Identification of microflora associated with dust falling on Karbala province and seasonal distribution

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Abstract. Dust is considered one of the most important and most dangerous atmospheric pollutants due to its physical properties and its chemical and biological contents. As a result, the study started in March 2017 and ended in February 2018 to diagnose the microflora associated with dust falling in Karbala province. The results showed that 18 species of fungi belonging to ten genera are Aspergillus, Fusarium, Penicillium, Rhizopus, Mucor, Alternaria, Cladosporium, Absidia, Candida, Trichoderma. The highest frequency was recorded for genera Aspergillus, Penicillium and Cladosporium, while the highest occurrence of fungi was in the spring. The results showed the occurrence of two genera of bacteria, Bacillus spp. and Staphylococcus spp., where the first genus dominant in most sites and seasons. Identification of microorganisms associated with dust and their temporal and spatial distribution is in great health and economic importance because of the role of these organisms to cause diseases to humans and plants.

Keywords: Dust, Microflora, Biological pollutants, Aspergillus, Air environment.

1. Introduction

No region of the globe is free of microorganisms, it is spreading in all environments, but environmental factors play an important role in their distribution within these environments. The air is not a viable environment for these organisms due to atmospheric fluctuations and their exposure to deadly UV radiation. It is often spread with airborne dust particles and is thus one of the air biological pollutants responsible for several diseases affecting humans, animals and plants [1].

Bioaerosol is made up of dust particles containing substances of animal, plant or microflora, as well as organic dust, which is defined as dust that consists of a living organism or some of its parts or products, such as fungi, bacteria, viruses, insects, plants and its parts or some of its metabolic products, such as pollen, hair particles, dead skin cells, waste and excesses such as mucus and saliva [2][3].

The properties of dust in terms of the size of particles and infections it causes are very important from a public health point of view, ranging from 0.5-15 microns for viruses, 0.1-4 microns for bacteria and 0.5-15 microns for fungal spores, while pollen ranges from 10-30 microns [4][5].

Dust is one of the most prominent pollutants in Iraq in general and particularly Karbala province, and most studies have focused on the physical properties of dust and its chemical mineral and elements [6][7], and there have been insufficient studies showing microorganisms related to dust and its seasonal distribution and its effect on public health, especially since Karbala province is one of the most important religious cities, which sees the arrival of more than 20 million visitors annually taking into account the diseases of some of these species and their ability to cause infection to humans as well as diseases caused by animals and the plants and toxins that they produce. The study, therefore, focused on its diagnosis and prevalence during the seasons.

2. Materials and Methods

Study area: Karbala province is located southwest of Baghdad, 105 km west of the Euphrates River on the edge of the Western Desert. The province is located at a longitude of 44 degrees and 40 particles and latitude
of 33 degrees and 31 particles. It has an area of 5034 km², which represents 1.14% of Iraq’s area (438317) km². Three different areas were chosen in Karbala province, the first rural area, the second urban area and the third industrial area.

Collection of samples: Samples of falling dust, which began from March 2017 to February 2018, were collected monthly by a polyethylene cylinder placed on the roofs of buildings approximately three meters high within the study areas.

Isolation of dust fungi: One gram of dust was weighted by using a sensitive balance and then mixed the suspension with 9 ml sterile distilled water well for 10 particles, then worked five decimal dilutions (10⁻¹, 10⁻², 10⁻³, 10⁻⁴,10⁻⁵) and then spread one ml of suspended in a container dish on a nutritious medium of Potato Dextrose Agar (PDA), then placed in an incubator temperature 25°C.

Diagnosis of fungi: Fungi were first diagnosed based on the occurrence characteristics of colonies and were examined microscopically by following diagnostic keys [8][9]. The percentage of frequency and occurrence were calculated, according to the following equations [10]:

\[
\text{Frequency} \% = \frac{\text{No. of isolated of fungus}}{\text{Total No. of isolated}} \times 100
\]

\[
\text{Occurrence} \% = \frac{\text{No. of Observation in which species appeared}}{\text{Total No. of samples}}
\]

Isolation and diagnosis of bacteria: One gram of dust was weighed and put in 9 ml distilled water and then conducted decimal dilutions in the same way as the fungus, then added nutrient agar medium to 1 ml of dilution 10⁻² and incubated for 24 hours at a degree of 37°C, and the number of colonies was calculated in the methods of counting dishes and diagnosed bacteria based on their characteristics occurrence and microscopy [11].

3. Results and Discussion

Fungi: The results of the study showed that the fungal species belong to three divisions and Ascomycota division were the domance, with 63%, followed by Zygomycota division, 22%, then the imperfect fungi (Deuteromycota) 17% (see Figure 1) and the domance of Ascomycota is attributed to being more development and their ability to adapt in dry environments compared to Zygomycota fungus, these results have been consistent with Xu [12] study. The results showed the presence of 18 species of fungi belong to 10 genera (see Figure 2) and the study in terms of the overall rate of frequency and occurrence of Aspergillus Niger fungus 19.25% and 27.75% respectively. Mucor racemosus recorded the lowest frequency and occurrence rate of 0.44% and 1.75% respectively (see Figure 2).

Rate of occurrence (%): The results of the study (see Tables 1, 2, 3, and 4) show that the highest rate of occurrence of all fungi was in the spring season while the lowest rate of occurrence in the summer, and that the highest rate of occurrence was 52% of the Aspergillus Niger in the urban area followed by Cladosporium herbarium by 43% during the spring in the rural area, while the study did not record in all locations any incidence or occurrence of Fusarium oxysporum, Fusarium solani and Mucor racemosus during winter (see Table 4). As well as the species of Absidia corymbifera and Candida albicans during the autumn and winter, respectively (see Tables 3 and 4).

Frequency ratio (%): Table 2 shows the highest frequency rate of 22.95% recorded in species A. Niger during the summer in rural areas, while the low frequency rate of 0.82% was recorded for A. corymbifera during the spring in the industrial area.

It is clear from the observation of the incidence of fungal species during the study areas and seasons that the highest occurrence rate was recorded in the urban area followed by the rural area and then the industrial area, due to the fact that most of the species appeared in the study are the Saprophytic fungi or facultative parasitism and most of them it is where the appropriate nutrient environments are available where waste accumulates in the city centre or the host plants in the rural area. This conclusion is confirmed by the fact that most of the fungi that have appeared in rural areas are those of pathogens of the plant R. stolonifer, R. arrhizus, F. oxysporum, C. herbarium and A. alternate [13]. While the highest rate of occurrence in the city
centre was species *A. fumigates*, *M. racemosus*, *A. corymbifera*, *C. albacans* and *A. Niger*, an opportunistic human pathogenic fungus such as Aspergillosis, a species that has the potential to produce dangerous and carcinogenic toxins, such as Aflatoxin toxins [14], the two species *A. alternata* and *C. herbarium* are fungi responsible for allergic diseases in humans, the majority of people are allergic to *A. alternata* [15], while species *C. herbarium* causes asthma and allergic diseases due to the fact that the spores of this species are high density in air [16].

The results showed that the highest rate of occurrence was in the spring while the lowest rate of occurrence of fungi was recorded in the summer, and this can be attributed to the fact that fungi tend to favour optimal environmental conditions for growth, especially humidity and temperatures, which are available in the spring and non-existent in the summer. These results agreed to a study conducted in Riyadh city [17] and differed with another study conducted in Egypt in which the incidence of fungi in the autumn was the highest [18]. *Aspergillus* genus showed the highest frequency rate and this result was consistent with several studies [17][19][20]. By comparing the results of this study with other studies (see Table 5), we find it compatible in recording the occurrence of most fungal genera and the occurrence of some species and the absence of others can be attributed to the availability of optimal environmental conditions for their growth in one region and their absence in another region as well as their ability to tolerate the air environment cruel and extreme and to the method of diagnosis followed.

**Figure 1:** Percentage of fungal divisions.

**Figure 2:** yearly average of occurrence and frequency of fungi.
Table 1: Fungal species and their frequency and occurrence in study areas during the spring season.

| Species of fungi | Rural | Urban | Industrial | Rural % | Urban % | Industrial % |
|------------------|-------|-------|------------|---------|---------|--------------|
| A. niger         | 43    | 52    | 34         | 16.96   | 13.38   | 14.5         |
| A. flavus        | 35    | 47    | 32         | 9.54    | 11.62   | 10.96        |
| A. fumigatus     | 33    | 40    | 30         | 5.22    | 6.61    | 8.14         |
| A. terreus       | 24    | 39    | 20         | 5.96    | 6.31    | 5.97         |
| A. parasiticus   | 30    | 43    | 28         | 5.06    | 7       | 6.66         |
| F. solani        | 42    | 12    | 8          | 6.8     | 2.62    | 2.99         |
| F. oxysporum     | 40    | 11    | 7          | 6.33    | 2.39    | 6.71         |
| P. italicum      | 30    | 35    | 23         | 4.75    | 5.77    | 6.51         |
| P. verrucosum    | 23    | 28    | 19         | 3.96    | 8.22    | 5.56         |
| P. expansum      | 22    | 33    | 17         | 3.64    | 5.46    | 5.29         |
| R. stolonifer    | 28    | 35    | 20         | 4.35    | 6       | 5.7          |
| R. arrhizus      | 11    | 23    | 7          | 2.37    | 3.46    | 2.71         |
| M. racemosus     | 3     | 8     | 4          | 1.05    | 1.69    | 1.22         |
| A. alternata     | 30    | 12    | 3          | 8.75    | 2.46    | 4.09         |
| C. herbarum      | 45    | 25    | 10         | 7.12    | 6.85    | 5.8          |
| A. corymbifera   | 2     | 5     | 2          | 1.02    | 1.7     | 0.82         |
| C. albicans      | 21    | 38    | 9          | 3.16    | 5.92    | 3.66         |
| T. harzianum     | 24    | 12    | 7          | 3.96    | 2.54    | 2.71         |
| Total            |       |       |            | 100     | 100     | 100          |

Table 2: Fungal species and their frequency and occurrence in study areas during the summer season.

| Species of fungi | Rural | Urban | Industrial | Rural % | Urban % | Industrial % |
|------------------|-------|-------|------------|---------|---------|--------------|
| A. niger         | 9     | 20    | 7          | 22.95   | 19.55   | 21.41        |
| A. flavus        | 8     | 14    | 4          | 10.36   | 12.24   | 14.33        |
| A. fumigatus     | 5     | 13    | 3          | 7.57    | 6.87    | 8.25         |
| A. terreus       | 4     | 10    | 3          | 6.68    | 5.5     | 7.29         |
| A. parasiticus   | 6     | 14    | 10         | 4.46    | 8.24    | 15.63        |
| F. solani        | 11    | 5     | 0          | 7.44    | 3.3     | 0            |
| F. oxysporum     | 12    | 4     | 0          | 6.55    | 2.75    | 0            |
| P. italicum      | 5     | 12    | 9          | 3.57    | 6.59    | 7.29         |
| P. verrucosum    | 3     | 10    | 2          | 2.68    | 5.49    | 5.31         |
| P. expansum      | 2     | 8     | 3          | 4.79    | 4.94    | 6.25         |
| R. stolonifer    | 4     | 7     | 4          | 2.68    | 4.4     | 8.33         |
| R. arrhizus      | 4     | 10    | 1          | 5.98    | 5.49    | 3.83         |
| M. racemosus     | 0     | 0     | 0          | 0       | 0       | 0            |
| A. alternata     | 0     | 0     | 0          | 0       | 0       | 0            |
| C. herbarum      | 14    | 5     | 0          | 8.93    | 5.3     | 0            |
| A. corymbifera   | 2     | 5     | 1          | 1.79    | 3.3     | 2.08         |
| C. albicans      | 0     | 0     | 0          | 0       | 0       | 0            |
| T. harzianum     | 5     | 11    | 0          | 3.57    | 6.04    | 0            |
| Total            |       |       |            | 100     | 100     | 100          |
Table 3: Fungal species and their frequency and occurrence in study areas during the autumn season.

| Species of fungi | Autumn Occurrence % | Winter Occurrence % |
|------------------|---------------------|--------------------|
|                  | Rural | Urban | Industrial | Rural | Urban | Industrial |
| A. niger         | 36    | 48    | 27         | 17.08 | 20.15 | 21.68      |
| A. flavus        | 28    | 43    | 24         | 12.45 | 9.95  | 11.62      |
| A. fumigatus     | 26    | 38    | 22         | 6.24  | 7.25  | 9.34       |
| A. terreus       | 19    | 36    | 13         | 3.73  | 6.8   | 6.37       |
| A. parasiticus   | 23    | 40    | 19         | 4.78  | 7.7   | 8.49       |
| F. solani        | 38    | 10    | 4          | 9.46  | 2.72  | 2.55       |
| F. oxysporum     | 33    | 8     | 3          | 6.5   | 2.27  | 2.87       |
| P. italicum      | 22    | 30    | 13         | 4.59  | 6.07  | 6.37       |
| P. verrucosum    | 16    | 23    | 10         | 3.16  | 4.99  | 5.45       |
| P. expansum      | 18    | 29    | 11         | 5.63  | 5.98  | 5.52       |
| R. stolonifer    | 23    | 32    | 12         | 4.78  | 6.35  | 5.73       |
| R. arrhizus      | 7     | 21    | 5          | 2.1   | 3.63  | 2.76       |
| M. racemosus     | 0     | 6     | 0          | 1.36  | 0     | 0          |
| A. alternata     | 23    | 9     | 0          | 4.78  | 2.36  | 0          |
| C. herbarum      | 40    | 22    | 10         | 7.65  | 3.81  | 5.94       |
| A. corymbifera   | 0     | 0     | 0          | 0     | 0     | 0          |
| C. albicans      | 15    | 32    | 6          | 2.96  | 6.35  | 3.19       |
| T. harzianum     | 20    | 8     | 3          | 4.11  | 2.26  | 2.12       |
| Total            | 100   | 100   | 100        | 100   | 100   | 100        |

Table 4: Fungal species and their frequency and occurrence in study areas during the winter season.

| Species of fungi | Winter Occurrence % | Frequency % |
|------------------|---------------------|-------------|
|                  | Rural | Urban | Industrial | Rural | Urban | Industrial |
| A. niger         | 19    | 23    | 15         | 21.11 | 20.42 | 21.76      |
| A. flavus        | 14    | 19    | 13         | 11.22 | 10.47 | 11.14      |
| A. fumigatus     | 11    | 15    | 12         | 8.4   | 6.94  | 8.56       |
| A. terreus       | 10    | 14    | 10         | 5.24  | 6.25  | 6.95       |
| A. parasiticus   | 12    | 17    | 11         | 7.71  | 7.87  | 8.02       |
| F. solani        | 0     | 0     | 0          | 0     | 0     | 0          |
| F. oxysporum     | 0     | 0     | 0          | 0     | 0     | 0          |
| P. italicum      | 11    | 14    | 9          | 6.79  | 6.48  | 6.42       |
| P. verrucosum    | 9     | 12    | 8          | 5.25  | 5.32  | 5.35       |
| P. expansum      | 8     | 13    | 8          | 6.01  | 7.79  | 6.88       |
| R. stolonifer    | 9     | 14    | 9          | 6.42  | 6.48  | 6.42       |
| R. arrhizus      | 4     | 9     | 3          | 3.71  | 3.94  | 2.67       |
| M. racemosus     | 0     | 0     | 0          | 0     | 0     | 0          |
| A. alternata     | 0     | 0     | 0          | 0     | 0     | 0          |
| C. herbarum      | 19    | 8     | 4          | 7.32  | 6.7   | 6.74       |
| A. corymbifera   | 2     | 3     | 2          | 0.93  | 1.39  | 1.07       |
| C. albicans      | 9     | 15    | 5          | 4.64  | 6.71  | 4.81       |
| T. harzianum     | 10    | 7     | 4          | 5.25  | 3.24  | 3.21       |
| Total            | 100   | 100   | 100        | 100   | 100   | 100        |
**Bacteria:** The results showed the presence of the two sexes of bacteria *Bacillus* spp. and *Staphylococcus* spp., the first genus was the dominance and most occurrences compared to the second genus that appeared rarely during the study months (see Table 6), and the results showed a clear variation in the numbers of colonies in locations as well as during the months. The numbers of colonies ranged from (0- greater than 300) colonies at the level of dilution $10^7$, and the highest rate of the number of colonies (greater than 300) in April and June in the rural area and the urban area respectively, and with the same result in August and September in the urban area and in January within the industrial area, while it did not appear at the same level of dilution in some of the most months and study area.

The dominance and occurrence of *Bacillus* spp. in most locations and months of study is due to the ability of this genus to adapt to the harsh air environment and some of its strains dominate high temperatures of up to $70^\circ C$ and its ability to produce internal spores and its ability to fix atmospheric nitrogen [1] and its composition of the internal spores also due to the low occurrence of genera compared to some studies (see Table 5) and the variation in the numbers of colonies due to the method of collection used, as the samples remain for a whole month exposed to extreme weather factors, particularly ultraviolet radiation and high temperatures.

**Table 5:** Shows the fungi genera and bacteria diagnosed infalling dust and compared with local and international studies

| Reference       | Location       | **Fungal genera**                                                                 | **Bacterial genera**                  |
|-----------------|----------------|-----------------------------------------------------------------------------------|---------------------------------------|
| Kwaasi [21]     | Saudi Arabia   | *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Mortierella*, *Mucor*,  | *Bacillus*, *Pseudomonas*, and        |
|                 |                | *Penicillium*, *Pythium*, *Ulocladium*, and *Verticillium*                        | *Staphylococcus*                      |
| Lyles [19]      | Kuwait         | *Alternaria*, *Cryptococcus*, *Mortierella*, *Penicillium*, *Phoma*, *Rhodotorula*, | *Arthrobacter*, *Bacillus*,           |
|                 |                | and *Stemphylium*                                                                  | *Cryptococcus*, *Flavimonas*,         |
|                 |                |                                                                                   | *Kurthia*, *Neisseria*, *Paenibacillus*, |
|                 |                |                                                                                   | *Pseudomonas*, *Ralstonia*, and       |
|                 |                |                                                                                   | *Staphylococcus*                      |
| Prospero [22]   | Barbados       | *Alternaria*, *Arthrinium*, *Aspergillus*, *Cladosporium*, *Curvularia*, *Microsporum*, | *Bacillus*                            |
|                 |                | *Penicillium*, and *Penicillium*, and *Periconium*                                 |                                       |
| Griffin [23]    | Turkey         | *Alternaria*, *Cladosporium*, *Microsporum*, and *Penicillium*                     | *Arthrobacter*, *Corynebacterium*,    |
|                 |                |                                                                                   | *Microbacterium*, *Nocardioides*,     |
|                 |                |                                                                                   | *Planococcus*, *Saccharothrix*, and   |
|                 |                |                                                                                   | *Streptomyces*                        |
| Al-Dabbas [20]  | Iraq           | *Aspergillus* and *Candida*                                                        | *Bacillus*, *Pseudomonas*,            |
|                 |                |                                                                                   | *Staphylococcus*, *Escherichias*,     |
|                 |                |                                                                                   | *Enterobacter*, *Proteus* and         |
|                 |                |                                                                                   | *Klebsiella*                          |
| Al-Barakah [17] | Saudi Arabia   | *Aspergillus*                                                                      | *Bacillus*, *Pseudomonas*, and        |
|                 |                |                                                                                   | *Staphylococcus*                      |
| The present     | Kerbala        | *Penicillium*, *Fusarium*, *Aspergillus*, *Rhizopus*, *Mucor*, *Alternaria*,       | *Bacillus and Staphylococcus*         |
| study (2018)    |                | *Cladosporium*, *Absidia*, *Candida*, *Trichoderma*                                 |                                       |
The rise in the number of colonies during the spring months was observed and can be attributed to the high concentration of falling dust during these months [7] and provides the right conditions for growth. The results of this study of Bacillus spp. and the high numbers of its colonies during the spring months were consistent with some studies [17]. Although this is an environmentally common genus, its large numbers, particularly in the urban area, make it very dangerous to public health.

| Table 6: Number of colonies for Bacillus spp. bacteria within areas during the study period. |
|-------------------------------------------------------|-------------------------------------------------|-------------------|
| Month | Rural | Urban | Industrial |
|---------|--------|-------|-----------|
| Mar. 2017 | 32 | 42 | 35 |
| Apr. 2017 | >300 | >300 | 110 |
| May. 2017 | >300 | >300 | 0 |
| Jun. 2017 | 8 | 0 | 0 |
| July. 2017 | 47 | >300 | 0 |
| Aug. 2017 | 26 | >300 | 8 |
| Sep. 2017 | 0 | >300 | 0 |
| Oct. 2017 | 0 | 0 | 31 |
| Nov. 2017 | 0 | 37 | 0 |
| Dec. 2017 | 0 | 0 | 15 |
| Jan. 2018 | 1 | 15 | >300 |
| Feb. 2018 | 1 | 1 | 0 |

4. Conclusions
Knowing the occurrence and frequency of fungi during the sites and seasons of the year is of great importance in terms of health and economic ways, as by determining this it is possible to alert health institutions about the time of activity of pathogenic fungi, especially since some of the fungi diagnosed are responsible for the cases of sensitivity suffered by many people. In addition, there are opportunistic fungi and other toxins capable of producing toxins, as well as warning farmers about the appearance of pathogenic species for prevention.

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