ORIGINAL ARTICLE

Investigation of Fungal Bioaerosols in Shiraz Composting Facilities During 2017

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Abstract

Background: Fungi are one of the pollutant emissions from the composting plants which change in variety during the composting process. They are predominant in stabilization stage.
Methods: This study assessed the thermotolerant airborne fungi based on NIOSH 0800 on 200 ambient samples from four composting processes and outdoor spaces in a composting plant.
Results: The concentration of fungi during shredding, separating and screening was higher than 1000 CFU/m^3. The level of fungi in all stages was higher than outdoor ($P<0.001$). The highest concentration was detected in shredding (6749±1347 CFU/m^3) ($P=0.007$), and the lowest concentration was related to screening (113±32 CFU/m^3) ($P=0.013$). The predominant species was varied during the stages. *Yeast* and *A. niger* were predominant species in shredding and separating.
Conclusion: The results of this study showed that the concentration of airborne fungi was decreased during composting process. The effect of these aerosols on indoor air was more than that in the outdoor space and workers at this site were exposed to high levels of thermotolerant fungi. Therefore, air-condition, ventilation system and safety operations such as respiratory masks are essential. The results of this study can be used in risk assessment.

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Introduction

About 68.81 percent of solid wastes in Iran are composed of biodegradable organic matter.[1] Therefore, the production of compost is on the agenda of the municipalities. Compostingis an aerobic degradation process. In this process, organic matters decompose by microorganisms. These microorganisms can become airborne which is called 'Bioaerosol'.[2] Bioaerosols include bacteria, fungi and actinomycetes. Among bioaerosols, microscopic fungi are predominant through microorganisms,[3] and they have been investigated as agents of special concern.[4] For that reason, they were identified as one of the main health risk sourcesfor the workers and occupational health issue and safety as well as environmental hygiene in composting plants.[5,6] They are involved in different airway symptoms, diseases and strong in flammogens, infectious diseases, respiratory diseases and cancer.[7] These health effects can be related to fungi.[8] Due to the high levels of exposure, it is necessary to control their levels to prevent adverse health effects.[4] Therefore, workers on composting sites are exposed to bioaerosols at various stages of composting process, such as shredding, separating, turning and screening. There is an ever growing concern regarding the effect of these activities on the occupational health and safety among waste collectors and employees.[9]

In previous studies, there were very high concentrations of fungi in theindoor air of solid waste sorting plant.[10] It is also known that workers in these types of plant usually complain about many different diseases.[11]

In addition, there are limited amounts of information on the concentration of bioaerosols in public places such as mixed composting plant in Iran and other waste sites and landfills.[12] Due to the importance of this issue, we need more information

with regards to bioaerosol concentrations in theses paces, where people are exposed to indoor air every day. This information on bioaerosol concentration can be useful to many countries where regulations on bioaerosols are being investigated. Therefore, this study is an attempt tocompare the concentration of airborne fungi in an indoor air with the nearby outdoor airborne fungi concentration to identify the effect of composting process on airborne fungi in an indoor air. Approximately 250 tons/day of total waste was separated for composting proposes in Shiraz composting site. It is predictedthat composting rates will be continuing to increase as the amount of waste entering the landfill is in decline.

Identification and enumeration of all compost organisms is not practical;[13,14] hence, one of the best identified microbial indicators is fungus thermomyces.[15] Therefore, it was suggested that the thermo tolerant fungi should be used as an indicator of organisms from air emission in composting plants,[3] and it is one of the main factors in risk assessment.[9] Analysis was also performed to investigate the level offungi which is a useful predictor for estimating the concentration of mycotoxins in composting plants. The aim of this study was to compare the concentration of airborne fungi during composting process includingseparating, shredding, turning and screening of waste in a composting plant and then tocompare it with that of the outdoor space.

Materials and Methods

Selection of Sampling Location

Shiraz is one of the major cities in Iran, located 300 kilometers north of the Persian Gulf and 900 kilometers south of Tehran. According to 2017 data, about 1000 tons of solid waste is produced daily in Shiraz. The selected composting plant was 18 km from Shiraz.[16] In the first phase, the wastes from the municipality were dumped in the site. Then, the sealed bags were shreddedand the recyclable materials were manually separated in sorting cabs.After that, compostable materials were transferred for decomposition. The capacity of this site wais 250 tons/day and the mixed municipal compostable and industrial wastes entered this site for producingtype 1 and 2 grade composts. At the time of observation, the plant was operating at full capacity. This sitewas considered to be a good representative of the composting facilities all over Iran. In this study, we divided the composting processes into 4 steps: (1) shredding, (2) separating, (3) turning, and (4) screening. Sampling was done on the days when composting process was operated.

Sampling and Identification of Fungal Species

Sampling was performed according to NIOSH 0800.[17] The samples were taken duringoperationof the composting process whichwas conducted one time a week mostly. Therefore, the total sample consisted of 200 which were taken from 9 a.m. to 3 p.m.from December 2014 until September 2015 in the composting plant. On site ambient air samples were collected at specific sampling locations(shredding, separating, turningand screening). The duration of on site measurements was initially ten minutes and then it was reduced to one minute due to saturation of some culture media.[3] The samples were taken in the respiratory height (1.5 m above the ground),[18] by Andersen single stage viable particle impactor, set at a flow rate of 28.3 l/min.[3] Sabouraud dextrose agar (SDA) was used as culture media. In order to prevent the bacteria from growing, chloramphenicol was used.[3] Following the sampling, theculture media were incubated in over 37°C (thermophilic temperatures) for 2–7 days.Then, the colonies were counted manually. For identification of fungal species, first the colonies were purifiedand thenidentifiedby slide culture. The concentrations were calculated after being divided by the number of fungi to air volume and expressed in CFU/m^3 (colony forming units per cubic meter). The fungi were identified to genus- and species-level for *Aspergillus*, by colony characteristics and microscopic examination with lacto-phenol blue stain.[3]

Statistical Analysis

Data analysis was performed using SPSS 16.0. In this study, non-parametric test (Kruskal-Wallis and Mann–Whitney test)was used. A significance level of $P<0.05$ was considered.

Results

In this study, the thermotolerant airborne fungi concentration was investigated during the composting process including shredding, separating, turning and screening at a mixed composting plant in southern Iran.

In Figure 1, the concentration of thermo tolerant airborne fungal was indicated in the four composting process. The highest concentrations were detected in the shredding phase, with 7862± CFU/m^3 ($P=0.007$) followed bythe separatingphase with 5397± CFU/m^3 ($P<0.001$) and the turning of composts (1772± CFU/m^3); also, the lowest concentration was found during screening of the stabilized compost ($P<0.001$). Moreover, there was a significant difference of concentration between screening and shredding ($P=0.002$) and this difference was high between indoor and outdoor air ($P<0.001$) in the composting site.

The average concentration in the turning phase was more than that of the screening phase (Figure 1), but there was no significant difference between the emission of thermotolerant fungi between these

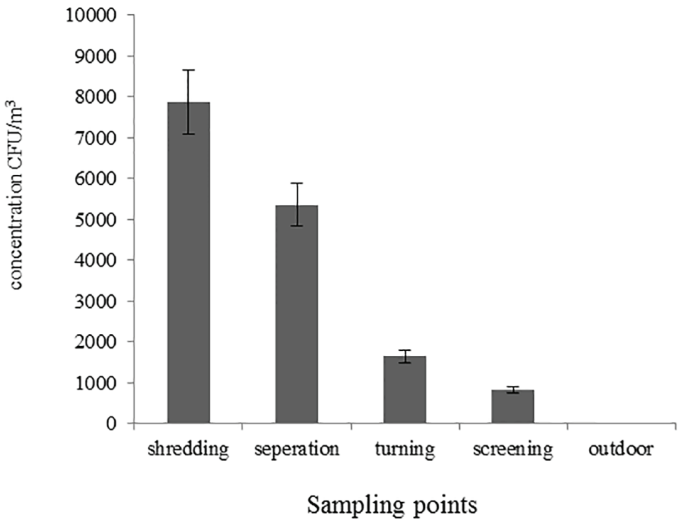

Figure 1: The average concentration of fungi during the composting process.

phases (P=0.114).

In Figure 2, the concentration of fungi was shownin indoor and outdoor space. As seen in this Figure, the indoor level was higher than the outdoor and upwind level and the different between them was significant (P<0.05).

In this study, 8 fungal species were detected when incubation temperature was over 37°C (thermotolerant species). Distribution and concentration of fungal genus are displayedin Table 1 and the predominantpercentage of fungi species is shown in Figure 3.

There are a variety offungal species during the composting process, as shown in Table 2. The highest concentration of fungal species was detected including *yeast* and *Aspergillusflavus* (*A. flavus*) and then *Aspergillusniger* (*A. niger*) in the composting plant (P<0.001); a similar difference was observed in the composting phases and the highest concentration of fungal species was *yeast* and *A.flavus* in shredding (P<0.001), *A. niger* and *A. flavus* in inseparating (P<0.001), and *Aspergillusfumigatus* (*A. fumigatus*) and *A. flavus* in turning phase (P=0.021). However,the highest concentration of fungal species was *Paecilomyces* and *yeast* in screening (P<0.001).

Discussion

In Iran, the number of occupational health investigations in composting was scarce. In other studies, the highest concentrations of fungi were found in the sorting area (412,000 CFU/m^3),[4] shredding and sieving phase; it

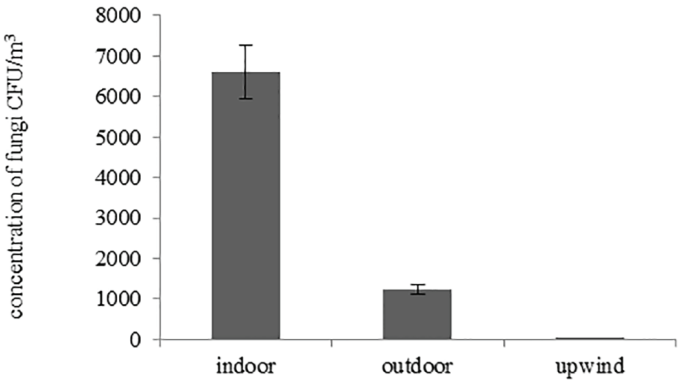

Figure 2: Concentration of fungi in indoor and outdoor areas.

Table 1: Description of the composting process and sampling points

Composting process	Condition	Description
Shredding	Semi-covered area(tunnels is covered but workers location is uncovered)	Shredding of sealed bags including mixed waste
Separation	covered area	Separation of recyclable waste such as plastic, papers and metals
Turning	Open area	Windrow including 50 mass and turning was performed weekly
Screening	Open area	Both manually and rotates screener, classification of compost according to pore size of the screen

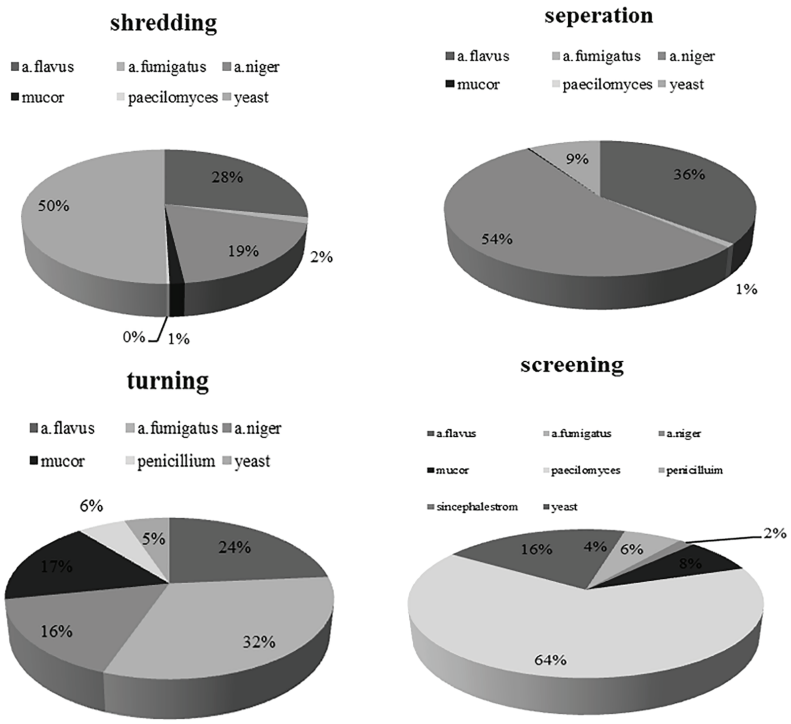

Figure 3: The percentage of fungi species during the composting process

Table 2: Average concentration of fungal species during composting process

	A.flavus	*A.fumigatus*	*A.niger*	*yeast*	*Mucor*	*Paecilomyces*	*penicillum*	*sincephalestrom*
Shredding	1453.69	115.48	340.45	2896.4	91.66	14.91	0	0
Separation	1210.24	17.61	1335.86	172.19	0.78	4.66	0	0
Turning	232.61	234.81	192.37	37.54	127.81	0	73.19	0
Screening	22.19	23.35	10.19	156.05	56	383.77	0.47	0.47

exceeded 10^5 CFU/m^3(3), 10^2-1.7×10^5 CFU/m^3 and 10^4 CFU/m^3 in green waste[3] and 220-412000 in a coffee-processing plant,[4] but there were lower concentrations of fungi during sorting the papers.[19] In Spain, the highest concentrations of the fungi were found in the sorting area (412,000 CFU/m^3, respectively), while the minimum levels were found around the facility (750 CFU/m^3, respectively).[4] Moreover, the lowest concentration was during turning of the compost (10^4 CFU/m^3) which was located in the nearby composting halls,[3] sorting cabin in 37°C.[4]

The composting process was related to microbial degradation and occurred in the windrow phase[13] Other processes such as shredding and separatingwere called sorting. Therefore, it was expected that bacteria in the turning phase weremore than other phases and airborne fungi was dominant in the stabilizingphase.[20,21] However, according to Figure 1, the highest concentration was detected in shredding of bags and lowest in the screening phase. Although organic matters do not degrade in sorting,this difference may be the result of collection system, waste type and composting system.[12] Because the collected wastes were transferred to composting facilities in sealed bags and they had long layover period before composting process, during shredding, high concentration of bioaerosols was released. Lower levels of airborne fungi in turning of the compost can be related to anaerobic condition in these masses;[4] the incubation temperature was 37°C in this study whereas the temperature of the stabilization phase was reduced below 23°C.[22]

The significant difference between screening and shredding can be related to thefactthat sorting is operated in covered spaces, but turning and screening is done in an open area. This was due to the fact that indoor space were not equipped with air-conditioner and ventilation system in this plant and it could be due to the high humidity levels and dusts at the indoor space becausethe humidity can accelerate the germination of the fungi. Moreover, dust was identified as another vital agent on the concentration of fungi because dust was emitted from the composting process. in another study,it was reported that the concentration was very high during loading, shredding, windrows turning and separating phases,[23] and the concentration of fungi washigher in the enclosed units such as waste storage composting halls.[4] Moreover, the lower concentration

was detected by Martı´Nadal (2009) at the indoor space equipped with double system of chemical washers and biological filtration.[4] Because the particles encompass airborne fungi trapped and controlled by filter,the use of ventilation and air-conditioning systems is not common in this site; thus, the occupational environment was ventilated by opening windows. Although application of ventilation, an exhaust system and central vacuum cleaners can significantly reduce bioaerosol concentration in working environments,[24] the effect of suchengineering measures such as air-conditions, efficient ventilation system and biofilters is not exactly recognized.[3] In order to avoid aerosolization of microorganisms, it is important to understand the main agent of the aerosolization of different bioaerosol components.[25] Therefore, indoor spaces equipped with a mechanical exhaust ventilation system can decline the bioaerosol.[12,24]

Although sorting is one of the most important phases among composting processes[25] and it has many advantages, but many hazardous agents such as bioaerosols and MVOC may be released during sorting, especially composting in enclosed units,[5] as this study indicated. In addition, the health effects were linked to bioaerosol emissions, especially for workers they were more related to the biological load of such emissions.[12] But this finding requires further studies to be conducted on the subject.

Although the concentration level in this study was 10–100 times lower than previously reported airborne fungi in other composting plants and the suggested guideline by EPA and WHO do not specify any proper level and threshold of bioaerosol concentration to prevent mold problems in an indoor environment,[3,26] the results of this study showed that the amounts of fungi were more than those explained in guidelines. Therefore, the potential exposure to pollutants in MSW management is quite important and specific regulation is clearly necessary.[4,14] Moreover, it is proposed that concentration of fungal flora can be 5×10^3-10^5 CFU/m^3.[24] However, IRSST proposed that the concentration of composting has not been more than the background areas.[10] In this site, some composting processes,such as separation and screening,were operated manually. Therefore, the risk of these phases was higher. More investigations are recommended to identify this risk for workers. It is suggested that all activities involved in the composting process should be controlled and the concentration of airborne fungi should be kept below Workplace Exposure Limits in the composting process;[2] also, personal protective measures are essential for workers in sorting and turning (leaving doors and window). Therefore, the main exposure was related to theindividual's behavior (opening cabin door or window).[12] Shredding was performed in tunnel 1 (covered) but the workers were working in semi-covered areas. Therefore, as expected, the exposure level of the workers was lower than that of this study.

Although it was expected that the concentrations of bioaerosol in a household composting was higher than other composts, such as green waste,[12] in this study the concentration of fungi was significantly lower than those of other studies because it was determined that the concentration of fungi was related to the nature of waste, season, process phase and sampling location.[3] In addition, other studies have investigated mesophilicfungi, butin this study only thermotolerant species was detected. These species can grow in specific thermal conditions (over 37°C). However, the growth of some other types of fungi species is suppressed in this temperature. Moreover, because pathogenic fungi grow in 37°C,[27] the rmomycetes fungi are one of the best microbial indicators for monitoring composting bioaerosols.[28] Therefore, it seems that further studies on these species are essential. Moreover, WHO guidelines noted the total concentration of fungi and not any specific ones such as the rmophilic and mesophilic fungi even though thermophilic fungi are more destructive than any other species. Consequently, it is notcomparable with the mentioned guidelines and other studies.

Although the average concentration of fungi in the turning phase was more than that of screening (Figure 1), there was no significant difference between emission of thermotolerant fungi between these phases (P=0.114). This result was not confirmed by other studies.[12] Moreover, the number of workers and drivers in screening wasmore than the turner drivers and the predominant fungal species during screening was *Paecilomycessp*; therefore, the potential health hazards for workers in screening weremore than those of turning since adverse health effects, such as eyes and nose infections, are related to this species and are confirmed.[27]

According to Table 2, fungal species were varied during composting phases. This vast variability was related to the high variability of indoor air in composting phases, different composting techniques, degree of decomposition, the condition and type of process engineering, composition of the compost, and sampling location.[3,29] Consequently, the presence of certain species can be an indicator of the degree of decomposition.

Amongthe detected fungal genus in this study, *Paecilomyces, A. niger, A. fumigates* and *Penicilliumare* classified as thermotolerant species[30] which can be more hazardous for human because they grow easilyin the body.

As shownin Table 2, predominant fungi species were varied during the composting process. In other studies, the predominant fungi were *Penicilliumsp*,[20] *Aspergillus* (especially *A. fumigatus* reached 10^5 CFU/

m^3) and *Penicillium*in shredding, *Aspergillussp* such as (*A. fumigatus, A. flavus, A. niger* and *A. clavatus*) in later stages[3] and *A.fumigatus* was predominant in the receiving area at 37°C.[4] The predominant *Aspergillussp* in indoor space indicated that there was a problem with water penetration or high humidity.[31] Therefore, operation for reduction of humidity in indoor space was essential. *Aspergillus* is a group of molds[32] themycotoxins of which are known to be present in the inhalable fraction of airborne component.[7] Moreover, these species are very common in the indoor space and they have small and dry spores that are easily emitted by the current air. Therefore, spores and mycotoxins in the indoor air of each three processes enter theworker's respiratory tract. Biomarker of *A. fumigatus* was detected in airway Epithelial Cell in a composting plant in Taiwan.[33]

One of the most important applications of airborne fungal contaminants in compost facilities was related to evaluation of building pollutant, microbiological indoor air quality[11] and assessment of the health hazards such as allergens and toxins.[30] Moreover, markers of microbial exposure were increased in people who live in homes with indoor storage of organic household waste.[12] Fungi produce mycotoxins and MVOC. Mycotoxins are one of the most important secondary metabolites identified as indoor pollution.[30] Aflatoxin is one of the mycotoxins produced by *A. flavus*. Aflatoxin can be a potent carcinogen.[7] With regard to predominant fungal species (Table 1), this species has a high risk for workers, especially in shredding. Moreover *A. niger* was the predominant genera in separation. Of *Aspergillussp*, *A. niger* produces much more mycotoxins[34] and is exposed to mycotoxins leading to hepato-cellular carcinoma and mycotoxicoses of the lung. Oxalic acid is another metabolite of *A. niger*. In addition, there are mycotoxins in both living and dead spores of fungi.[30] Therefore, periodical clinical tests are required for workers. With regard to, Anderson impactor only collected viable microorganisms, so that the risk of mycotoxins was not determined in this site.

According to the result, *A. fumigates* was detected in 50% of the total samples. This species was found in all phases except for during shredding, where *A. flavus* and *A. niger* were also predominant. It can depend on synergistic characteristic of these species. Because the presence of *A. fumigatus* during the composting was related to operational treatment processes, the meteorological conditions, kind of organic matter and the used technology.[23] The major agent in worker's exposure to *A. fumigatus* was presence of workers in outdoor composting process. In addition, working in outdoor space was the major factor in concentration of airborne fungi in the composting process. In the current study, concentration of *A. fumigatus* was lower than 300 CFU/m^3. According to EPA (2010), the concentration of fungi must be lower than 1000 CFU/m^3 in the composting plant. In another study, the concentrations of these species were more than this limitation.[20] Moreover, the metabolites of this species were found when their concentration was above 10^7 CFU/m^3.[30] In this study, the concentration of *A. fumigatus* was much lower. Therefore, the production of metabolites from these species is impossible and the potential risk of this genus is very low for workers in this plant, especially in sorting. Thesespecies are parasites only for immuno-suppressed patients and fortunately there was no worker with this characteristic. The growth of some thermotolerant species, such as *A.fumigates,* is accelerated by high ambient temperatures;therefore,a high concentration of spores was already detected during the high temperature of biodegradation, especially windrow mass (60°C, respectively).[30] Then, they were aerosolized easily during turning. Moreover, one of the agents which decrease these species in screening can be lower temperature of this phase (23°C respectively).

Penicillium is one of the other saprophyte species, but it was not detected during separation and shredding. In other studies, *Penicillium* was isolated widely. *Penicilliumsp* are omnipresent saprophytes in temperate soils. Due to their pronounced variable enzymatic ability these species can be isolated from almost all organic materials.[20] Airborne levels of produced extracellular polysaccharides (EPS) by *Penicilliumsp* are a good marker for common fungal exposure. Although EPS has no pathogenic role in inflammatory or allergic reactions to fungal components,[12] the analysis of non-volatile secondary metabolites can lead to detection of some species, especially *Penicillium*.[30] In spite of the low concentration of *Penicillium* in this plant, further studies are suggested.

Conclusion

This study was completed based on the impact of air on agar enumeration. The highest concentration was related to shredding phase because this phase was done in a closed space.Moreover, there weretoo many workers in the shredding area. It is suggested that wearing respiratory masks is essential for workers. Moreover, equipped turner to anti-fungal filters can be useful in preventing their entrance. Concentration of fungi was decreased during the composting process. The predominant species varied during the composting plant. *Aspergillussp* was predominant in the majority of different phases. With regards to high concentration of *Aspergillus* in this workplace, it would bebetter that the composting plant be convertedfrom manual to automatic. Therefore, the number of exposed workers will decrease. Moreover, proper air-conditioning system and proper ventilation can reduce the fungi level. Because pollutantswere

lower in the outdoor areas in comparison to anindoor space such as screening and turning. Therefore, operational conditions are vital factorsin concentration of fungi, furtherstudieson other bioaerosols, such as actinomycetes, bacteria and metabolites (mycotoxins, endotoxin and dusts), are required.

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