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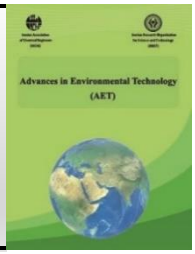

Assessment of bioaerosol emissions from composting application in the urban green space of Kermanshah province in Iran

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ABSTRACT

The vast majority of microorganisms in composting sites produce aerosols, which can cause respiratory difficulties. The objective of this study was to evaluate the emission of bioaerosols from compost applications in urban green space and assess their potential health hazards. The biological samples of bacteria and fungi in the air were collected in 20 points of the urban green space. Fungal medium and bacterial medium has been used as two plates containing the same medium to identify the bacteria and fungi in the air. The ambient temperature and humidity were measured at each of the 20 points of the sampling sites. The obtained results showed that the average concentration of bacteria and fungi in the background samples (before compost application) were 1108 and 122 CFU/m^3, respectively. The bacterial and fungal concentration increased three times in the main samples (bacteria: 8393 CFU/m^3 and fungi: 1659 CFU/m^3) and increased relatively two times in the downwind samples at a distance of 10 m. Although the airborne fungal concentration in the main samples increased three times more than the background samples, a significant statistical difference was not verified between these values. As a result, the increasing of airborne fungi from compost application cannot be proven with certainty. Compost application in the urban green space is considered as the potential source for pathogenic bacteria emission.

1. Introduction

Composting is a natural biological process in which organic waste is turned into a stable product with a high nutrient content through existing microorganisms [25]. The products are rich in nutrients and valuable microorganisms, improving soil quality as a soil conditioner and fertilizer [23]. Notwithstanding the above, there are many potential hazards and health risks related to compost application. Déportes, Benoit-Guyod, and Zmirou [8] summarized the hazardous effects of waste compost into three parts: 1. waste compost containing heavy metals, organic hazards, and microorganisms that can be adsorbed through direct contact with the body, 2. accumulation of hazardous material in agricultural productions when compost is applied as a fertilizer in farmlands, and 3. dispersion of compost particles by wind and atmospheric flows that cause microorganisms and toxicants to be transferred as aerosols that come into contact with individuals. Contact with compost microorganisms can potentially cause illnesses for those exposed to it and manifest in various forms, such as infections and allergic reactions [13]. Furthermore, the microorganisms involved in the degradation of organic waste, including some pathogens such as coliforms in the initial organic waste, can be quickly destroyed due to heat generated by the proper operation of the composting process. The lack of this condition during the composting process can lead to bioaerosol emission in the surrounding environment [16,20]. In particular, these biological aerosols or bioaerosols contain a large number of microorganisms that are either pathogenic or non-pathogenic and dead or alive, which present a health risk

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when exposed to one's skin or inhaled [4]. The airborne microorganisms ranging from 0.001 to 100 m are suspended in the air because of their low weight. They may include a variety of algae, bacteria, fungi, pollen, protozoa, virus, and fragments as well as released molecules like endotoxin, 1-3 -glucans, spores [32], and biological substances such as microbial enzymes [31]. Particles less than 2 µm can enter into the bronchial, and particles less than 1 µm can get into the alveolus [7]. Generally, exposure to compost causes infectious diseases, fatigue, headache, respiratory diseases, and cancer; the inhalation of bioaerosols produce respiratory symptoms and lung inflammation [1,12]. Legionnaire's disease and Pontiac fever are infectious diseases caused by exposure to bioaerosol [27-28]. The respiratory symptoms and airway inflammation caused by bioaerosol with a high concentration of microorganisms have been confirmed in many studies [1,12]. Airway inflammation from toxins, pro-inflammatory agents, or allergens lead to respiratory diseases like asthma, mucous membrane irritation, chronic bronchitis, and hypersensitivity pneumonitis [22,24,30]. Many microorganisms are known as respiratory sensitizers generated by dust during the composting process. Fungi such as *Aspergillus* spp, *Penicillium* spp, *Cladosporium* spp, *Rhizopus* spp, and *Alternaria* spp are well-recognized allergens, while Gram-negative bacteria are the source of endotoxin [14-15]. Breathing organic dust can cause respiratory and immunological symptoms, which are generally divided into four reaction categories; rare cases can lead to infection [26], fatigue, and headaches. In many urban areas of arid and semi-arid regions, processed compost from manure and feedstock is used in parks and green spaces as a fertilizer, aiming to minimize water loss. The release of organic dust due to wind and loss of water content in arid and semi-arid regions is a frequent occurrence. Furthermore, dust storms in the Middle East in the last decade are a recurring phenomenon and has increased the hazardous effects of organic dust on the health of those living in these areas and similar areas. Also, many biological agents in such aerosols that may cause health problems are currently not well identified. Therefore, the objective of this study was to evaluate the bioaerosol (fungi and bacteria) emissions from compost application in the urban green space in the city of Kermanshah, Iran, which is intensively exposed to organic dust and dust storms.

2. Materials and methods

2.1. Sampling and procedure

The study area was located in Kermanshah, Iran (central coordination: 34° 22' 31.00" N, 47° 7' 48.00" E) and based on metrology data from 1951-2015. The city has a moderate to warm climate with a mean annual temperature of 14.4 ºC, average wind speed of 4.9 knots, and mean annual precipitation of 439.5 mm. The humidity varies between 15.5-32.5%. The airborne samples containing bacteria and fungi were collected from 20 points before composting application (background sample), and after compost application (main samples) from April to September in 2017. The location of the sampling points and adjacent green space is shown in Figure 1.

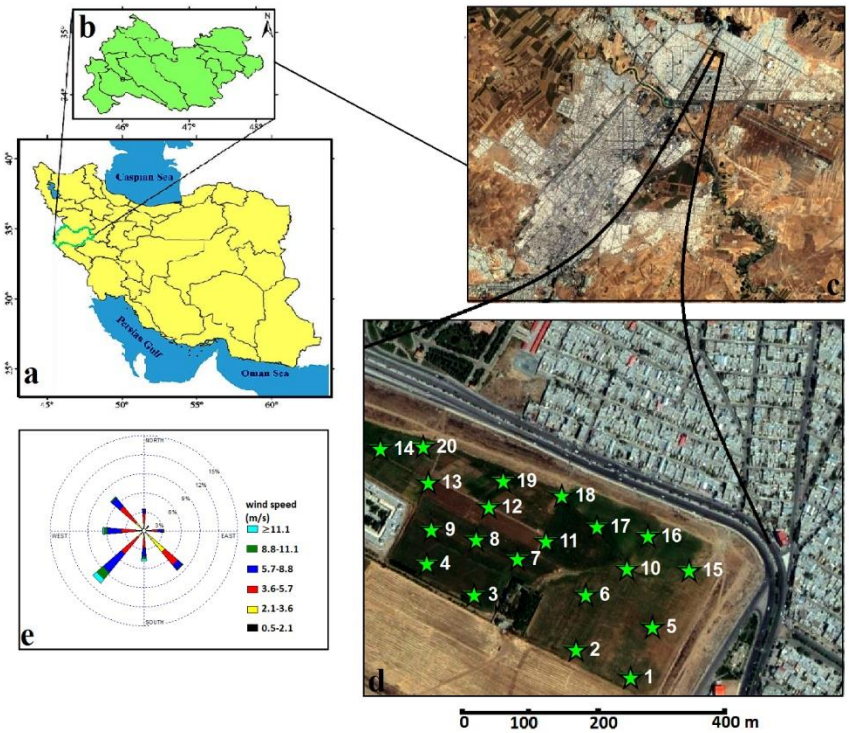

Fig. 1. Sampling points a: Iran, b: Kermanshah, c: study area, d: google earth map of study area, e: wind rose diagram of study area

This study was a descriptive analysis. The biological samples (bacteria and fungi) were collected from 20 points in the air using a single-stage Anderson cascade impactor before compost application (background sample), after compost application (main samples), upwind at the distance of 10 m from the compost application site, in contrast of the dominant wind direction, and downwind at the distance of 10 m and 20 m (dominant wind direction) (Figure 2).

Fig. 2. Point of sampling and distances of sampling in that point along wind direction

The ambient temperature (Laser Infrared Thermometer-AIR-801) and humidity (RH297 moisture meter) were measured simultaneously at each sampling site. The malt extract agar (MEA) and tryptic soy agar (TSA) medium were transferred to the sampling site to examine airborne bacteria and fungi, respectively. From each point, four air samples were taken by the dual method (for each sample, two plates containing the same culture medium) and transferred to the laboratory via a cold box [3].

2.2. Identification of bacterial and fungal bioaerosols.

The biological samples were transferred to the laboratory according to the standard method and incubated. The bacterial medium (TSA) was incubated at the temperature of 35 ± 0.5 ˚C for five days, and the fungal medium (MEA) was incubated at the temperature of 25 ˚C (environmental temperature) for seven days. Then, the bacterial colonies on the TSA medium and the fungal colonies on the MEA medium were counted and reported as the colony forming unit (CFU/m^3). In each sampling, two plates containing the same medium (duplicate) were used, and the average of the counting colonies was reported as the bacterial and fungal concentration in the air. It should be noted that the Gram staining method was performed to identify the bacteria. The bacteria were surveyed in terms of shape, structure, and type of gram reaction. For fungal identification, the appearance of colonies in terms of color, growth, and topography were surveyed; afterward, they were identified based on microscopic morphology such as shape, amount, and color of the conidia [28]. The mechanism of the device is based on microorganism impaction with the medium in the plate inside the device. In this method, the intended medium was poured on the collective plate under sterile conditions and kept at 4 ˚C until sampling time; it was transferred to the sampling sites through a cold box and placed in the device, and air sampling was carried out. The data were analyzed by SPSS (t-paired test and Pearson correlation). The results in this section are presented based on the statistical results from 20 sampling points in different urban green spaces in Kermanshah, Iran.

3. Results and discussion

3.1. Effect of the environmental factor on bioaerosol emission

The Pearson correlation test results showed that there was a significant statistical difference between the bacterial concentration in the main samples with humidity ($P < 0.05$, r: 0.516). This means that by increasing humidity, bacterial emission was reduced. The results also revealed an inverse relationship between the average concentration of bacteria in the main samples and the downwind samples at a distance of 10 m and 20 m with humidity ($P > 0.05$, r: -0.562). But a significant statistical difference was not verified between temperature and bacterial density or temperature and humidity with fungal density. By surveying different studies, it was found that the weather conditions, wind speed and direction, and compost moisture content affected the concentration of the generated bioaerosols [16]. The results of this study showed that ambient humidity affected the bacterial emission from compost application. It must be noted that the isolated Gr- bacteria was lesser than the Gr+ bacteria in different samples. Brooks, Tanner, Josephson, Gerba, and Pepper [5] reported that aerosol Gr- bacteria was no longer active under the conditions of high temperature, low humidity, and UV light. The wind flow is an environmental factor that affects bioaerosol emissions. Bioaerosols can be transferred over long distances through wind and thermal current as well due to their low mass. The pattern of bioaerosol emissions around a composting site depends on several factors: emission rate, atmospheric condition (wind speed and direction, solar radiation, temperature gradient, and relative humidity), and area topography [10]. Additionally, bioaerosol emission is related to the type of system applied for composting organic waste, the moisture content of the compost, and the microbial content of the initial waste [6,17,18]. Recent studies showed that the concentration of *Aspergillus fumigatus* spp and total mesophilic bacteria reached the background concentration at the distance of 200 m in composting organic waste [9]. In this study, the t-paired test results verified a significant statistical difference between the bacterial and fungal concentration in downwind samples (dominant wind direction) in the distance of 10 m; however, the number of counted bacteria and fungi in upwind samples were less than the downwind samples at the same distance. The same results were reported by Hryhorczuk, Curtis, Scheff, Chung, Rizzo, Lewis, and Moomey [19]; they found that the fungal cell was 3098 and 2448 CFU/m^3 in downwind and upwind samples, respectively. Gladding, Rolph, Gwyther, Kinnersley, Walsh, and Tyrrel [17] identified that the *Staphylococcus* spp.

downwind was at least 61 times higher than the background at the boundary and at least 8 times higher 70 m downwind on the four farms tested.

3.2. Bacterial concentration

Table 1 shows the average of bacterial colonies in each 1 m^3 of air (CFU/m^3) in the 20 points, including background and main samples, the different distance of 10 m (upwind samples), and 10 m and 20 m (downwind samples). Based on the results, the lowest average bacterial concentration was related to the background points (1108 CFU/m^3), and the highest bacterial concentration was related to the main point (8393 CFU/m^3). The average of downwind bacterial concentration in the distance of 10 m was 6217 CFU/m^3, which increased notably as opposed to the background samples. But the bacterial concentration in the distance of 10 m upwind and 20 m downwind was not remarkably different than the concentration of the background samples. Sánchez-Monedero, Stentiford, and Urpilainen [29] studied green waste composting. Bacterial and fungal concentration in the background samples was less than 1000 CFU/m^3 when the special activity was not performed. But processes such as shredding, turning of compost piles, and final screening caused the high value of bioaerosols relatively two times more than increasing than the background value in the distance of 40 m in the downwind samples (dominant wind direction). Various bacterial and fungal concentrations have been reported in composting plants by different studies [11]. The results showed a significant difference between the background bacterial concentrations with the main concentration ($P < 0.05$). Hryhorczuk, Curtis, Scheff, Chung, Rizzo, Lewis, and Moomey [19] studied bioaerosol emissions on the inside and outside of a composting plant as well as the significant differences observed between the bacterial concentrations in both places. It was also found that the downwind value was more than the upwind value. Figure 3 compares the average bacterial concentration in the various samples. As shown, the bacterial concentration order is as follows: main, 10 m downwind, 20 m downwind, 10 m upwind, and background. Generally, among the sampled sites, the bacteria concentrations of samples 10 to 18 were higher at various distances. It seems that this trend is in line with the wind direction.

Table 1. The number of counted bacterial colonies in air (CFU/m^3)

Samples	Minimum	Maximum	Average
Background	35	1130	1108
Main	493	19893	8393
Downwind 10 m	347	14459	6217
Downwind 20 m	104	7869	3332
Upwind 10 m	94	5968	2518

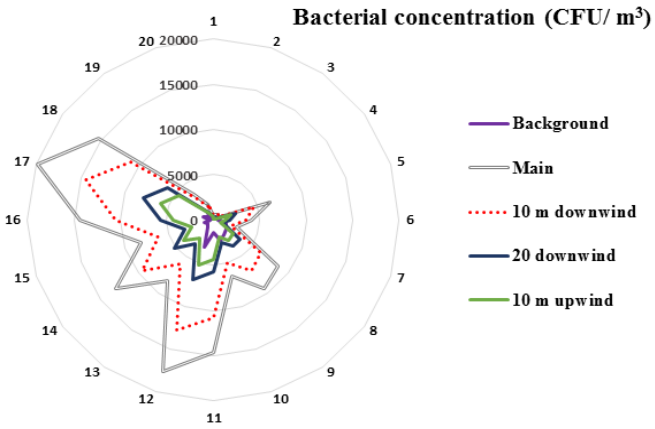

Fig. 3. Comparative study of bacteria concentrations

3.3. Fungal concentration

The average of the fungal colonies in each 1 m^3 of air (CFU/m^3) in the 20 points, including background and main samples, the different distance of 10 m (upwind samples), and 10 m and 20 m (downwind samples), are shown in Table 2. Based on the results, the lowest average fungal concentration was related to the background points (122 CFU/m^3), and the highest fungal concentration was related to the main point (1659 CFU/m^3). The variation of fungal concentration was relatively the same as the bacterial concentration. The fungal concentration in the main samples increased about ten times more than the background samples; the background concentration was 122 CFU/m^3, which increased to 1659 CFU/m^3 in the main samples. The downwind samples in the distance of 10 m (1199 CFU/m^3) were remarkably different than the background concentration. The fungal concentration showed a notable difference in the upwind samples in the distance of 10 m (318 CFU/m^3), but there was no notable difference in the downwind samples in the distance of 20 m (778 CFU/m^3). The average concentration of bacteria and fungi in the organic composting waste in Oklahoma were respectively 5059 and 972 CFU/m^3 in the downwind samples (dominant wind direction) in the distance of 30 m [19], which was consistent with the present study. The results of other work reveal that the value or specific limitation cannot be presented for bioaerosols concentration in composting and its related process. But there was no significant difference between the background fungal concentrations with the main concentration ($P > 0.05$). Although it was found that the main fungal cell concentration was about three times more than the background concentration, a significant statistical difference was not verified between these data. Also, no significant difference was observed between the background bacterial and fungal concentrations with average concentrations of bacteria and fungi (the average concentration in main samples, downwind samples in the distance of 10 m and 20 m in each sampling site). Brooks,

Tanner, Josephson, Gerba, and Pepper [5] studied the use of biological solids in an American desert area and showed that their application did not create health risks. Also, the numerous microorganisms could be trapped inside the biological solid and failed to convert into aerosols.

Table 2. The number of counted fungi colonies in the air (CFU/m^3).

Samples	Minimum	Maximum	Average
Background	29	326	122
Main	91	3962	1659
Downwind 10 m	69	2565	1199
Downwind 20 m	19	2574	778
Upwind 10 m	62	944	318

The average fungal concentration in the various samples are compared. As shown, the fungal concentration order is main, 10 m downwind, 20 m downwind 10 m upwind, and background. At the sampled sites, the concentrations of fungus are predominantly higher at various distances and show a similar trend with the bacteria's distribution (Figure 4).

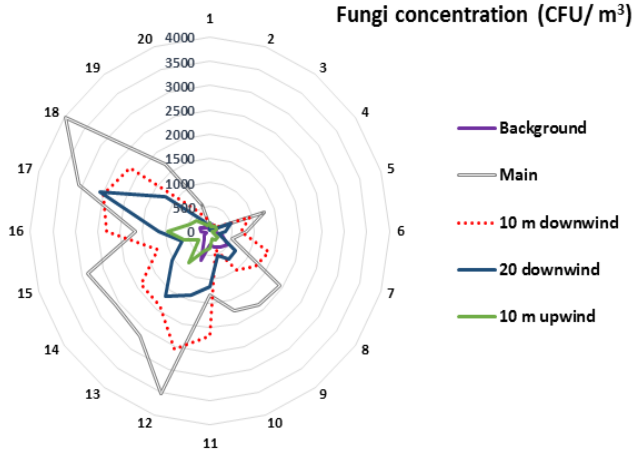

Fig. 4. Comparative study of fungi concentrations.

3.4. Type of identified bacteria and fungi

The bacteria were separated into four categories and included gram-positive bacilli based on the Gram staining method and the shape of the bacteria. Figure 5 shows the various types of bacteria in the studied samples. As shown, the Gram-positive bacteria, including Cocci and Bacilli, were predominant in all the samples with 73-85 percentage abundances.

The results showed that the isolated fungi belonged to four main categories, including *Dematiaceous* fungi, *Penicillium* spp, *Aspergillus* spp, and yeast, according to appearance and morphology (microscopic observation). As shown, *Dematiaceous-fungi* was the most predominant spp in all the samples in the 30-42 percentage range, and followed by *Penicillium*, yeast, and *Aspergillus,* respectively. Additionally, *Fusarium*, *Mucor*, *Rhizopus*, *Glicocladium*, *Chrysosporium*, *Acremonium*, *scopulariopsis*, and sterile mycelium were identified in some samples (Figure 6).

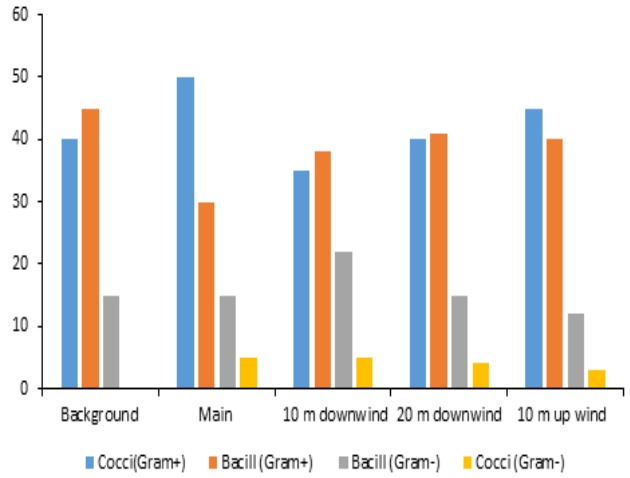

Fig. 5. The percent of identified bacteria in each sample fungal concentration.

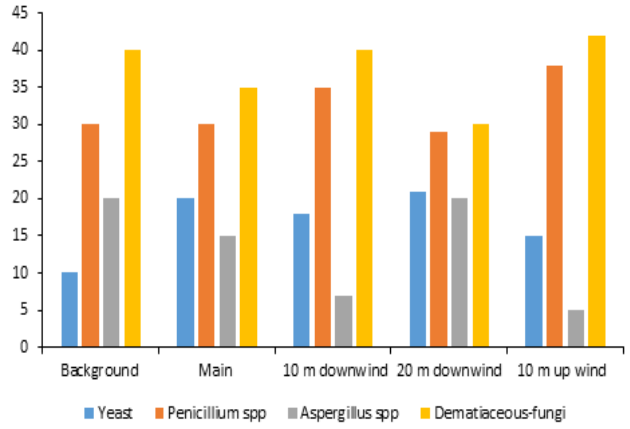

Fig. 6. The percent of identified fungi in each sample.

The type and percentage of isolated bacteria and fungi in different samples did not show great variation. But generally, all of the samples showed a higher concentration of Gr+ bacteria in comparison to Gr- bacteria. The highest concentration of isolated fungi in terms of frequency was related to *Dematiaceous* fungi, *Penicillium* spp, yeast, and *Aspergillus* spp, respectively. A literature review of other works demonstrated that compost application and related processes significantly caused the emission of *Aspergillus* spp, which belongs to the thermophilic fungi species [3,18,21]. But in this study, the percent of *Aspergillus* spp in the background samples was even higher than the main samples. The unpleasant smell resulting from compost application in the urban green space of Kermanshah indicates that the composting process has not passed all of the stages properly, indicating that the thermophilic phase has not likely started. And this leads to the low proliferation of thermophiles fungi, such as *Aspergillus* spp, in bioaerosol emission. The threshold values of airborne microorganisms or their toxins have not been published, even for occupational exposures, due to some reasons: 1) Limitation in the sampling method for all biological components, 2)

Lack of information about the relationship between contact with bioaerosols and allergic reactions, and 3) Vast variation in the susceptibility of individuals to biological agents. Although some researchers have tried to establish the occupational and environmental exposure limitations based on health factors, the scientific basis is not characterized by some presented data. It was found that fungal spore emissions and related health risks with fungal spores from compost application do not cause the specified problems. But there is a possible public health risk in terms of the bacteria cell.

4. Conclusions

This study revealed that compost application in urban green space could be considered a potential source for pathogenic bacteria emission. The findings showed that compost application increased the main bacterial concentration compared to the background samples with a significant difference. Although the average concentration of airborne fungi in the main samples increased three times more than the background samples, a significant difference was not observed. As a result, the increase of airborne fungal from compost application cannot be proven with certainty. There was no significant difference between the concentration of bioaerosols (bacteria and fungi) in the upstream samples than the downwind samples in the distance of 10 m. Also, there was no significant difference between the concentration of bioaerosols (bacteria and fungi) in the background samples with the average concentration in the main samples and also the downwind samples in the distance of 10 and 20 m. An inverse relationship was observed between ambient humidity and bacterial concentration in the main samples, indicating that high humidity led to lesser bioaerosol emissions from compost piles. A relationship was not observed between the ambient temperature with a number of airborne bacteria and also the ambient temperature and humidity with a number of airborne fungi from compost application. In most cases, the isolated bacteria from the environment were Gr + bacteria. The isolated fungi in terms of frequency were *Dematiaceous* fungi (*Cladosporium*, *Alternaria*, *Ulocladium*, and so on), *Penicillium* spp, yeasts, and *Aspergillus* spp, respectively. Generally, it was concluded that compost application in the urban green space in Kermanshah could be considered as a potential source for bioaerosol emissions and subsequently increase allergic reactions, asthma, and respiratory problems.

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