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Determination and Distribution of Allergenic and Fungal Bioaerosol in Composting Facilities

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Abstract: The presence of fungi on respiratory tract with contaminated air can play an important role on the occurrence of respiratory diseases such as bronchial asthma and allergic reactions. The purpose of this survey was to determine the prevalence of different fungal concentrations in the composting facility and to compare air quality in indoor and outdoor environment. In this analytical- cross sectional study, the presence of fungi was analyzed using fungi samples were taken from different parts of the composting facility (13 sampling location) using settle plate (open plate method), at a height of 1.5m from ground level. Results showed that the main percentage of each fungal genus were Penicillium (48.3%), Aspergillus (10.2%), Mucor (7%), Alternaria (6.8%) and Rhizupus (4%) species, respectively. Mean of fungal concentrations in outdoor environment was observed higher than indoor environment. However, the differences between viable fungal species as well as concentrations observed in environment can be too large to be a reason of significantly higher risk for allergic asthma symptoms in any group of workers. The statics analysis showed that the fungal counts correlated significantly between indoor and outdoor environment ($p<0.001$).

Key words: Bioaerosol · Fungi · Composting Facility

INTRODUCTION

Accumulation of a large amount of waste may create several problems to inhabiting populations [1]. According to World Bank study, urban per-capita waste management rate for most of the low-income countries will increase by approximately 0.2kg per day by 2025[2]. With increasing evidence of adverse health effects of outdoor bioaerosols, the effects of ambient bioaerosols have drawn much attention in recent years. Among various bioaerosols, fungal spores are of major concern because of their abundant sources and ubiquitous presence in environments [3].Composting is biological decomposition of organic waste material which necessarily leads to proliferation of microorganisms within the composting substrate. Whenever composting materials are handled, for examples during the shredding, turning and screening, the microorganisms could be aerosolized, forming what is termed bioaerosol [4].

Airborne biological contaminants known as bioaerosols include bacteria, fungi, viruses and Pollens [5]. Occupational health and safety concerns and public health issues are varied. Epidemiological studies show that high concentration of bioaerosols can result in several adverse health effects such as respiratory disorders, allergic reactions, infections and toxic responses [6-8]. Therefore, the exposure may be a cause of allergic and immunotoxic diseases such as: bronchial

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asthma, allergic alveolitis, allergic rhinitis, atopic conjunctivitis, organic dust toxic syndrome, chronic fatigue-like syndrome. Mould fungi of Aspergillus, Penicillium, Alternaria, Cladosporium and Mucor genera were identified as the etiologic factors of the above-mentioned diseases and plays an important role in human health, especially in indoor and environments [9].

Three major groups of diseases associated with bioaerosol exposure can be distinguished: 'infectious diseases', 'respiratory diseases' and 'cancer'. Infectious and respiratory diseases are most common [10]. There are, however, very limited information and studies related to airborne biological contaminants, especially airborne fungi, in composting facilities. Since concentration of airborne fungi is an important factor influencing Indoor Air Quality (IAQ) and the prevalence of bronchial asthma and other hypersensitive lung disorders has steadily increased in the last decade, In this paper we present results of a one-year survey on aerial prevalence of fungi bioaerosol in Gorgan, Iran.

MATERIALS AND METHODS

Sampling: This study was conducted at a full-scale composting facility in Gorgan City, Iran. In this analytical-cross sectional study, fungi samples were taken from different parts of the composting facility during 2012. In this study, using settles plate (open plate method), at a height of 1.5m from ground level, sampling was performed from different parts of the compost facilities [9, 11, 12]. The sample size was calculated from the formula ratio. According to a study conducted on bioaerosol in landfill at 2001, the maximum frequency of studied fungal bioaerosol was 81% respectively in comparison with the total bioaerosol[13]. Therefore, values of p and (1-p), respectively, 81% and 19% was considered. Considering d=0.05, $Z_{1-\alpha/2}$= 1.96 and using formula of $n = \frac{z^2 p(1-p)}{d^2}$, the total number of 236 samples were obtained. Sampling, multi-stage was selected. Thirteen sampling locations were selected. Also, sampling was conducted in indoor and outdoor air of composting facilities. These sites were denoted as Site 1 (S.1) to Site 13 (S.13). S.1 near the entrance to the compost facilities, S.2 in scale, S.3 in waste reception hall, S.4 in near the grinder machine, S.5 in entrance of composting facility hall, S.6 in leachate treatment plant, S.7 in car repair shop, S.8 in laboratory, S.9 in wastes separation hall, S.10 in plastic baggage landfill, S.11 in exit of composting facility hall, S.12 in leachate landfill and S.13 in open landfill were located.

Sample Analysis: The sampling was repeated three times at each sampling location during (7-8 a.m.), (11-12 a.m.) and (4-5 p.m.). Limitation culture plates when the microorganisms concentration is less than 100 CFU/100m^3. In this research, the presence and amounts of bioaerosol in the air were assessed by exposing the plates containing Sabouraud's Dextrose Agar (SDA) (Oxiod Ltd., Basingstoke and Hampshire, U.K.) medium for 15 minutes. After collection of airborne particulates containing viable fungi, the plates were incubated at 25°C for 6-7 days until abundant growth was noted [13]. During incubation, the plates were reviewed for the growth of fungal colonies once every 24 hours. Finally, the numbers of colonies were counted after each incubation period and a fungal concentration were expressed in terms the percent of the number of colony-forming units (CFU/m^3) to total forming colonies in cultural medium. To identify the type of fungus grown in culture medium, fungal colonies were then examined microscopically and classified as to genera.

Statistical Analysis: Descriptive summary statistics (mean, median, range and percent) of study variables were generated using PC-SPSS.18. The concentration difference of airborne fungi between the outdoor and indoor area of the stations was calculated with one-way ANOVA test by SPSS version 18.

RESULTS

During 10 months, the total samples were taken from each of the thirteen sampling sites. After the determination of fungal genus using macroscopic/ microscopic characteristics and counting the number of fungal colonies, the following results were obtained: Due to the percentage of each fungal genus, the most identified fungi were: Penicillium (428 CFU/m^3±21.1), Aspergillus (90CFU/m^3±4.8), Mucor (62CFU/m^3±5.2), Alternaria (60CFU/m^3±1.8) and Rhizupus (35CFU/m^3±2.3) species, respectively as showed in Table 1. The results showed that Penicillium S.p. had high percentage and the lowest of percentage belonged to Mucor S.p. A comparison of the fungi recorded in the different stations including to outdoor and indoor air during the study period using 1 h averages are given in Table 2. It appears that the concentrations of total particles and bioaerosols were highly dependent on the type of operation might readily cause any particles or microorganisms to suspend in the air. The results of sampling for total viable fungi (Aspergillus, Alternaria, Penicillium, Mucor and Rhizopus)

Table 1: Colony Count and percentage of the identified fungi

Fungi	percentage	Colony Count	SD	(Min.)	(Max.)	(Median)
Aspergillus Sp.	10.2	90	4.8	0.0	14	5
Alternaria Sp.	6.8	60	1.8	1	7	5
Penicillium Sp.	48.3	428	21.1	7	67	23
Mucor Sp.	7.0	62	5.2	1	20	3
Rhizupus Sp.	4.0	35	2.3	1	8	2
Other[1]	23.8	211	11.3	2	37	14
Total	100	886	20.4	45	104	62

[1]= the fungus were not identified in cultural media

Table 2: A comparison of the fungi recorded in the different stations

Sampling of Location	Aspergillus Sp.	Alternaria Sp.	Penicillium Sp.	Mucor Sp.	Rhizupus Sp.	Other[1]	Total	SD(±)	Percentage (%)
S.1	5	5	23	2	2	12	49	8.1	5.5
S.2	8	3	16	2	1	22	52	8.6	5.9
S.3	0	1	59	20	8	2	90	22.8	10.2
S.4	1	4	67	4	6	2	84	26	9.5
S.5	4	2	42	2	3	2	55	16.1	6.2
S.6	9	5	60	2	1	27	104	23	11.7
S.7	5	6	15	3	2	14	45	5.6	5.1
S.8	14	6	7	4	1	37	69	13.2	7.8
S.9	14	5	58	11	5	9	102	20.4	11.5
S.10	10	6	16	3	3	27	65	9.3	7.3
S.11	4	4	26	3	1	10	48	9.3	5.4
S.12	13	6	18	1	1	22	61	8.9	6.9
S.13	3	7	21	5	1	25	62	10.1	7
Total	90	60	428	62	35	211	886	20.4	100
(±)SD	4.8	1.8	21.1	5.2	2.3	11.3	-	-	-
Percentage(%)	10.2	6.8	48.3	7	4	23.8	100	-	-

[1]= the fungus were not identified in cultural media

S.1= entrance to the compost facilities, S.2= in scale, S.3 = in waste reception hall, S.4= in near the grinder machine, S.5= in composting facility hall, S.6= in leachate treatment plant, S.7= in car repair shop, S.8= in laboratory, S.9 =in separation of wastes hall, S.10= in plastic baggage landfill, S.11= in infection solid wastes landfill, S.12= in leachate landfill and S.13= in open landfill.

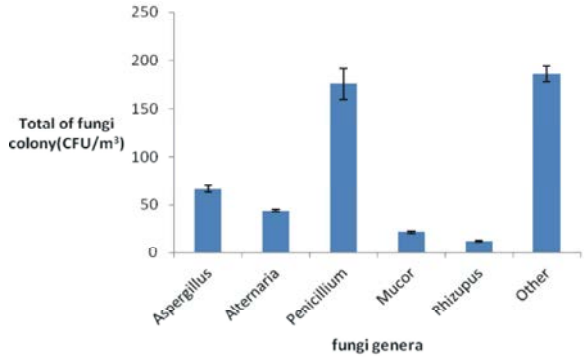

Fig. 1: Mean concentrations of fungi in outdoor environment

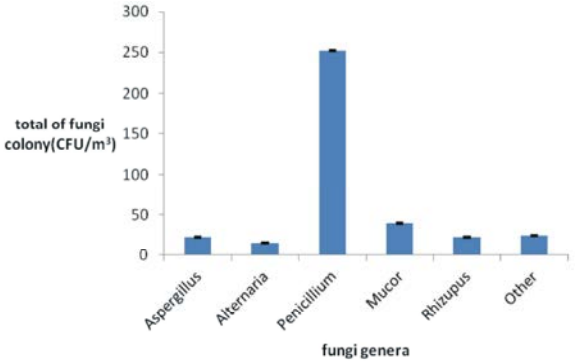

Fig. 2: Mean concentrations of fungi in indoor environment

at the indoor and outdoor environment of different sampling locations are shown in Figures 1 and 2. Accordance to Figure1, the most identified fungi were: Penicillium (34.7%), Aspergillus (13.2%), Alternaria (8.7%), Mucor (4%) and Rhizupus (2.4%) species in outdoor environment, respectively. The maximum concentration of fungal aerosol reached almost 104 CFU/m^3 ±23in S.6 areas (leachate treatment plant) and the minimum concentration of fungal was almost 45 CFU/m^3±5.6 in S.7 (car repair shop) in outdoor environment.

As well, Figure 2 shows that the most identified fungi in indoor environment were: Penicillium (66.5%), Mucor (10.6%), Aspergillus and Rhizupus (6.1%) and Alternaria (4.2%) species, repectively. The maximum concentration of fungal aerosol reached almost 102 CFU/m^3±20.4 in S.9 area (separation of wastes hall) and the minimum concentration of fungal was almost 48 CFU/m^3±9.3 in S.11 (Exit of composting facility hall) in outdoor environment. To evaluate the relationship between the recorded parameters (location of stations as indoor and outdoor environment) and the concentration of airborne fungi (As CFU/m^3), Bivariate Correlations study (Pearson's correlation coefficient test) was performed. The statistical analyses showed that some of the fungi genera as Aspergillus and Alternaria in term of CFU/m^3 had significant correlation with local of sampling station ($p<0.05$). Also, there was a significant correlation between outdoor environment and indoor environment of fungi ($p<0.01$). Levels of fungal contamination in different parts of composting facility and local of station were compared separately with one-way ANOVA test. Between the two groups of fungal genera as Penicillium and Mucor, a significant difference was observed ($p<0.05$).

DISCUSSION

Composting of MSW reduces the volume of the wastes, kills pathogens that may be present, but fungi have a wide presence in composting facility and by entering into the respiratory system, a large number of them are the ability to create an allergy-prone individuals [14]. It is notable that fungal spores are a significant component of bioaerosol and are also considered to act as a marker of the level of atmospheric bio-pollution. Therefore, better understanding of this phenomenon demands a detailed survey of the airborne particles. Some studies have emphasized that fungal spores' concentration can be a scientific indicator of indoor air quality and that it is necessary to deepen the studies of indoor atmospheres in order to promote the air quality, the health/hygiene and a better consideration of the biology of indoor fungi [15, 16]. Workers in composting facility are constantly threatened by emerging and recurring asthma, rhinitis, bronchopulmonary disorders, mycoses and hypersensitivity pneumonitis epidemics [17].

According to the results, maximum fungal concentration was in near the grinder machine station (S.4) with an average of 104±23 CFU/m^3 (11.7%) and minimum was in car repair shop (S.7) with an average of 45±5.6 CFU/m^3 (5.1%). High concentration of fungal in S.4 can be attributed to the grinding of solid wastes and poor ventilation in this station. One of the most prominent findings of this study was the high level of Penicillium S.p emitted by the composting facility. In located stations, the concentration of Penicillium aerosol was about 428±21.1CFU/m^3 (48.3%) and was distinctly higher than the other fungi aerosol. In contrast to the results for penicillium, Rhizupus concentrations were 35±2.3 CFU/m^3 and were less than of other fungi. According to the obtained results, various species of Penicillium, Aspergillus contain the highest percentage of fungal aerosol in the studied area (Table 2). According to the previous studies on this subject, Penicillium Sp., Chrysosporium Sp., Cladosporidium Sp., Candida Sp., Alternaria Sp.Fusarium Sp., Ulocladium Sp., Geotrichum Sp. and Zygomycota (Mucor & Rhizopus spp.) were the most common fungal air flora in various regions of Iran and have mainly shown similar results, as mentioned above [18]. Those were revealed that Penicillium was significantly higher in the indoor and outdoor environments.

Totally, the results showed that in the outdoor environment, the frequency of fungi are similar to indoor environment. Also, in this study, the results showed that fungal concentration in outdoor environment was higher than indoor environment (Figure1, 2). In outdoor environment, the high concentration of fungi is related to S.6 area (In leachate treatment plant). One of the major causes of high concentration in this site, is the near it to composting facility. Also, the process of wastewater treatment such as aeration and mechanical agitation could be effective (Figure1). In indoor environment, the high concentration of fungi is related to S.9 area (Wastes separation hall). In this area, all of solid wastes are separated from each other and the airborne fungi were speared in ambient air. This problem could be the high risk for composting facility workers. So, to considering healthy laws should be enforced in order to safety employers.

Few studies have been done to measuring fungal contamination in environment and their results were similar to this study [10]. Daily activities at the sampling sites were also associated with changes in during day. In this study, the higher levels of fungal seen at noon. Since different activities affects airborne fungi in environment, so in this study, in addition to the correlation between environment and fungi, significant difference was observed between fungi and activities

during different time ($p<0.01$). Comparison of this static analysis showed that type of activities could be affect in distribution of fungi.For this reason, to dominance of Penicillium in composting facility can be attributed to the ability of these fungi in contrast adverse environmental conditions. Development of methods to measure long-term concentrations of fungal spores from composting and other sources are needed. This work is probably the first study which measured fungal levels in and around composting facilities in Gorgan City. Since fungal generation is significant at the composting sites, speciation of fungi would also be very useful. Apart from the gaseous pollutants, there are some biological contaminates like bacteria, molds, virus and yeasts are usually found in moist indoor environment. Therefore, adequate ventilation of the indoor environment can be recommended for optimum penetration of sunlight as well as proper dilution of air pollutants under indoor environment [19]. Due to high concentrations of airborne fungi in composting industry, it is necessary to perform cross sectional surveys and monitor ventilation systems in these industries.

CONCLUSIONS

The presented data demonstrate that the composting facility were a significant source of concentrations of fungi. An assessment of the airborne fungi in the indoor and outdoor environment was experimentally investigated. Experiments were carried out at thirteen varying types of areas during study. This study indicates that there is significant assessment of the indoor and outdoor airborne fungi. The following conclusions can be made:

- Penicillium show higher growth comparing to other growing fungi.
- Mean of fungi in outdoor environment was made higher than indoor environment.
- The most identified fungi were: Penicillium, Aspergillus, Mucor, Alternaria and Rhizupus species, respectively. There was a significant correlation between outdoor environment and indoor environment of fungi ($p<0.01$).

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