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RESEARCH ARTICLE

Determination of bacterial and fungal bioaerosols in municipal solid-waste processing facilities of Tehran

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Abstract

Purpose The concentration and distribution characteristics of airborne bacteria and fungi were assessed at municipal solid-waste processing and disposal facilities (SWPDFs) of Tehran (Arad Kouh Site).

Methods Air samples were obtained from the indoor air of a laboratory and refectory as well as from the air surrounding a conveyor belt, rotary screen, and bailer. Sampling was conducted according to the standard procedure to determine two bacterial species (*Klebsiella spp.* and *Staphylococcus aureus*), total bacteria and fungi, and *Aspergillus fumigates*.

Results The maximum concentration of *Staphylococcus aureus* was in the air surrounding the conveyer belt with the average of 993.2 CFU/m^3. The highest concentrations of total fungi (4958.8 CFU/m^3) and *Aspergillus fumigates* (2114 CFU/m^3) were measured in the air surrounding the rotary screen. The mean concentrations of bacterial bioaerosols in a more contaminated sampling location in summer and winter were 1687.6 and 1479.4 CFU/m^3, respectively.

Conclusions There were significant differences between the concentration of bioaerosols in cold and warm seasons. The concentrations of bioaerosols in the air surrounding the rotary screen and conveyer belt were significantly more than those in other sampling locations, but were within the recommended maximum of 10^3 and 10^4 CFU/m^3.

Keywords Fungal bioaerosol · Bacterial bioaerosol · Solid-Waste Processing Facilities · Tehran

Introduction

Solid-waste processing and disposal facilities (SWPDFs) are sources of different kinds of diseases Microorganisms from different parts of facilities are transported with the wind and can cause different health problems [1–4]. This transportation is influenced by microorganisms resistance, meteorological conditions, sunlight intensity and time [4]. During different processes in solid-waste processing facilities, for example, during the collecting, shredding, turning, screening and material transport by loaders, bioaerosol is formed when microorganisms are aerosolized [4]. Many studies have been conducted on the measurement of air pollutants and their impact on human health [5–10]. One of the air pollutants is bioaerosols. Bioaerosols may include pathogenic or non-pathogenic, live or dead bacteria, fungi, viruses and pollens [2, 11, 12]. Bioaerosols and airborn microorganisms may cause different diseases in facility workers and residents in vicinity of SWPDFs [1, 2, 12, 13]. This is why bioaerosol measurement and monitoring have gain interest over the last few decades. Three major groups of diseases related to boiaerosol exposure are discussed as "infectious diseases", "respiratory diseases" and "cancer", in which respiratory and infectious diseases are the most common diseases [12, 13]. Fungi and bacteria are the most important elements in diseases related to bioaerosols [12–19]. Occupational exposure to biological agents is a

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major problem of occupational medicine and public healthcare, because it is associated with detrimental health effects ranging from simple irritation and discomfort to allergic reactions, infections, infectious diseases, and toxic reactions [1, 2, 19, 20]. In the workplaces of Municipal Solid-Waste Processing, harmful biological agents most commonly contain bioaerosols. Bioaerosols carry pathogens and allergens and also they are transmitted in the air and penetrate the body through nasal and mouth mucosa, the skin or an insect bite [1, 19]. All activities in solid waste disposal and processing management involve risks, either directly to the worker or indirectly to the resident [19, 21]. Health problems are important at every step of processing and disposal including producing at home, household composting, collecting, recycling and final disposal [22]. Emissions from municipal solid-waste processing facilities are issues related to occupational health and safety as well as to environmental hygiene[1, 4, 10, 16]. Bioaerosols or organic dusts including bacteria, microscopic fungi, viruses, plants pollen and different substances are produced by microorganisms being carried by dusts or droplets [22–24]. A large group of thermophilic and thermotolerant microorganisms have been observed to be spreading in the surrounding of residential places from composting with open-air turned windrow facilities [13, 20, 25]. Several bioaerosols including Aspergillus fumigatus, actinomycetes and endotoxins known for their infectious or allergenic properties are emitted through composting [20, 23, 25–27].

Environmental agency has defined acceptable levels as bioaerosol levels during the composting process including bioaerosol levels before the start of the composting process or bioaerosols levels less than 1000 colony forming units (CFU) m^{-3} total bacteria, 500 CFU m^{-3} A. *fumigatus* and 300 CFU m^{-3} Gram-negative bacteria. The 500 CFU m^{-3} A. *fumigatus* limit has replaced the previous 1000 CFU m^{-3} total fungi limit [25, 28]. However, evidences show that bioaerosols may travel further than 250 m from the emission site [25]. Studies have shown that facility size, compost pile agitation frequency, compost moisture levels, local meteorological conditions and local topography may all affect the downwind distance, at which bioaerosol emissions can be detected [25]. Even though the direct link between emission peaks and downwind peaks has not been demonstrated to date, bioaerosol emissions are known to be strongly influenced by processing activities [25]. Several health hazards have been produced by poor indoor air quality. The inhalable size range of airborne bacteria and fungi can deeply penetrate into human lungs (< 10 μm) [29]. Despite the correct management and proper maintenance of landfills, they are a source of emission and dispersal of bacterial and fungal aerosol [19, 21, 24, 29, 30].

Mycotoxins or fungal toxins are toxic to both animals and humans due to the evidence of carcinogenicity in some of them (e.g., aflatoxin from *Aspergillus*). Thus, it is recommended to monitor solid-waste processing facilities. Douwes et al. discussed about exposure to bioaerosols and wide range of health effects in solid-waste management facilities and found that respiratory symptom and lung function impairment are the most widely studied [12]. There is lack of data about bioaerosol content in the air surrounding SWPDFs of Tehran both indoor and outdoor. Therefore, it is required to measure the air quality at these sites in terms of bioaerosol content and its public health impact.

According to the ASTM .E 884–82 method, Klebsiella *spp*., Staphylococcus *aureus*, total bacteria, total fungi and *Aspergillus fumigatus* were selected to be measured [31]. *Aspergillus fumigatus* is a fungus that induces allergic sensitization and infectious mycosis [12]. Many fungal species described as type I allergens endotoxins (only in Gram-negative bacteria) and peptide-glycans (most prevalent in Gram-negative bacteria) may cause respiratory symptoms.

The aim of the study was to assess bioaerosols in the air in a laboratory and conveyor belt, as well as in the air surrounding a rotary screen, bailer and refectory. The study also considered the seasonal variation of the air microbes at the same sites.

Materials and methods

Sampling location

SWPDFs of Tehran are located in the south of Tehran Province. The mean temperature of this area in warm and cold season is 43°C and − 4°C, respectively. This processing and disposal site is located in the plain where is far from flood path and underground water. The nearest residential place to the SWPDFs of Tehran is 10 Km. Processing, composting and disposal of waste are performing in this site frequently. This site consists of one laboratory, three processing salon, one refectory and office department. The study was performed on the air quality at the indoor environment of a laboratory and refectory as well as on the air surrounding three operational units including a conveyor belt, rotary screen, and bailer which are located in the processing salon (Fig. 1); all the five study sites are located at SWPDFs of Tehran. Moreover, office department was selected as control. The main characteristics of SWPDFs, sampling points and time are shown in Tables 1 and 2.

For each sampling location, five replicate samples were collected in the morning (9 am) from Oct 2012 to Sep 2014. The sampling time was chosen to avoid overloading of the impaction plates; accordingly, the loading on any of the plates should not exceed 300 colonies per plate [31].

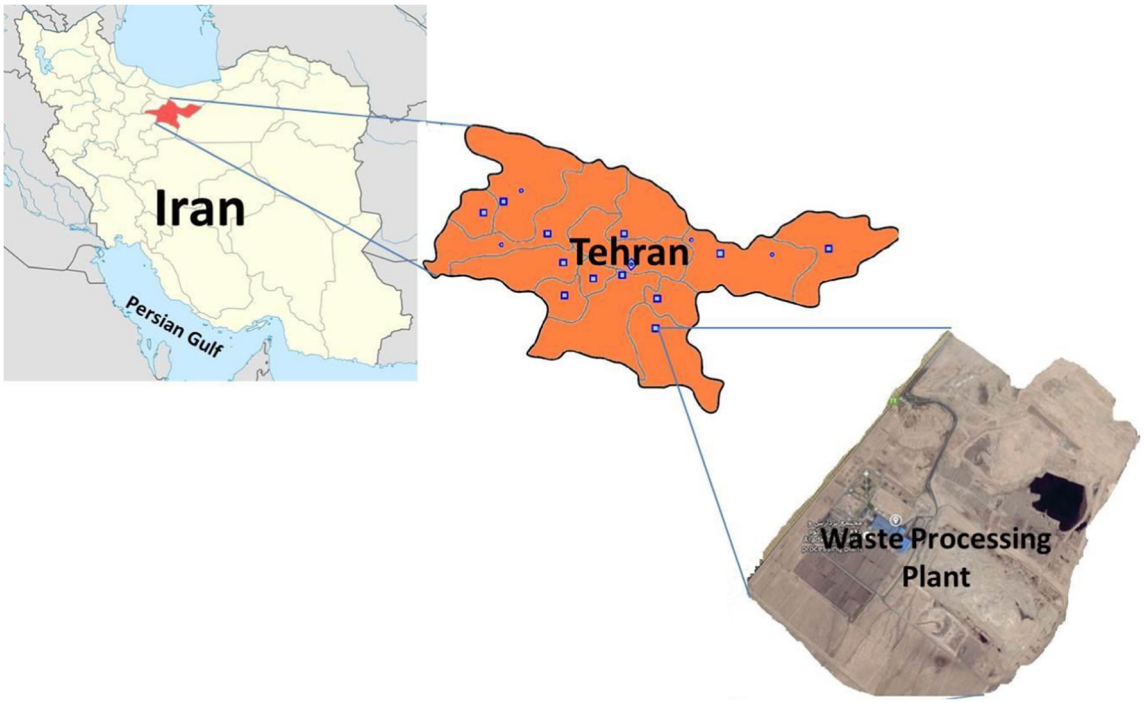

Fig. 1 Location map of the study area to show the sampling site

Sampling procedure and measurement equipment

All samples were obtained according to the standard practice for sampling airborne microorganisms at municipal solid-waste processing facilities (ASTM. E 884–82) [28] using the QuickTake® 30 Sample Pump equipped with a single-stage cascade impactor (Eighty four, PA 15330 USA). The pump was set for an air-flow rate of 28 L/min, and the flow rate was calibrated by a manometer. The sampling height was 1.5 m (5 ft) above the floor level to approximate the breathing zone of workers or other individuals exposed to bioaerosol. All the internal parts of the sampler were sterilized with 70% alcohol before collecting the samples.

Identification of bacterial and fungal flora

According to ASTM .E 884–82 [28], the microbiological air testing included determining the number of Klebsiella *spp*., Staphylococcus *aureus*, total fungi and Aspergillus *fumigates*. Levine Eosin Methylene Blue and Vogel and Johnson were used to growth Klebsiella *spp*. and Staphylococcus aureus as the culture medium with 50 mg/L Cycloheximide to inhibit the growth of fungi [31]. Sabouraud Dextrose Agar (SDA) was used as the transfer culture medium to growth airborne fungi. In order to prevent bacterial growth, 50 mg/L of Chloramphenicol was added to fungal culture media. The culture media were prepared in laboratory and transferred to the sampling location under the sterile condition. After the samples were collected, the media were transferred to the laboratory. The bacterial media were cultured for 24–48 h at 37 °C and airborne fungi were incubated for 7 days at room temperature (20 °C).

After the incubation, the grown colonies were counted and the results were converted to the colony forming units in 1 m^3 of air (CFU/m^3) by dividing the values counted for the colonies which were formed in the media by the air volume (m^3). To identify the fungal species, the simple method of slide culturing was established by performing some levels of the microscopic study.

Statistical analysis

Descriptive statistics (mean ± SD) were used to report the concentrations (CFU/m^3) of bacteria and fungi. The one sample Kolmogorov-Smirnov test was performed to determine the normality of the data. The concentration difference of bioaerosol between different sampling locations was

Table 1 Main characteristics of Tehran municipal solid-waste processing facilities

Details	Number
Daily solid-waste production in Tehran (ton/day)	8000
Number of site workers	400
Composting site area (ha)	40
Biomechanical processing capacity (ton/day)	500
Karco composting capacity (ton/day)	300
Number of transport stations in Tehran	14

Table 2 Details of measurements

Sampling location	Sampling location description	Sampling time
laboratory	In the center of laboratory	2 min
near a conveyor belt	1 m horizontal distance	30 s
near a rotary screen	1 m horizontal distance	30 s
near a bailer	1 m horizontal distance	30 s
refectory	In the center on refractory	2 min

calculated with the Kruskal–Wallis *H* test. The concentration difference between the two seasons was analyzed for statistical significance using the Mann–Whitney *U* test. All the analyses were performed using SPSS software version 21. For all the statistical procedures, significance was assessed at $p < 0.05$.

Results and discussion

Concentration of bacterial bioaerosol

The concentrations of bacterial bioaerosol in two different seasons are presented in Table 3. As shown in Table 3, the mean concentrations of bacterial bioaerosolat the more contaminated sampling location in summer and winter were 1687.6 and 1479.4 CFU/m3, respectively. Based on the results of the statistical analyses, a significant difference was observed in the concentration of bacterial bioaerosol (CFU/m^3) between the two seasons (Mann–Whitney *U* test, $p < 0.05$). The concentrations of bacterial bioaerosol varied widely at different sampled locations, and ranged from 4 CFU/m^3 in the indoor air of the refectory to 1687.6 CFU/m^3 in the air surrounding the conveyer belt. The concentrations of Klebsiellain the air surrounding the conveyor belt, rotary screen and bailer (the indoor air of the operational units) were significantly higher than those in the indoor air of the laboratory and refectory (Kruskal–Wallis H $p < 0.05$).

Based on the results obtained from the Kruskal–Wallis *H* test, there were no significant differences between the concentration of airborne bacteria (both Klebsiella *spp.* and staphylococcus *aureus*) in the indoor air of the operational units. There were, however, significant differences between the concentration of bacterial bioaerosol in the indoor air of the operational units, laboratory and refectory (Kruskal–Wallis H $p < 0.05$).

Regarding staphylococcus *aureus*, the maximum concentration was in the air surrounding the conveyer belt with the average of 993.2 CFU/m^3.

Concentrations of total fungi in the air

Table 4 represents the concentration of total fungiin summer and winter. The statistical results showed that there was a significant difference in the concentration of total fungi (CFU/m^3) between cold and warm seasons (Mann–Whitney *U* test, $p < 0.05$).

Table 5 presents the concentrations of total fungi and Aspergillus *fumigates*at different sampling locations in summer and winter, respectively. According to the results obtained from the Mann–Whitney *U* test, ($p < 0.05$), there were significant differences between the concentration of Aspergillus *fumigates* in cold and warm seasons.

The highest concentrations of total fungi (4958.8 CFU/m^3) and Aspergillus *fumigates* (2114 CFU/m^3) were measured in the air surrounding the rotary screen. The two-way analysis of variance showed that the concentration of microorganisms (bacteria and fungi) in the indoor air depended on the sampling site ($p < 0.05$) (Tables 3 and 4).

There is no uniform international standard on acceptable levels of bioaerosols. Thus, according to the Polish Standard [19], a majority of the indoor air samples collected from the air surrounding the conveyor belt, rotary screen and bailer (operational units) were classified as uncontaminated with bacteria. Accordingly, 34.2%, 28.9% and 12.5% of the air samples collected from the air surrounding the conveyor belt, rotary screen and bailer were classified as medium contaminated, respectively. According to Table 6, only 0.1% of the air samples collected from the air surrounding the rotary screen was classified as heavily contaminated. According to the Polish standard [19], 100% of all the indoor air samples collected from the air surrounding a rotary screen can be classified as

Table 3 Bacterial concentrations in the indoor air of the municipal solid-waste processing site

Places	Summer				Winter			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Laboratory	18	20	19	1	3	15	8.8	5.84
Adjacent to conveyer belt	1659	1722	1687.6	25.46	1358	1578	1479.4	96.47
Adjacent to the rotary screen	1250	1771	1419.4	234.08	1125	1155	1135	11.72
Adjacent to the bailer	840	1543	1156.2	250.75	636	661	647.2	9.62
Adjacent to the refectory	14	19	16	2.12	4	6	5.2	0.83

Table 4 Fungal concentrations in the indoor air of the municipal solid-waste processing site

Places	Summer				Winter			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Laboratory	800	810	805.4	3.64	632	670	646.4	14.65
Adjacent to conveyer belt	2750	3640	3295	402.24	1015	1035	1022	8.36
Adjacent to the rotary screen	4890	5004	4958.8	58.37	2176	2230	2195.2	21.56
Adjacent to the bailer	1950	2591	2417.8	265.5	873	1000	914.6	49.65
Adjacent to the refractory	597	640	623.4	15.97	48	210	88.6	68.7

averagely contaminated (1st degree) with fungi. No dangerous contamination for the environment was detected at the sampling sites throughout the entire research period (Table 6).

Figures 2 and 3 show bioaerosols proportion at different sampling locations in winter and summer respectively. According to Figs. 2 and 3, the concentrations of bioaerosolsin the air surrounding the rotary screen, conveyer belt and bailer were significantly more than that at the laboratory and refectory.

According to the occupational and environmental health issues of solid waste management [11], every step in municipal solid-waste management systems from collecting to the point of final disposal includes risks, either directly to workers or indirectly to people living in the vicinity of waste management systems.

All the components of the environment including the air are influenced by landfill sites. The presence of bacteria and fungi on petri dishes with contaminated air can play an important role in the probability of occurrence of respiratory diseases.The organic matter in the waste is the best source of nutrition for microorganisms. There are no internationally accepted standards for exposure to bioaerosols. Scandinavian studies suggest that occupational exposure limits should be 5 to 10×10^3 CFU/m^3 for total microorganisms and1×10^3 CFU/m^3 for Gram negative bacteria[8]. The measured concentrations of bacterial and fungal aerosols in the present study (between 10^3 and 10^4 CFU/m^3) were within the range normally obtained by other researchers[19, 21, 26, 30, 32].

Outi K Tolvanen (2004) reported that a real problem in waste processing was high concentration of microbes [33]. Relatively similar levels of bacterial and fungal microorganisms were observed by Birgitte Herbert Nielsen et al. [34] to be 9.2×10^5 CFU/m^3in total microorganisms, 7.8×10^4 CFU/m^3infungi (moulds), 2.9×10^3 CFU/m^3 in Aspergillus fumigatus, 9.0×10^2 CFU/m^3 in mesophilic actinomycetes, and 1.0×10^4 CFU/m^3inbacteria. In this study, seasonal variation of microbes showed high concentration of A. *fumigatus* during summer ($p < 0.05$). However, no differences were observed between the three operational units. According to Birgitte Herbert Nielsen et al. (1998) and Borrello et al. (2000) [32, 35], the concentration of microorganisms ranged from 5.0 to 12×10^9 cells ml-^{1} during storage of bio waste [29, 32]. In comparison with other studies, the concentration of microorganisms was higher in this study. In different kinds of windrow during different turning frequencies, Johanna Lott Fischer et al. (1998) [36] observed that higher concentration of A. *fumigatus* dispersed in the air in the more frequently turned composts. The initial concentration of A. *fumigatus* was more than 10^6 CFU/g-dw which was reduced after two weeks of composting. However, the concentration remained more than 10^4 CFU/g-dw, even in less frequency turned windrow. The emission of bioaerosols from different waste storage systems was assessed by Kari Kulvik Heldal et al. (2001) [22]. In closed containers, the microbial concentration was higher than compostainers, and a majority of emitted aerosols were fungal spores, especially A. *fumigatus*. However, there was no

Table 5 Aspergillus *fumigates* concentrations in the indoor air of the municipal solid-waste processing site

Places	Summer				Winter			
	Min	Max	Mean	SD	Min	Max	Mean	SD
Laboratory	48	56	51.6	3.28	8	18	12.2	4.14
Adjacent to conveyer belt	1968	1980	1973.6	4.72	841	910	880.2	28.8
Adjacent to the rotary screen	2100	2120	2114	8.2	1200	1335	1247	54.26
Adjacent to the bailer	1300	1340	1325	15.8	268	300	281.6	13.5
Adjacent to the refractory	18	23	20.2	1.78	2	4	2.6	0.89

Table 6 Bacterial and fungal concentrations (in percent by sampling site)

Microorganisms	Range of values (CFU/m3)	Pollution degree	Percent of samples by sampling site				
			Near a conveyor belt	Near a rotary screen	Near a bailer	Laboratory	Refectory
Total Bacteria	< 1000	Not polluted	65.8	71	87.5	100	100
	1000–3000	Medium	34.2	28.9	12.5	0.0	0.0
	> 3000	Heavy	0.0	0.1	0.0	0.0	0.0
Total Fungi	3000–5000	I	100	98.7	100	100	100
	5000–10000	II	0.0	1.3	0.0	0.0	0.0
	> 10000	III	0.0	0.0	0.0	0.0	0.0

significant difference between different waste storage systems. The indoor and outdoor exposure of bacterial and fungal bioaerosols in workers of municipal landfills was assessed by Agnieszka Kalwasinska et al. (2014) [19]. In the outdoor, the highest concentration of molds was 1179 CFU/m^3 at the technological square. The indoor exposure of workers was 10707 CFU/m^3 for bacteria and 12471 CFU/m^3 for mold in the sorting facility. In the present study, the level of the bioaerosol contamination of the indoor air varied significantly between the seasons. However, no such difference was reported in other studies [19, 27, 37]. The results obtained in this study confirmed that people working near a conveyor belt, rotary screen and bailer are more exposed to harmful bioareosols than people working at a laboratory or refectory.

Conclusion

Moreover, the results indicated that landfills are a source of emission and dispersal of bacterial and fungal aerosols. In this study, a higher number of bioaerosols was observed in the air surrounding the rotary screen, conveyer belt and bailer compared to the indoor air at other sampling locations. Amongst fungal microorganisms, Aspergillus *fumigatus* was found to be a real problem at the workplaces, because it was associated with allergic reactions. To solve bioaerosols issues at such workplaces, radical improvements are needed. New bioaerosols collection systems can be among the best methods to solve bioaerosols issues. To decrease or eliminate health risks associated with bioaerosols at risky workplaces, workers

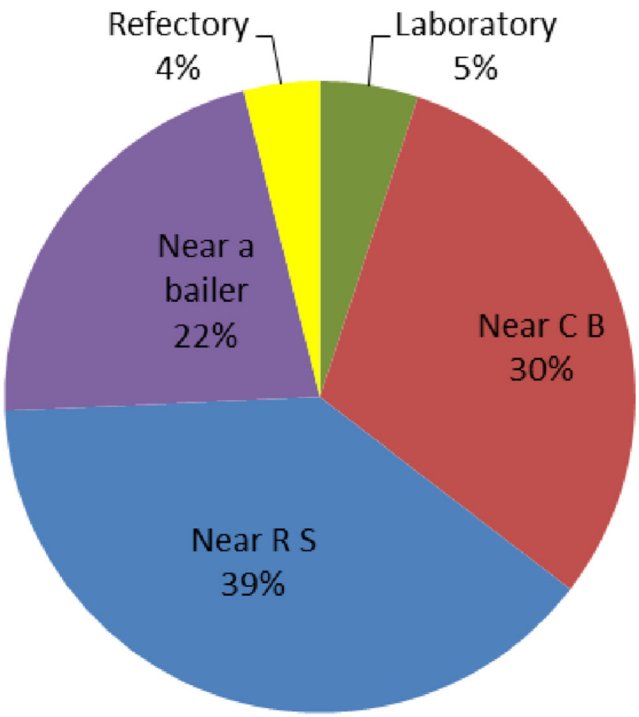

Fig. 2 Bioaerosols proportion at different Sampling locations in winter

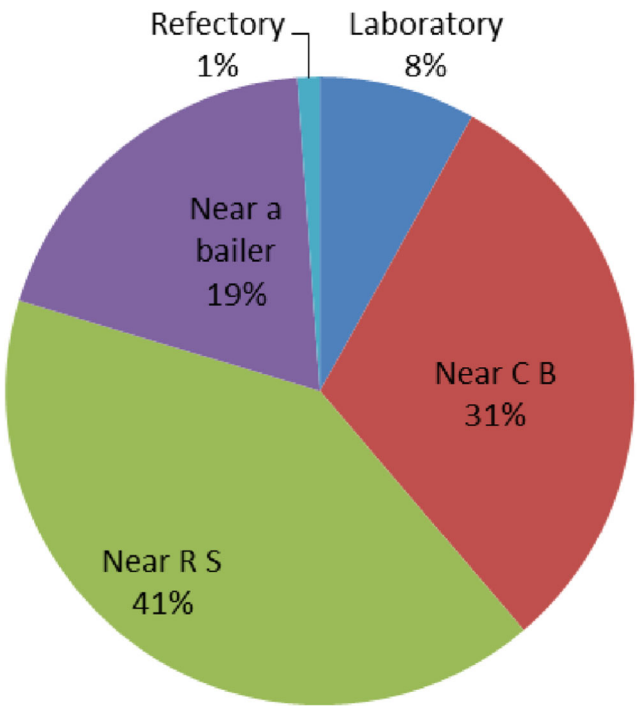

Fig. 3 Bioaerosols proportion at different Sampling locations in summer

must be obligated to wear overalls, gloves and masks, observe the personal health care and so on.

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Compliance with ethical standards

Conflict of interest All authors contributed sufficiently to the study and read this final manuscript and gave their approval for the manuscript to be submitted in its present form.

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