

THE AIRBORNE MYCOBIOTA OF A DUST STORM IN COMPARISON WITH A CALM CLIMATE IN ERBIL CITY-IRAQ

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ABSTRACT:

A series of dust storms stroked Iraq from April to May 2022. These abnormal environmental events hold and transfer microorganisms far from their original sites. Mycobiome is a main part of the aerobiological aerosol, including agents of respiratory harm, opportunistic mycosis, as well as mycotoxin producers. 32 Petri dishes were exposed to the falling down dust for two days during dust storm (hence, S1). The time of exposure was 10 minutes per hour, a total of 8 samples/ day since 9:00 am. Sabouraud's dextrose agar and pollen grains agar media were used. Sampling was identically repeated after two days during a calm climate (hence, S2). Fungal genera, frequency%, and total similarity% were calculated for S1 and S2 samples.

257 colonies (143CFU/m³) occurred in (S1) samples, in contrast, a total of 110 colonies from (S2) samples (59CFU/m³). The statistical analysis showed a highly significant difference between CFU for S1 and S2 ($p \leq 0.0001$). Fifteen isolates were identified from (S1), and (5) from (S2). The highest occurrence O% and frequency F% genera in S1 were *Aspergillus* (65.6%; 15.9%), *Alternaria* (56.6%; 11.2%), *Cladosporium* (46.8%; 13.6%), *Penicillium* (37.5%; 8.1%), besides sterile mycelia, while in S2 were (40.6%; 30.6%) ; (25.0%; 17.2%) ; (32.0%; 26.3%) (34.3 %; 12.7%) respectively. The similarity between the fungal communities of the two times was low =35.7%.

Results showed that air mycobiota on stormy days have a higher fungal abundance and diversity. All isolated fungi are adverse to humans, and animals. Individuals who have respiratory problems or with weak immunity showed careful to protect themselves.

KEYWORDS: airborne fungi, outdoor, storm, dust, air mycobiota, Iraq

1. INTRODUCTION

1.1 Studies of air-borne

Aerobiology deals with several airborne microorganisms and biological particles. Fungi are the main part of the air microbiome. Studies have been conducted worldwide to explain their effects on health, and agriculture. Most studies of air mycobiota around the world focused on calm climate. They investigate the diversity of outdoor and indoor airborne fungi and their health impacts as well as factors that affect their population (Savković et al., 2021; Xin et al., 2021). (Liu et al., 2021; Mendell, 2009; Al-Badr et al., 2018). Most of the predominant airborne fungi are allergens and agents of human health disorders (Kim et al., 2018; Garga et al., 2019).

1.2 Dust storm events

Official meteorological agencies and studies mentioned serious changes in the Middle East's climate (Waha et al., 2015; Ahmadalipour and Moradkhani 2018). An official report prepared by Michael Carlowicz, the earth observatory and managing editor of *NASA*, explains that since the beginning of April 2022, Iraq and other parts of the Middle East region will be struck by a sequence of dust storms.

1.3 Study airborne fungi in Iraq

Several studies aimed to identify and discuss the fungal air-spores in Iraq; they covered outdoor, indoor, and devices of air conditioning. Al-Bader (1995) listed (34) genera by monthly outdoor sampling over a year, *Cladosporium* and *Alternaria* were predominant. Abdullah and Al-Ani, (2003) recorded 29 genera from hospital indoor air samples. Badran et al. (2018) recorded 11 genera from indoor and outdoor samples, *Cladosporium*, *Penicillium*, and *Alternaria* were predominant. Al-Bader et al. (2013) screened indoor airborne fungi and their relationships with respiratory disorders. The fungal contamination of air conditioner units in five hospitals in Erbil city was investigated by Al-Bader et al. (2018). They recorded 13 genera, where *Penicillium*, *Aspergillus*, and *Alternaria* showed the highest occurrence.

1.4 Storm dust mycobiota

Microorganisms attached to dust particles have been recognized to travel far away from their original habitat (Favet et al., 2013). Dust storms hold and transmit fungi. They are a probable source of asthma and several respiratory disorders. In Iran, Nourmoradi et al. (2015) mentioned increasing colony-forming units (bacteria + fungi) directly by increasing dust particle concentration. The common fungus was *Mycosporium*. In KSA, Rajendran et al. (2017) observed that the most important fungal allergens associated with storm dust

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were, *Fusarium*, *Cladosporium*, *Ulocladium*, *Aspergillus*, and *Alternaria*. A study conducted by (Moataz et al., (2010) in Iraq showed that *Aspergillus* and *Candida albicans* were common after storm dust loads. In a study of the west Texas air dust microbiota, the common genera were *Cryptococcus*, *Aureobasidium*, *Alternaria*, *Cladosporium*, and *Filobasidium* (Elmassry et al., 2020).

Due to the frequent drought and desertification in Iraq and the frequent dust storms and the lack of information on the fungi associated with dust, this study was conducted to reveal the fungal community associated with dust and its difference from its counterpart in the calm climate in Erbil city.

2. MATERIALS AND METHODS

2.1 Sample collection

On May 23rd and 24th, 2022, thirty-two samples from Erbil city were collected (S1). The source of storms were southwest arid areas of Iraq which expand to KSA desert. The gathering place is an outer edge of a window that is 15 meters above the ground and in a direction that is blocked from direct air movement. The samples collection site was absolutely free from trees in the vicinity of the place. The plating exposure method was followed to collect the settle-down dust. The gathering place is an outer edge of a window 15 meters above the ground. 16 Petri plates were used daily. Two plates were exposed per hour starting from 9:00 am to 4:00 pm. The time of exposure was 10 minutes, and the plates were wrapped with paraffin film and incubated at 25± 2. Two plates were used as controls. Two types of culture media were used, Sabouraud's dextrose agar and pollen grain agar (Al-Bader, 2019). According to Erbil Metrologic agency (<https://www.timeanddate.com/weather/iraq/erbil/historic>), the mean temperature during sampling was 25-27°C, the mean humidity was 25%, and with different wind directions (SW, W, WN). Samples were recollected after the storm passed for 48 hours and the procedure of S1 was identically followed for a calm climate (S2).

2.2 Colonies counting and identification

The plates were checked after 3 days to count the number of colonies. Omeliansky's equation was used to calculate the colony forming units/m³ ($N = 5a \times 10^4 (bt)^{-1}$) where N= microbial CFU/m³ of air. a= number of colonies / Petri dish. b= dish surface, cm². t: exposure time, minutes (Fekadu and Getachewu, 2015)

Petri plates were checked periodically for 14 days to identify fungal genera, then fungi were distinguished either directly or after the preparation of a pure culture. The microscopic characteristics were confirmed according to (De Hoog et al., 2000; Ellis et al., 2007).

2.3 Analysis of the fungal population of S1 and S2

Total occurrence% (TO) and total frequency% (TF) for fungal isolates were calculated for both S1 and S2. Also, The total similarity (TS) between genera of S1 and S2 was explained via the Jaccard index (Singh, 2012) according to the following:

$TO\% = (\text{No. of times fungal appear} * 100) / \text{No. of collected samples}$

$TF\% = (\text{No. of fungal isolates} * 100) / \text{No. of total fungallisolates.}$

Jaccard's Index = $2 \times (\text{the number in both sets}) / (\text{the number in either set}) \times 100.$

2.4 Statistical analysis

Statistical analysis was performed by paired t-test to compare the mean of the number of isolates, occurrence, and frequency of fungal genera between a dust storm and a calm climate. The SPSS software (Windows Version 23.0) was utilized for all statistical processing. When the p-value was <0.05, the difference was judged statistically significant.

3. RESULTS

The total colony count for 32 Petri dishes of S1 was 257 related to 13 genera and 2 unidentified yeasts and mold. (143CFU/m³) while they were only 110 colonies related to 5 genera in S2 (59CFU/m³) The statistical analysis showed a highly significant difference between CFU for S1 and S2 ($p \leq 0.0001$), while it was less and negative for O% and F% respectively (Table 1). The isolated genera viz *Alternaria*, *Aspergillus* (5 sp.), *Botrytis*, *Cladosporium*, *Fusarium*, *Monilia*, *Mortierella*, *Mucor*, *Penicillium*, *Phoma*, *Stemphyllum*, *Ulocladium*, *Verticillium*, and Yeast (creamy color), as well as sterile mycelia(white) The predominant group was Hyphomycetes (10genera.) followed by Zygomycetes (2 genera) and Coelomycetes (1 genus). 50% of Hyphomycetes were dematiaceous (Table 1, Figure 1) The abundance of isolates in the S1 samples (isolate/plate= 8,3) was more than that of the S2 samples (isolate/ plate =3.43) (Figure 2).

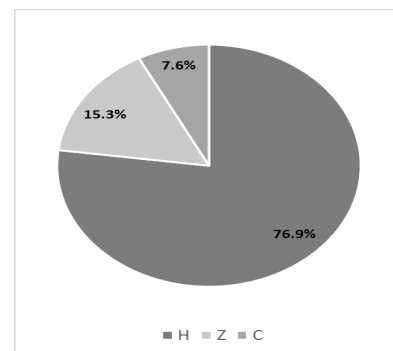


Figure 1. Occurance% of Hyphomycetes (H), Zygomycetes (Z), and Coelomycetes (C)

The predominant airborne genera of S1 include *Aspergillus*, *Alternaria*, *Cladosporium*, and *Penicillium* besides the sterile mycelia. They are the only genera investigated in normal climate samples (S2). The results showed low total similarity between S1 and S2 fungal populations (TS=35.7%). In S1, *Aspergillus* was represented by *A. fumigatus*, *A. niger*, *A. terreus*, *A. ochraceus*, and *A. flavus*. The genus has the highest occurrence (65.6%), and also it had the highest frequency (15.9%), followed by *Alternaria*, *Cladosporium*, and sterile mycelium (Table 1). In S2, *Aspergillus* is the common genus with fewer species (2 sp. only), followed by *Cladosporium*.

Table 1. Fungal genera of dust storm S1 and calm climate S2 samples. No= Number of isolates, O%=occurrence% , F%= frequency %(F%) . TG= taxonomic group, DH= dematiaceous Hyphomycetes, HH= hyaline Hyphomycetes, Z = Zygomycetes, C=Coelomycetes.

	Fungi	No. S1	No. S2	O% in S1	O% in S2	F% in S1	F% in S2	TG
1	<i>Alternaria*</i>	29	19	56.2	25	11.2	17.2	DH
2	<i>Aspergillus*</i>	41	37	65.6	40.6	15.9	33.6	HH
3	<i>Botrytis</i>	10	0	9.3	-	3.8	-	DH
4	<i>Cladosporium*</i>	35	29	46.8	32	13.6	26.3	HH
5	<i>Fusarium</i>	19	0	34.3	-	7.3	-	HH
6	<i>Monilia</i>	7	0	9.3	-	2.7	-	HH
7	<i>Mortierella</i>	8	0	9.3	-	3.1	-	Z
8	<i>Mucor</i>	9	0	25	-	3.5	-	Z
9	<i>Penicillium*</i>	21	14	37.5	34.3	8.1	12.7	HH
10	<i>Phoma</i>	11	0	9.3	-	4.2	-	C
11	<i>Stemphylium</i>	6	0	12.5	-	2.3	-	DH
12	<i>Ulocladium</i>	14	0	9.3	-	5.4	-	DH
13	<i>Verticillium</i>	6	0	6.25	-	2.3	-	DH
14	Yeast (creamy)	13	0	34.3	-	5.0	-	-
15	Sterile mycelia*	28	11	40.6	34.3	10.8	10.0	-
Mean		17.13	7.3	27.04	11.08	6.61	6.65	
Mean of differences (95% CI)		9.8 (7.4 – 12.2)		15.96 (9.94 - 21.99)		-0.04 (-3.99 - 3.91)		
P value		≤ 0.0001		≤ 0.0001		0.98		

*Genera which occur in calm climate samples S2.



Figure 2. Stormy climate (1+2); Air sample of stormy dust (3); Calm climate (4+5) ; Air sample of calm climate(6)

4. DISCUSSION

The present study distinguishes the fungal components of one of the most severe dust storms that hit Iraq and Erbil city during the previous years. The completely identical methods of S1 and S2 showed a big difference between the total colony count in the dust storm samples and their counterparts after passing through the storm. The concentration (143CFU/m³) was higher than the level of microbial contamination given by American Conference of Governmental Industrial Hygienists Committee (100 CFU/m³) and lower than the guidelines provided by WHO (500 CFU/m³). The low significant and non-significant differences for O% and F% were due to the low number of genera (low diversity) in the S2 fungal community. The increase in the total airborne fungi of dust storms was reported by several workers (Griffin et al., 2001; Kellogg et al., 2004).

Also, Nourmoradi et al. (2015) indicated a highly positive relationship between a dust storm and aerobiological particles. The relatively minor differences among previous studies are due to the original regions of the storms, which are regarded as the main effective factor in air dust mycobiota (Peng et al., 2021). Furthermore, the fungal populations in S1 and S2 showed a great dissimilarity after Jaccard's index that ranged from (0-1). The current study showed an increase in the number of genera in S1 than in the S2 samples, while the dominant genera were the same in both cases.

The same result was reported in the north of Iran by Neisi et al. (2019), who listed *Cladosporium*, *Aspergillus* and *Penicillium* as common genera.

The predominant genera of the present study were recorded by Al-Bader et al (2016) in Kuwait. They identified 17 genera viz

Fusarium, *Alternaria*, *Ulocladium*, *Phoma*, *Aspergillus*, *Acremonium*, and *Penicillium* were predominant. The similarity with the present study results relates to meteorological factors rather than biological ones. Iraq and Kuwait are located in the same geographic region and are approximately affected by the same environmental factors, including dust storms, and this may explain why the air dust mycobiota was the same. The similarity of current results with such a study in KSA may refer to the fact that the origin of dust storms that hit Iraq is the KSA deserts. A 50% of fungi represented in the current study were identified in KSA sand storms; moreover, the predominate genera are the same in both studies (Rajendran et al., 2017).

The predominant genera in the current study (*Alternaria*, *Aspergillus*, *Cladosporium*, and *Ulocladium*) are common airborne in the Mediterranean region, they showed a significant increase in air mycobiota of dust storms. And that may refer to several characteristics that help them survive as aerobiological particles. *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Stemphylium*, *Botrytis*, and *Phom* were described as xerophilic and xero-tolerant fungi (Petrović et al., 2000) Moreover, they are dematiaceous and can produce a great number of dry conidia. Such traits enable them to survive along with the storm's dust (Peng et al., 2021).

There is a lack of information about the aerobiology of dust storms in Iraq. Al-Dabbas et al. (2010) discussed it according to a geological view, where the study covered the middle part of Iraq, and the dust storms for 4 months (12/2008-3/2009) were analyzed. They counted a total of 647 particles from 8 dust storms. The largest isolates were related to culturable bacteria and fungi. They counted 310 fungal colonies, and *Aspergillus* was predominant. Khider et al. (2012) studied bacteria and fungi of cloud dust in Erbil city. They depend on the ratio (no./wait) to explain and compare among samples. The fungal population of the current study showed similarity ($S = 87.55\%$). The high similarity reflected the similarity of dust origin, which plays an important role in fungal diversity. Al-Oumari and Mohammad (2013) focused on the fungi of inpatient individuals before and after exposure to the dust storm. They recognized the high incidence of *Aspergillus*, *Candida*, *Penicillium*, *Alternaria*, and *Fusarium* in the nose, ear, and oral pharynx.

5. CONCLUSION

The results indicated that the total colony count of air mycobiota and the fungal population diversity in a dusty climate is markedly increased during the dust storms, yet the dominant genera remain the same. The spatial factors affect on air fungal population more than the temporal one.

Hence, *Aspergillus*, *Candida*, *Penicillium*, *Alternaria*, and *Fusarium* were predominant in dust storms and were well-known allergens. Further studies should be done on the significance of the dust storm in terms of living and non-living particles of mycobiota.

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