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Identifying and Determining Dispersion Boundary Bio-aerosols of Bacterial and Fungal Pathogens from Municipal Waste Collection Containers

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Background & Aims of the Study: Identifying and determining the dispersion boundary bio-aerosols of bacterial and fungal pathogens for locating containers of municipal waste in accurate management to prevent from health damage and enhance society health is essential. The aim of this research is to identify and assess the radius of bacterial and fungal bio-aerosol emissions from municipal waste collection containers.

Materials and methods: This was a cross- sectional study in Gonabad city in which air sample prepared from around the containers from July to October 2015. Sampling was carried out by active Zefon A6 Impactor samplers at a distance of 1.5 m from surface of earth and in several horizontal distances up to 2 m from containers. Totally, 360 samples were gathered and the bacteria and fungi were identified by microbiology tests. Also, temperature, humidity and wind speed was measured.

Results: Aspergillus, yeast and Alternaria were the most dominant species of fungi in around of containers. Among bacteria, gram-positive bacilli and Staphylococcus epidermidis were dominant. Most of bio-aerosols in a distance of 25 cm from the containers (228.00±167.12 CFU/m^3) and the lowest were observed at a distance of 2 m of the containers (223.86±204.72 CFU/m^3).

Conclusion: The findings indicated that municipal waste collection containers released the hazardous biological agents as Aspergillus and Staphylococcus aureus that are very pathogenic for human in environment. So the position of the garbage containers for decline the environment health effect, it should not be less than 2 m from human exposure.

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Background

Although air at ground level is more available and accessible, it contains large amounts of impurities. Several types of contaminants from human activities on the earth, enter to the atmosphere. Bio-aerosols are one of contaminants (1). Bio-aerosols are airborne particles in an abundant spectrum of forms and sizes that include germs, viruses, and related factors. Bio-aerosols can produce health effects such infections or allergies in people. The result of microorganism's growth of human body is infection but allergies are a consequence of contact with antigens. The immune system responses microorganisms and makes sensitivity. Bio-aerosols are divided into living and non-living. Live microorganisms as bacteria, fungi, yeasts, and molds that are released in the environment. Some of them to

be in workplace that causes various diseases. Non-living bio-aerosols as pollen, fragments of insect body and fine particles of herbal that are released into the environment and causes sensitivity and disease some cases (2). According to research, one gram of dust can contain up to about one million bacteria (3). Contact with contamination caused by bio-aerosols in today's urban life is inevitable. In each occupation, various harmful agents are generated, according to activity. Air is the most common and important intermediary in the workplace. Breathing polluted air in the workplace is as the main source of exposure to airborne contaminants. Sampling from the air in the work environment has two general purposes: monitoring of occupational exposures and health researches with the approach of protecting the health of staff in the work environment (4). Bio-aerosols transmit transmissible infections through the air. The risk of developing an infection following inhalation of these agents depends on the number of live organisms that enter in to the respiratory tract (5). contact with bio-aerosols is associated with an abundant spectrum of health effects including infectious diseases, acute toxic effects, allergies, and cancer (6). Reviewing waste background shows that waste management has engineering performance (7). Because of expansion of urbanization and increasing population, the optimal management of production waste issue is one of the essential subjects that the majority of urban management activities in municipalities are dedicated to this (8). People and workers breathe polluted air around garbage containers of roadside during garbage collection and storage time in garbage containers. Concerns about effect of correct waste management on health and safety of pedestrian and worker that exposure to bio-aerosols arising from maintenance and collect of waste are growing. There is little information are available about the extent of exposure to bio-aerosols during garbage collection. However, recent studies have proven the side effects such as respiratory problems, attacks of fever, allergic diseases such as asthma and problems with the digestive system in people who have been exposed to a pile of trash (9). In addition, daily active workers who have been exposed to polluted air around garbage containers without the proper use of personal protective equipment, biological aerosol emissions are most probably affect residents of the region.

Aims of the study: The aim of this study is to identify and determine the radius of bacterial and fungal bio-aerosol emissions from municipal waste collection containers.

Materials & Methods

In this cross-sectional study, garbage containers were collected under random selection method by 145 containers with a volume of 700 L in Gonabad city (Iran), from July to October 2015. In this research, the quality status of air adjacent containers was tested and bio-aerosols that include different types of bacteria and fungi tested. A sampling of bio aerosols done using by active sampler at a distance of 1.5 m from surface of earth which is considering breathing height. In active air sampling methodology, plates inside Zefon A6 Impactor contact the air around of container for a few minutes (average flow rate is 15 L/min and duration of air passing through the plate was 6 min, respectively, in other words, 90 L of air is passed each plate). To investigate bio-aerosols density in the air around of containers, samples were taken at different horizontal distances of 0.25, 1 and 2 m. To determine relationship between number of colonies with environmental conditions, temperature, and relative humidity was measured by digital devices Temp. & Humidity Meter (precision of temperature ±10 °C and precision of humidity ±5% RH). Anemometer measured wind direction and speed. In microbiology laboratory, sabouraud dextrose agar (Merck, Germany) containing chloramphenicol antibiotic (Sigma, Germany) (0.25 mg/mL) and

trypticase soy agar (Merck, Germany) containing nystatin antibiotic (Sigma, Germany) (0.25 mg/mL) used for cultivating bacteria. Bacterial samples were placed at 37 °C for 48-24 h. Fungi samples were placed in ambient temperature at 28 °C for 5-7 days. Then, colony counting was done using Colony Counter (Stuart, UK). Diagnosis of fungal and bacterial species was done by bacterial diagnostic tests. The density of counted colonies was calculated based on the CFU/m3 (number of counted colonies in the volume of air passing) and thus at every turn were reported as the mean. The average temperature (27.582±6.567 °C), with an average air humidity (20.10±5.359%) and the average wind speed (0.775±0.723 m/s) in the sampling were measured. analysis of data done by the ANOVA and Tukey tests.

Results

Analysis and counting colonies Show that most biological agents were isolated from bacterial species (table 1).

Table 1) The number of counted colonies of biological agents (CFU/m^3)

Microorganisms	Min	Max	Average ± SD
Bacteria	0	755	99.35 ± 108.320
Fungi	0	400	30.43 ± 50.014

Table 2 shows the frequency of fungal and bacterial species in the air around garbage container respectively. According to the table 2, Aspergillus, yeast, and Alternaria were the most dominant species of fungi around the containers and in bacteria, gram-positive bacilli and Staphylococcus epidermidis were dominate.

Based on the Figure 1, there were significant differences in density of bio-aerosols in different temperature ranges (p=0.001). Figure 2 demonstrated that by increasing humidity, the average concentration of bio-aerosols has increased and state graph was ascending. There was the significant difference in terms of bio-aerosols in different periods of relative humidity (p=0.000). Tukey test showed a significant difference between bio-aerosols in interval 10-20% with the range of 21-30% and 31-40%.

Table 2) Frequency (Percent) of fungus and bacterial species in the air around the garbage containers

No	Fungus species	Frequency (%)	Bacteria species	Frequency (%)
1	***Alternaria***	9.44	*Micrococcus*	12.37
2	**Yeast**	13.89	Gram-positive bacilli	41.47
3	***chrysosporium***	2.78	*Staphylococcus saprophyticus*	6.7
4	***Aspergillus***	44.44	*Staphylococcus aureus*	14.05
5	***Aureobasidium***	6.67	*Staphylococcus epidermidis*	22.41
6	***Cladosporium***	6.67	*Diphtheroid*	1.5
7	***Mucor***	2.78	*Streptococcus*	1.5
8	**sterile hyphae**	8.33		
9	***Fusarium***	3.33		
10	***Ulocladium***	2.22		
11	***Acremonium***	3.89		
12	***Penecillium***	2.78		

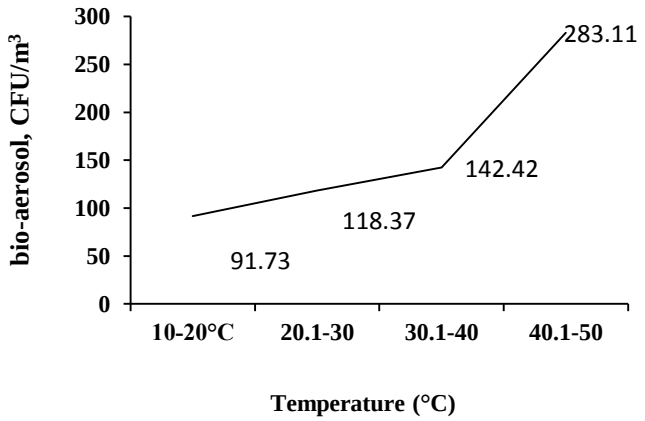

Figure 1) The concentration of bio-aerosols (CFU/m^3) in different temperature interval

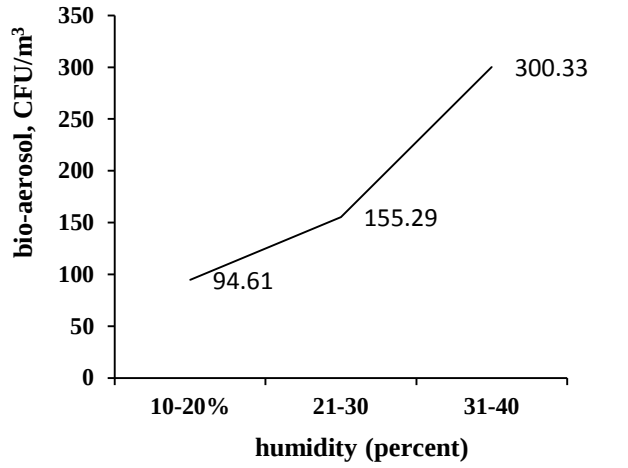

Figure 2) The concentration of bio-aerosols (CFU/m^3) in different relative humidity interval

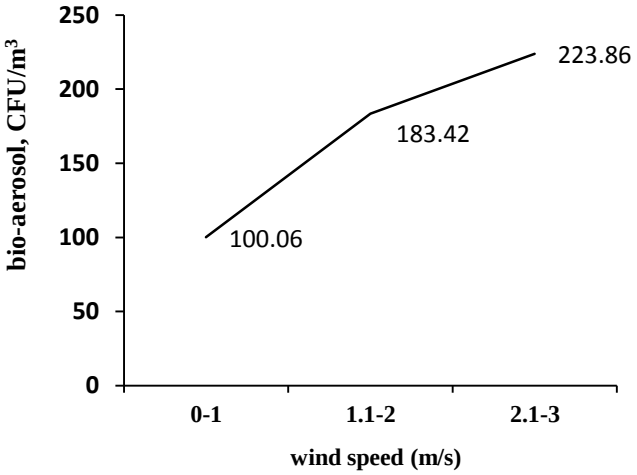

Figure 3) The concentration of bio-aerosols (CFU/m^3) in different wind speed interval

Also between the bio-aerosols in interval 30-21% with the range of 31-40% there was a significant difference (p=0.21). With increasing wind speed, an average concentration of bio-aerosols in the container increased (Figure 3) and ANOVA test demonstrated a significant difference due to the amounts of bio-aerosols at different times wind speed (p=0.000). But there wasn't significant difference between the amounts of bio-aerosols in the range 1.1-2 m/s to 2.1-3 m/s (p =0.488).

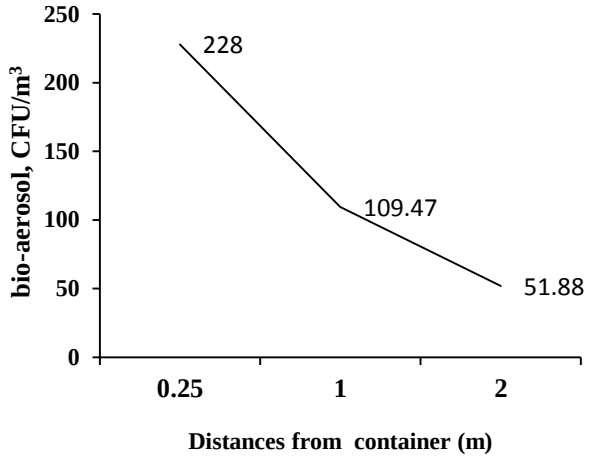

Figure 4) The concentration of bio-aerosols (CFU/m^3) at different distances from the container

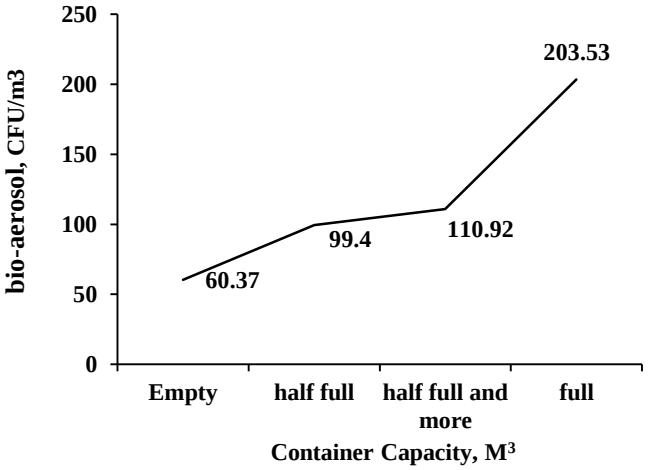

Figure 5) The concentration of bio-aerosols (CFU/m^3) based on the capacity of container

According to Figure 4, by increasing the horizontal distance of garbage container, the average concentration of bio-aerosols declined and the state diagram was descending. According to the results, there was the most amounts of bio-aerosols in the entire volume of the container (203.53±187.72 CFU/m^3) and the lowest in empty containers (60.37±47.83 CFU/m^3) (Figure 5). ANOVA test indicated a significant difference in various capacity of containers in terms of bio-aerosols (p=0.000). In terms of differences between the interval

time, Tukey test showed significant difference between the bio-aerosols in completely full container with other three capacities, but there wasn't significant difference between the empty container with a capacity of less than half, half and more, as well as the capacity of less than half with half and more ($p > 0.05$). As seen in table 3 there is a significant correlation between fungi and bacteria also bacteria with temperature ($P<0.05$). The relationship between bio-aerosols and humidity is significant ($P<0.01$).

Table 3) Spearman rank correlation coefficient matrix between the different parameters ** significance level of less than 0.01 (ANOVA P <0.01)

Parameters	Bacteria	Fungus	bio-aerosol	Temperature	Humidity	Wind speed
Bacteria	-	-	-	-	-	-
Fungus	0.448 **	-	-	-	-	-
bio-aerosol	0.947 **	0.663 **	-	-	-	-
Temperature	0.247 **	- 0.015	0.201 **	-	-	-
Humidity	0.127	0.145	0.171 *	-.226 **	-	-
Wind speed	0.129	0.222 **	0.947 **	0.204 **	-.005	-

** Significance level of less than 0.01 (P <0.01)
* Significance level of less than 0.05 (P <0.05)

Discussion

As in the findings, the most common fungi found in the air around the garbage containers in the city were Aspergillus, yeast and Alternaria respectively. Although the emergence of allergic reactions doesn't require a large number fungus, touching a large number of fungal spores could be a prelude to the making fungal infection. Due to this fact that among the inhalation allergens, fungi have the specific situation and as allergens plays the major role in the development of allergic rhinitis, asthma, extrinsic allergic alveoli, and sinusitis (10). The exact identification of allergens would contribute to better management of allergens, including diagnosis, prevention, and treatment of related diseases (11). Due to the presence of melanin pigments and resistance to sunlight, there is a wide range of black fungus species such as Alternaria (12). In this study, gram-positive bacilli, Staphylococcus epidermis, and Staphylococcus aureus have been identified the most abundant bacteria in the air around garbage containers. Bacillus species were dominant bacterial groups in the current research. The reason for this issue is the potency of bacteria to form spores and their resistant to harsh environmental conditions so their survival in the air is so much. Most bacillus species are opportunistic and cause gastroenteritis, eye infections and sepsis in association with the catheter into the vein. Also, they have involved two forms of food poisoning (13). Staphylococcus aureus may be symbiotic on the skin there. When the skin barrier is destroyed, the bacteria attack the tissues. These bacteria are one of the main pathogens causing food poisoning. The incidence of antibiotic resistance may increase among bacteria has made an important problem in health care (14). Staphylococcus aureus and Staphylococcus epidermidis in most frequent microbiology case in normal circumstances are not as the threat to human health and are safe. It can be as a positive factor (15). The results of a study in Shahroud, showed that the average number of colonies of bio-aerosols in the air adjacent the garbage containers was 334 CFU/m^3 (305 bacterial colonies, 29 colonies fungus) but in the present study, this number was less

(129.78±134.248 CFU/m^3). Type of bacteria found in Shahroud study included: Staphylococcus, Bacillus cereus, Bacillus subtilis, Streptococcus, Lactobacillus and Micrococcus and fungi such as Alternaria, Penicillium, Aspergillus, and Cladosporium. But in this study, the amount of horizontal distance from containers is not mentioned for sampling the air around (16). Abundance and variety of bio-aerosols in various studies aren't the same patterns which can be involved several reasons such as geographical location, season of sampling, kind of garbage, weather conditions and time intervals of waste collecting (17).

In a study, conducted in Denmark, findings showed that the mean extent of exposure of workers who collect garbage with the microorganisms between 106-105 cell/m^3, average exposure to cultivate fungi between 105-104 CFU/m^3 for arable bacteria 104-103 CFU/m^3. One of the affecting factors on the rate of exposure is the type of waste. The workers who collect compostable waste, mixed waste and classified waste had the same exposures to fungi, while workers who collect bulky waste, the sheet had low exposure. The exposure affected both by the carrier and the season so that total density of microbes in the winter was low (18). Lavoie and et al showed that the highest exposure to bacteria in workers was in compost garbage with the average 5030 CFU/m^3 (19). In cross-sectional study in Alexandria, 346 municipal workers with one-year employment and more were divided into 2 groups with direct and indirect exposure. The findings showed that direct exposure (including garbage collection workers) were at greater risk in comparison with the second group (20). The results of a study in Taiwan, showed that household waste collection is a risk factor for chronic respiratory symptoms (a cough, phlegm, dyspnea and chronic bronchitis), respectively (21). In the cross-sectional study in Kerala, India, 313 workers (77%) from the solid waste management sector entered in this research. The finding showed that 21% of workers suffer from respiratory diseases (infections and allergies) and 2.33% of them had eye problems (22). As the charts of this study, with increasing temperature, relative humidity, wind speed and the amount of waste in the container and reducing the distance from the container, average concentrations of bacteria and fungi in the air adjacent the containers has increased. Due to the relation between bio-aerosols and these variables, it can be decided on the suitable location of containers. Because there is a possibility of biological particulate emissions and affect the region's residents. The results of a research to determine parameters for the safety and health of workers during the process of collection, transport and disposal of urban waste in Ardabil, showed that poor quality of individual equipment, non-use of protective equipment such as masks, shoes, glasses, etc. were the main problems of health and safety among workers (23). The results of a study about of the possible release of bio-aerosols from containers, show that significant concentrations of bio-aerosols and particularly endotoxin can be produced during prolonged residual waste storage and collection (24). Based on the results of studies conducted, we find that all activities in association with waste management, including collection, aside from high expenses, if they associate with non-compliance with technical, safety and health principles leads to serious risks for people and the environment. Therefore, raising awareness about this issue is more crucial than ever and informing the managers and municipal officials leads to the prioritization and proper planning. Developing useful content, scientific reports and practical training can create a good platform to promote knowledge of human resources in municipalities, especially those who are in association with waste management. It is hoped that the increasing awareness, proper use of safety equipment and the utilization of new technologies, leading to increasing performance

in the optimal management of municipal waste. The workers used in the waste collection sector expose to biological particles directly and basically their distance from waste and contaminated martial is low. Undoubtedly extended exposure to these particles during work will have adverse effects on their health and they have health problems as eye irritations, allergies, skin and respiratory problems. So, the use of appropriate protective equipment and appropriate human resource training in the section are required for adhering to safety and health tips. Custodians would also provide equipment and personal protective equipment with high quality to waste collection sector employment.

Conclusion

The findings are shown that municipal waste collection containers released the hazardous microorganisms as Aspergillus and Staphylococcus aureus that they are very pathogenic for human in the environment. So the position of the garbage containers for decline the environment health effect, it should not be less than 2 m from human exposure.

Footnotes

Acknowledgment

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Conflict of Interest:

The authors declared no conflict of interest.

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