plant residues during sampling period may be a reason for its disappearance in present samples [28].

The comparison between the 4 sampling periods showed the highest CFUs/m3 are during midday, with the highest temperature and wind speed, while the lowest CFUs/m3 are during midnight and early morning, with the lowest temperature and wind speed.

The WHO standard (500 CFUs/m3) is exceeded by the total culturable CFU count, and if non-living fungal aerosols are taken into account with bacteria and other non-biotic air pollutants, health hazards will undoubtedly rise. Environmental characteristics, vegetation cover, and the height of the sample collecting location have a significant impact on the components and density of OAF, which has resulted in various fungal community [29].

The present study showed statistically significant correlation between total CFUs and temperature, and it is consistent with earlier studies. Dryness of plant and soil surfaces actually promotes the aerosolization of microbial structures [30, 31]. The statistical studies carried here revealed that the fungal community was significantly influenced by wind speed; wind facilitated the release and dissemination of fungal structures by removing them from mycelia and promoting their movement and suspension in the air [32].

Low humidity during the sampling period and a simple difference between samples are to blame for the non-significant connection between humidity and total CFUs.

CONCLUSIONS

It is important to note the lack of research on the daily profile of airborne fungal CFUs in Iraq, and the findings of the current study could be a useful source of data for Erbil City's air quality monitoring.

The ability of Hyphomycetes to produce vast quantities of tiny, easily-separated dray spores that can be aerosolized is connected to their high level of dominance. In addition to being hot and dry, the prolonged sunlight during the collection period may have raised the O% and F% of dematiaceous SM compared to earlier research.

The non-significant link with humidity may be due to the timing of the investigation, as there is a substantial correlation between CFUs and wind speed and temperature.

The fluctuation of CFUs deserves to be studied from a pathological point of view due to the potentially hazardous effect of these fungal taxa on human health. Studies with long-term sampling are needed to get additional data and increase our understanding of aeromycoflora composition and dynamics in Erbil City throughout different seasons.

The current results indicate that outdoor air is a potential threat to public health, and the number of fungal CFUs exceeds the limit set by the WHO. The fungal community includes pathogenic and allergenic airborne fungal spores, which can serve as the main source of contamination in indoor environments.

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