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Calculating the Percentage of Air Pollution with Fungi Through Rainwater

Huda Raheem Hashim*, Wisam Jasim Abed Ali[1] and Zina Abdulhussein Jawad
University of Al-Muthanna, Al-Muthanna, Iraq
[1]University of Sumer, Sumer, Iraq
✉ hudaraheem@mu.edu.iq

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Abstract: The first location where the frequency ratios of the isolated fungi were 73.78% - *Aspergillus niger*, 66.87% - *Aspergillus flavus*, 34.69% - *Alternaria* sp., 22.99% - *Fusarium* sp., 15.65% - *Rhizopus* sp., 11.54% - *Penicillium* sp. and *Cladosprium* sp. - 5.87% was in the north of Al-Diwaniya city. Whereas the percentage of fungi frequency in the air before the rains occurred in the same location was 41.77% *Aspergillusniger*, 43.56% *Aspergillusflavus*, 11.56% *Alternaria* sp., 8.95% *Fusarium* sp., 4.64% *Rhizopus* sp., 4.55% *Penicillium* sp. and *Cladosprium* sp. was 0% (while the frequency ratio for fungi isolated from the air after the rain was 3.45% *Aspergillus niger*, 2.63% *Aspergillus flavus*, 0% *Alternaria* sp., 0% *Fusarium* sp., 0% *Rhizopus* sp., 0% *Penicillium* sp. and *Cladosprium* sp. was 0%). In the second location in the south of Al-Diwaniya city the frequency ratios of isolated fungi was 77.45% *Aspergillus niger* , 71.64% *Aspergillus flavus* , 40.18% *Fusarium* sp., 30.24% *Rhizopus* sp., 23.85% *Alternaria* sp. and *Penicillium* sp. was 14.74%, respectively, while the fungus *Cladosprium* sp. did not appear. The results showed that the percentage of fungi contamination was more in the southern region of the city. Whereas the percentage of fungi frequency in the air before the rains occurred in the same location was 50.43% *Aspergillus niger*, 48.54% *Aspergillus flavus*, 22.36% *Fusarium* sp., 18.19% *Rhizopus* sp., 13.54% *Alternaria* sp., 11.67% *Penicillium* sp. and *Cladosprium* sp. was 0%. While was the frequency ratio for fungi isolated from the air after the rain was 3.46% *Aspergillus niger*, 3.53% *Aspergillus flavus*, 0% *Alternaria* sp., 0% *Fusarium* sp., 0% *Rhizopus* sp., 0% *Penicillium* sp. and *Cladosprium* sp. was 0%).

Key words: Air pollution with fungi, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria* sp.

Introduction

Fungi are microorganisms that are found everywhere in the environment, be it air, water, and soil (Watanabe, 2002). Most are restored, some are pathogenic, and some are opportunistic (Uzochukwu and Nkpouto, 2013). The pathogenic ones infect people with a weak immunity (Pavan and Manjunath, 2014). It leads to air pollution, dust laden or dust-filled with fungal spores, which it carried and transported by wind from one place to another (Hedayati et al., 2005). Fungi have become pathogens that affect the respiratory system due to their presence in the air (Charles et al., 2009). When fungal spores is inhaled in high quantities during breathing, they cause allergies and irritation of the respiratory system (Abdulrahman et al.,1999). Three mechanisms by which fungi can infect our body are allergies, infection and food poisoning (Begum et al., 2009). Also published research indicated that increased exposure to fungi in the air causes asthma (Bhatnagar et al., 2002). All parts of fungus cause allergies when inhaled (spores or mycelium). Moreover, it has the

*Corresponding Author

ability to induce antibodies in the body (Black et al., 2000). Some fungal strains include 40 or more allergens in humans (Hogaboam et al., 2002). Therefore, the research aimed to identify the fungi that pollute the air and the role of rain in reducing this pollution.

Material and Methods

Samples were collected from two locations in Al-Diwaniyah city, where a region in the north of the city was chosen, samples were collected from ten sites and three replicates for each sample, and another site south of the city collected samples from ten sites and three replicates as well. The study period was during March and April.

Samples were collected in three stages, which are from the air before the rains occurred, by the opening of Petri dishes which contain PDA medium for five minutes in the air. Then, rain samples are collected at the specified sites and aseptic containers. Then samples are collected from the air after the rain occurs in the same way as opening the petri dishes in the air for five minutes.

Prepare the Culture Medium

The medium of potato and dextrose was prepared by dissolving 39 g of ready-made powdered media from the Indian company Himedia, adjusting the pH to 6.5, and then adding 50 ppm of streptomycin. It is placed in a conical flask and sterilised with a sterilizer under the pressure of 1 atmosphere and at a temperature of 121°C and then distributed in Petri dishes (Panagopoulo, 2003).

Isolation and Diagnosis of Fungi

Samples were incubated under 28°C, and after growth, the fungi were diagnosed by using the classification keys (Al-Bader et al., 2007; Kim and Kim, 2007).

Result and Discussion

The results showed fungi isolation from 20 samples of rain water, taken from two location of Al-Diwaniyah city. The fungus *Aspergillus niger* formed the highest percentage in isolation and for all two sites, it was followed by the fungus *Aspergillus flavus* in the ratio, while the rest of the isolates were graded according to the site as follows:

The first location in the north of Al-Diwaniya city, the frequency ratios of the isolated fungi were 73.78% *Aspergillus niger*, 66.87% *Aspergillus flavus*, 34.69% *Alternaria* sp., 22.99% *Fusarium* sp., 15.65% *Rhizopus* sp., 11.54% *Penicillium* sp. and *Cladosprium* sp. was 5.87% (Figure 1). Whereas the percentage of fungi frequency in the air before the rains occurred in the same location was 41.77% *Aspergillus niger*, 43.56 % *Aspergillus flavus*, 11.56% *Alternaria* sp., 8.95% *Fusarium* sp., 4.64% *Rhizopus* sp., 4.55% *Penicillium* sp. and *Cladosprium* sp. was 0%. The frequency ratio for fungi isolated from the air after the rain was (3.45% *Aspergillusniger*, 2.63 % *Aspergillus flavus*, 0% *Alternaria* sp., 0% *Fusarium* sp., 0% *Rhizopus* sp., 0% *Penicillium* sp. and *Cladosprium* sp. was 0%) (Table 1).

While for the second location in the south of Al-Diwaniyah city the frequency ratios of isolated fungi was 77.45% *Aspergillus niger*, 71.64% *Aspergillus flavus*, 40.18% *Fusarium* sp., 30.24% *Rhizopus* sp., 23.85% *Alternaria* sp. and *Penicillium* sp. was 14.74%, respectively, while the fungus *Cladosprium* sp. did not appear. The results showed, that the percentage of fungi contamination was more in the southern region of the city as shown in Figure 2.

Whereas the percentage of fungi frequency in the air before the rains occurred in the same location was 50.43% *Aspergillus niger*, 48.54% *Aspergillus flavus*,

Table 1: Frequency ratios for fungi isolated from air before rain, from rain water samples, and from air after rain in the north of Al-Diwaniyah city

No.	*Isolated fungi*	*The frequency of fungi in rain water*	*The frequency of fungi in the air before the rain*	*The frequency of fungi in the air after the rain*	*L.S.D.*
1	*Aspergillus niger*	73.78	41.77	3.45	2.75
2	*Aspergillus flavus*	66.87	43.56	2.63	1.99
3	*Alternaria* sp.	34.69	11.56	0	1.34
4	*Fusarium* sp.	22.99	8.95	0	1.15
5	*Rhizopus* sp.	15.65	4.64	0	1.11
6	*Penicillium* sp.	11.54	4.55	0	1.09
7	*Cladosprium* sp.	5.87	0	0	1.23

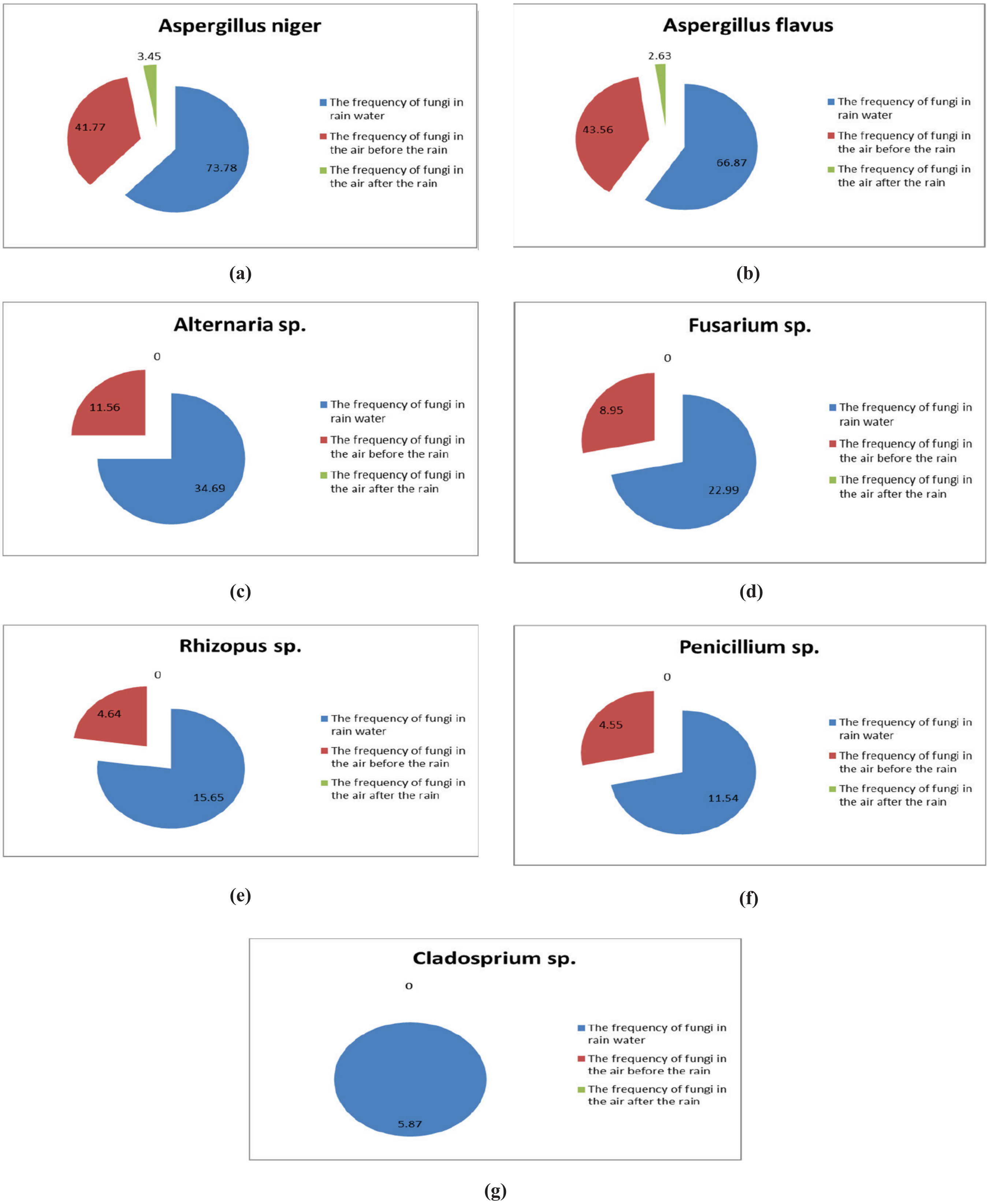

Figure 1: (a-g) Frequency ratios for fungi isolated from air before rain, from rain water samples, and from air after rain in the north of Al-Diwaniyah city.

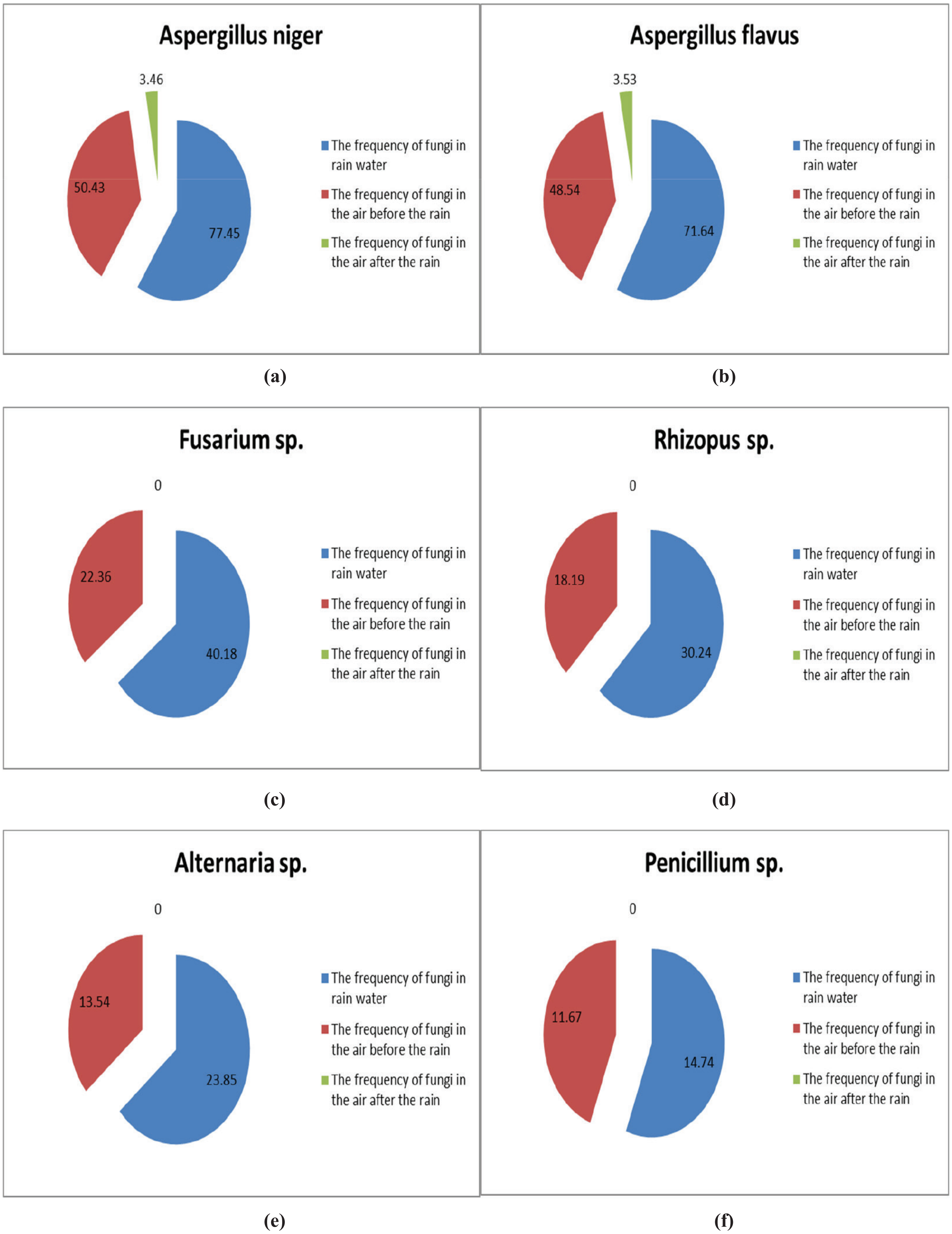

Figure 2: (a-f) Frequency ratios for fungi isolated from the air before rain, from rain water samples, and from air after rain in the south of Al-Diwaniyah city.

22.36% *Fusarium* sp., 18.19% *Rhizopus* sp., 13.54% *Alternaria* sp., 11.67% *Penicillium* sp. and *Cladosprium* sp. was 0%. The frequency ratio for fungi isolated from the air after the rain was 3.46% *Aspergillus niger*, 3.53% *Aspergillus flavus*, 0% *Alternaria* sp., 0% *Fusarium* sp., 0% *Rhizopus* sp., 0% *Penicillium* sp. and *Cladosprium* sp. was 0% (Table 2).

The results showed the great role of rain in reducing the rate of air pollution by fungi and thus the safety of the human respiratory system from any possible injury by opportunistic fungi (Shelton et al., 2002).

These results are consistent with his findings (Levetin and Vande water, 2001), which indicated that the fungus *Aspergillus* sp. is the most common fungus causing respiratory tract allergy. The results also agree with his findings (Haas et al., 2007) that the fungi in the air are an essential factor for the occurrence of anaphylaxis and the production of antibodies in the body. While Walli and Ali (2022) indicated that fungi in dust cause respiratory diseases such as bronchopulmonary aspergillosis. Air does not consider the environment for the growth of microorganisms, including fungi (Horner et al., 2004). Rather, it is present in them and their numbers are affected by the speed of wind, humidity, temperature, distance and proximity to agricultural areas and foodstuffs that are consumed in buildings (Basilico et al., 2007).

Air is an important environmental component that is susceptible to fungal contamination, especially in areas where organic waste is available. Fungi are spread by air currents in the form of fungal hyphae and spores. Air fungi have become increasingly important because they have harmful effects on human health, as exposure to fungi through their entry through the respiratory system causes allergies, irritations, and other toxic effects (Basilico et al., 2007).

There is a lot of research that has proven that the presence of fungi in the air represents one of the causes of asthma, which results from allergies and the presence of allergic antibodies to fungi. All parts of mushrooms (fungal spores and filaments) are allergenic and have the ability to induce antibodies. In addition, some fungal strains contain more than 40 factors that cause allergies in humans. Most scientific studies concerned with the study of fungi indicate that the process of inhaling parts of fungal filaments or spores causes allergic infections and other diseases, including poisoning. This depends on the type of fungus and the time period of exposure to it. Also, allergic responses (rhinitis, pneumonia, hypersensitivity, and asthma) can be considered normal and widely common problems and are related to inhaling fungi in the air (Lehrer et al., 2013).

The air is not considered an appropriate environment for the growth of fungi, but rather they exist in it, and their numbers are affected by humidity, wind speed and temperature (Lee and Lin, 2004).

Based on the period of sample collection March and April months, this period is characterized by fast winds and low humidity in the study area due to high temperature and winds. Therefore, it was the reason for the low percentage of fungi being isolated from the air, especially the *Cladosporium* spp.

Another thing, we noticed was that there was no isolation of the *Cladosporium* spp., and the reasons above are one of the factors in addition to the role of agricultural lands and pesticides used by farmers, which had a major role in reducing the spread of the fungus during this period.

Table 2: Frequency ratios for fungi isolated from air before rain, from rain water samples, and from air after rain in the south of Al-Diwaniyah city

No.	*Isolated fungi*	*The frequency of fungi in rain water*	*The frequency of fungi in the air before the rain*	*The frequency of fungi in the air after the rain*	*L.S.D.*
1	*Aspergillus niger*	77.45	50.43	3.46	3.88
2	*Aspergillus flavus*	71.64	48.54	3.53	2.40
3	*Fusarium* sp.	40.18	22.36	0	1.20
4	*Rhizopus* sp.	30.24	18.19	0	1.98
5	*Alternaria* sp.	23.85	13.54	0	1.23
6	*Penicillium* sp.	14.74	11.67	0	1.11
7	*Cladosprium* sp.	0	0	0	0

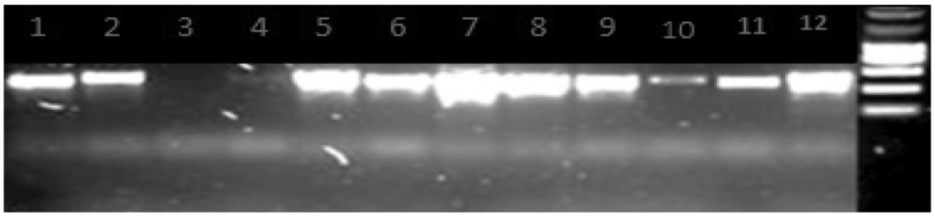

Figure 3: PCR results of *Aspergillus* sp. (sample 12) on agarose by electrophoresis.

Aspergillus sp. was a genus of fungi including some of the most common in air molds These isolations in the current research might be explained by the fact that these months (April and March) had lower humidity levels (Newson et al., 2000). may grow on surfaces when moisture is present. *Aspergillus* spores were wind-dispersed and they were often extremely abundant in the air (Pavan and Manjunath, 2014). DNA was extracted from a pure culture of *Aspergillus* sp. as shown in Figure 3, and then the concentration and purity was measured by using Nanodrop meter, as shown in Table 3. Subsequently, amplified by PCR method and the product of this reaction was analysed by agarose gel electrophoresis method (Table 3).

Table 3: Measured of purity and concentration by using Nanodrop meter

Genus	*ng/µl*	*A260-A280*
Aspergillus sp. (Sample 12)	6.0	1.16

There was no band for some genes (sample 3 and 4) of isolated *Aspergillus* sp. on an agarose gel plate after electrophoresis due to unsuccessful DNA amplification by PCR, as in Figure 3. The ratio (1.16) is slightly lower than the ideal value of 1.8-2 for pure DNA, this could indicate presence of contaminate.

Conclusion and Recommendation

The results showed contaminated fungi in the air in the study area, and emphasised on the role of rain as an effective factor in reducing the percentage of pollution in the air. Through this study, it is recommended to expedite the treatment of organic waste and not leave it for long periods without treatment, as well as increase green spaces, which have a role in raising the percentage of air humidity through the process of transpiration, which achieves great benefit in reducing the rate of pollution and the spread of fungi.

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