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Density assessment and mapping of microorganisms around a biocomposting plant in Sanandaj, Iran

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Abstract Exposure to microorganisms can cause various diseases or exacerbate the excitatory responses, inflammation, dry cough and shortness of breath, reduced lung function, chronic obstructive pulmonary disease, and allergic response or allergic immune. The aim of the present study was to investigate the density of microorganisms around the air of processing facilities of a biocomposting plant. Each experiment was carried out according to ASTM E884-82 (2001) method. The samples were collected from inhaled air in four locations of the plant, which had a high traffic of workers and employees, including screen, conveyor belt, aerated compost pile, and static compost pile. The sampling was repeated five times for each location selected. The wind speed and its direction were measured using an anemometer. Temperature and humidity were also recorded at the time of sampling. The multistage impactor used for sampling was equipped with a solidified medium (agar) and a pump (with a flow rate of 28.3 l/m) for passing air through the media. It was found that the mean density of total bacteria was $>1.7 \times 10^3$ cfu/m^3 in the study area. Moreover, the mean densities of fungi, intestinal bacteria (*Klebsiella*), and *Staphylococcus aureus* were 5.9×10^3, 3.3×10^3, and 4.1×10^3 cfu/m^3, respectively. In conclusion, according to the findings, the density of bacteria and fungi per cubic meter of air in the samples collected around the processing facilities of the biocomposting plant in Sanandaj City was higher than the microbial standard for inhaled air.

Keywords Compost · Airborne microorganism · Exposure · Workers · Bacteria

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Introduction

Increased urbanization and intensive industrialization along with developed agriculture have resulted in the generation and accumulation of large amounts of waste throughout the world (Korzeniewska 2011). One of the unavoidable components of the waste processing is the spread of pathogenic microorganisms, endotoxins, odors, and dust particles in the surrounding air (Miaśkiewicz-Pęska and Szyłak-Szydłowski 2015). As a part of solid waste management, composting is the decomposition of organic matter by microorganisms including bacteria, fungi, and yeast that are present in compost pile with a density of $>10^{11}$–10^{10}/g of dry matter of garbage (Albrecht et al. 2007). Generally, composting and vermicomposting are commonly used to transform organic solid waste into fertilizer (Lim et al. 2016; Wu et al. 2014). Collection of solid waste is associated with numerous chemical, biological, physical, and ergonomic health hazards (Jerie 2016). When current practices are examined, it can be seen that, even in developed countries, the solid waste collection is often carried out in an unhealthy and inefficient way (Cavdar et al. 2016). These hazards involve microbiological exposure associated with the collection of decaying material, chemicals from the waste itself and from its decomposition, truck exhausts, temperature extremes, and ultraviolet radiation (Lavoie et al. 2006). During operational activities, the emission of bioaerosols increases the concentration of microorganisms in the air by several orders of magnitude (Wéry 2014).

Bioaerosols released from the processing and handling of composted materials may establish a potential health risk to exposed individuals, which depends on the composition of the microorganism in the basic material (Nielsen et al. 1997a, b). Therefore, garbage is a potential source of biological hazardous materials through the release of microorganisms and toxins in relation to the microbial metabolism that are produced in their cellular structure (Coccia et al. 2010). Exposures to bioaerosols in the occupational environment are linked with a wide range of health issues with major public health impacts. Some of these health effects are infectious diseases, acute toxic effects, allergies, and cancer (Douwes et al. 2003). Among the most studied and important bioaerosol-associated health effects are respiratory symptoms and lung function impairment. Despite these adverse health effects, some protective effects of microbial exposure on atopy and atopic conditions have been suggested (Douwes et al. 2003). Microbiological exposures related with waste can occur indoors (waste storage) or outdoors (waste collection), and may be influenced by sorting, transferring, and cleaning processes (Ivens et al. 1997).

Contamination of the air by pathogenic microorganisms generates from various sources, both natural, such as water, soil, or rotting plants and animal remains, and anthropogenic, including municipal landfills and sewage treatment plants (Korzeniewska 2011). Such hazardous compounds can attach to dusts during processing and recycling of waste materials (Thirumala et al. 2012; Pillai et al. 1996). However, there are no exact thresholds for adverse health effects due to exposure to endotoxins, airborne bacteria, and fungi (Korzeniewska 2011).

Municipal waste consists of biodegradable waste of animals and plants as well as diverse flora. In this regard, aerosols with high microbial content can be generated during waste processing (Lembke and Kniseley 1980). Daily, 220 t of mixed waste are transferred to a composting plant located at a distance of 8 km from Sanandaj City. This amount is 60% of the total waste generated in this city. Municipal solid waste approximately contains 70% organic waste, 10–12% recyclable solid waste, and 18–20% non-recyclable waste (Ghavami et al. 2010; Zazouli et al. 2010). Aerosols can be produced from non-pathogenic and pathogenic microorganisms during processing and disposal of waste materials. The aerosols may include more than 8×10^6 cfu of bacteria and more than 2×10^7 cfu of fungi, which can be breathed by workers and employees in their workplace (Albrecht et al. 2007). Bacterial cell wall components, such as endotoxin and peptidoglycans, are agents with important pro-inflammatory properties that may induce respiratory symptoms (Poole 2012). The involved species include many common genera such as *Penicillium* and *Aspergillus*, which occur in some work environments usually at very high levels (e.g., composting facilities, farms, etc.) (Douwes et al. 2002). In recent years, studying airborne pathogenic microorganisms including *Aspergillus flavus*, *Neisseria meningitidis*, *Serratia*, *Streptococcus pneumonia*, and *Tuberculosis bacillus* (bacillus tuberculosis) has been a focal point of research (Prussin and Marr 2015; Ogunshe et al. 2015; Licina et al. 2016). These microorganisms could be found on the skin, hair, clothes, and interior spaces of houses (Jedlicka et al. 2012). *Aspergillus fumigatus* is often present in the compost produced from plant material (Deacon et al. 2009). It could cause invasive aspergillosis in people with impaired immune systems (Dagenais and Keller 2009). Therefore, it can be used as an indicator for exposure to fungus in the compost sites.

Processing, recycling, and disposal sites of municipal wastes are considered as one of the potential sources for releasing the airborne bioaerosols or microorganisms that are usually not included in the previous studies. Releasing can occur in all stages of the composting process, from crushing after receiving to screening the mature compost (Pankhurst et al. 2011).

The existence of bioaerosols and the level of bioaerosol spreading are articulated by biotic factors controlling the sustainability of the aerosolized organisms, as well as the abiotic factors limiting release, transport, and dissemination of organisms. The most important physical characteristics are the size, density, and shape of the droplets or particles, while the magnitude of air currents, relative humidity, and temperature are the significant environmental parameters. The transport of bioaerosols can be defined in terms of distance and time. Sub-microscale transport involves very short periods of time, under 10 min, as well as relatively short distances, under 100 m. This type of transport is common within indoor environments (Korzeniewska 2011). Microscale transport of bioaerosol could be less than 1 h and 1 km, which is significant from a human health standpoint (Pillai and Ricke 2002).

In recent years, trying to recycle, and save energy, has led man to use their waste for achieving this goal. The relationship between municipal waste and occupational health of workers and employees of collection, processing, and disposal of waste has been discussed in so many studies (Cointreau 2006). The enormous development of aeromicrobiology is indebted to recent awareness about the risks posed by airborne microorganisms. However, it seems that there is no internationally accepted threshold limit value for biological contamination of air (Turner et al. 2008). Few health-based occupational exposure limits (OELs) are available for risk assessment (Eduard et al. 2012).

Health, education, and welfare group of the US Environmental Protection Agency (USEPA) has published its manual on the relationship between waste materials and disease (solid waste/disease relationship) (Hanks 1967). In this manual, the disease was introduced as hazardous diseases or incurable diseases. Density of microorganisms can be measured by several methods including counting colonies formed after culture on solid medium (Brugger et al. 2012), cell counting under a microscope after DNA staining, or more recently by counting PCR (Nadkami et al. 2002). The specific objectives of the study would determine which measurement method is the most appropriate ones. Currently, quantitative detection of airborne microorganisms is often based on the culture method on the solid medium after sampling. Culture method has some drawbacks such as poor repeatability, identifying specific species, and depending on the chosen medium and temperature. Moreover, the dead microorganisms and cellular debris will not be recognized by this method.

In order to obtain reliable, comparable, and interpretable data, it is essential that the measurements be performed according to standard procedures and instructions. The American Society for Testing and Materials (ASTM) instruction is one of the introduced and acceptable guidelines for sampling the air around the processing facilities and waste disposal sites (ASTM E884-82 2001). Hence, the aim of this study was to determine the type and concentration of microorganisms in the air around the processing facilities of a biocomposting plant in Sanandaj City based on the ASTM E884-82 (2001) instructions.

Materials and methods

The study area

The Sanandaj composting plant is located at a distance of 8 km from Sanandaj, Iran. Figure 1 shows the geographical location of the biocomposting plant and its units. This plant receives municipal wastes collected from residential areas of the city. The waste is mainly composed of food waste, paper, wood, street wastes, ferrous and non-ferrous metals, glasses, bottles, plastics, etc.

Sampling

In order to determine the airborne microorganisms in the air around processing facilities of the composting plant, sampling of inhaled air of workers and employees was conducted during both summer and winter seasons, 2015. In this study, each sample was collected in operation mode from inhaled air in four locations of the plant, which had a high traffic of workers and employees including screen, conveyor belt, aerated pile, and static pile. The sampling was repeated five times for each selected location. The wind speed and direction was measured using an anemometer (TAM618-Terminator, Japan). The real-time temperature and humidity were also recorded at the sampling points. Sampling height was about 1.5 m, which there is the most likely for inhalation of air by employees and workers. According to the international guidelines (ASTM E884-82 2001), which correspond to the sampling of municipal

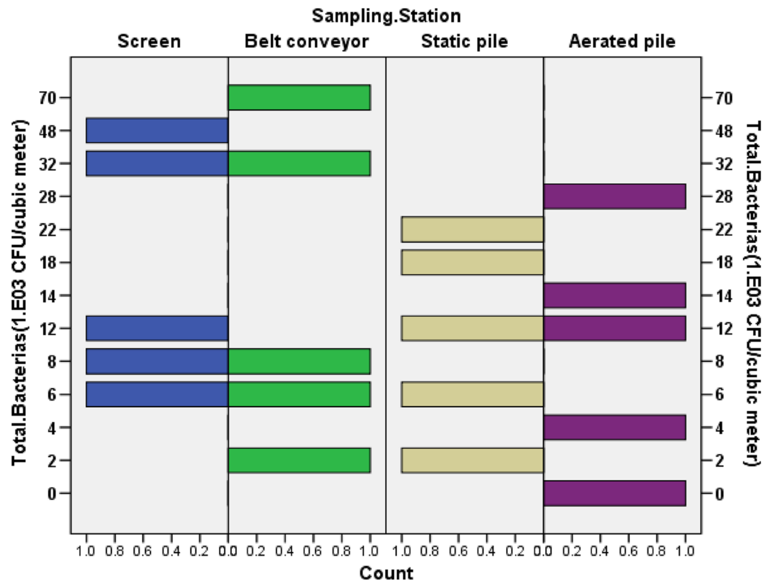

Fig. 1 Comparison of the density of total bacteria at different stations

solid waste processing and disposal facilities, the multi-stage impactor (QuickTake 30) used was equipped with a solidified medium (agar) and a specified pump (flow rate 28.3 l/m) for passing air through the agar media. The media were applied to identify fungi, *Staphylococcus aureus* bacteria, *Klebsiella* bacteria, and intestinal bacteria. The media used were Sabouraud dextrose agar for fungi identification, selective medium Vogel and Johnson for *S. aureus*, eosin methylene blue for *Klebsiella* intestinal bacteria, and trypticase soy agar for total count of bacteria. The samples were transported to the microbiology laboratory and were incubated at 37 °C for 3 days, and results were reported based on the number of colonies per cubic meter of air (cfu/m^3 of air).

Results and discussion

Sampling of the processing units' screen, conveyer belt, and composting site from aerated and static pile was carried out in accordance to the ASTM E884-82 (2001) guidelines. The samples were collected in two consecutive winter and spring seasons. The risk potential of the airborne microorganisms within the processing facility was plotted using the Surfer software.

Statistical analysis

The normal distribution of data in each data group (*Klebsiella*, total bacteria, total fungi, and *Staphylococcus aureus*) was controlled using the one-sample Kolmogorov-Smirnov test at a significance level ($\alpha = 0.05$). Obtained results showed that the distribution of data is normal ($P > 0.05$). As a result, in order to compare the data among the four sampling stations, the parametric tests (ANOVA) were used through the SPSS software. The statistical analysis revealed that in the four groups (*Klebsiella*, total bacteria, total fungi, and *S. aureus*), data distribution is identical and there was no significant difference between the averages of samples at four evaluated stations ($P > 0.05$). The results of the microorganism in the air around the processing facility of biocomposting plant in Sanandaj are summarized in Table 1.

The comparison of obtained data from different sampling stations, by considering the same data distribution in the four stations ($P > 0.05$), showed that the highest density of microorganisms was contributed to the total bacteria. Moreover, the lowest density was related to the *Klebsiella* bacteria, except in a static pile that the lowest density was contributed to the total fungi (Figs. 1, 2, 3, and 4).

The distribution of the microorganisms and their density mapping is a useful tool for predicting the microorganisms and the protective measures required for workers dealing with waste processing. The results of measuring *S. aureus* in a composting hall within a shredder, screen, and conveyor belt are mapped in Fig. 5. The contour lines indicates the severity of risks involved. The number of bacteria at the sampling stations was measured in the range of 1–10. The contour

Table 1 The average density of airborne microorganism in solid waste processing facilities

		No.	Mean	Std. deviation	Minimum	Maximum
Total fungus (1.E03 cfu/m^3)	Screen	5	6.40	4.980	2	14
	Conveyor belt	5	11.60	9.529	2	26
	Static pile	5	2.00	2.449	0	6
	Aerated pile	5	5.60	2.608	2	8
	Total	20	6.40	6.278	0	26
S. aureus (1.E03 cfu/m^3)	Screen	5	4.00	1.414	2	6
	Conveyor belt	5	6.40	5.367	2	14
	Static pile	5	2.80	2.280	0	6
	Aerated pile	5	3.20	1.789	2	6
	Total	20	4.10	3.210	0	14
Total bacteria (1.E03 cfu/m^3)	Screen	5	21.20	18.199	6	48
	Conveyor belt	5	23.60	28.475	2	70
	Static pile	5	12.00	8.246	2	22
	Aerated pile	5	11.60	10.807	0	28
	Total	20	17.10	17.598	0	70
Klebsiella (1.E03 cfu/m^3)	Screen	5	5.20	3.633	2	10
	Conveyor belt	5	4.40	4.336	2	12
	Static pile	5	2.00	1.414	0	4
	Aerated pile	5	5.60	4.336	2	12
	Total	20	4.30	3.629	0	12

lines on the map indicate that the highest contamination load of this bacterium is around the conveyor belt.

Figure 6 shows the *Klebsiella* distribution in composting hall within the vicinity of the shredder, screen, and conveyor belt. The number of *Klebsiella* at the sampling stations was measured in the range of 1–10. The contour lines on the map indicate that the highest contamination load of this microorganism occurs at around and end of the conveyor belt and screen.

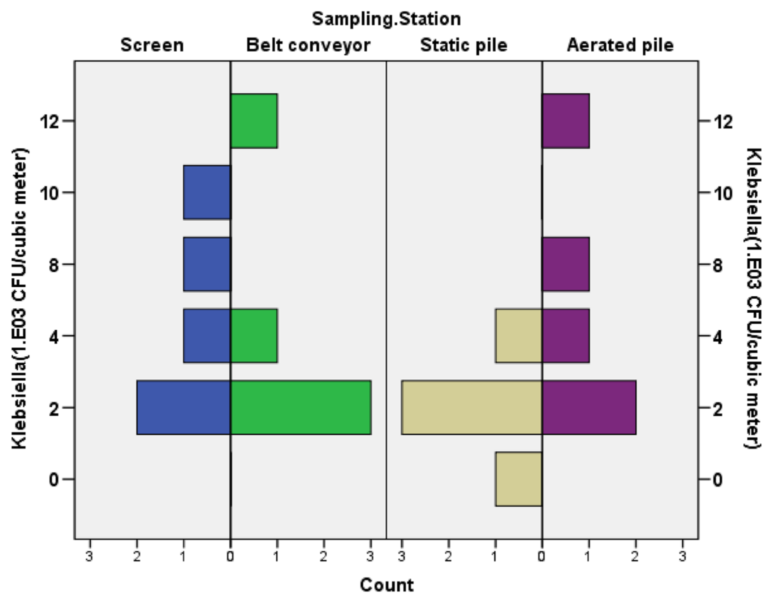

Fig. 2 Comparison of *Klebsiella* bacteria density at different stations

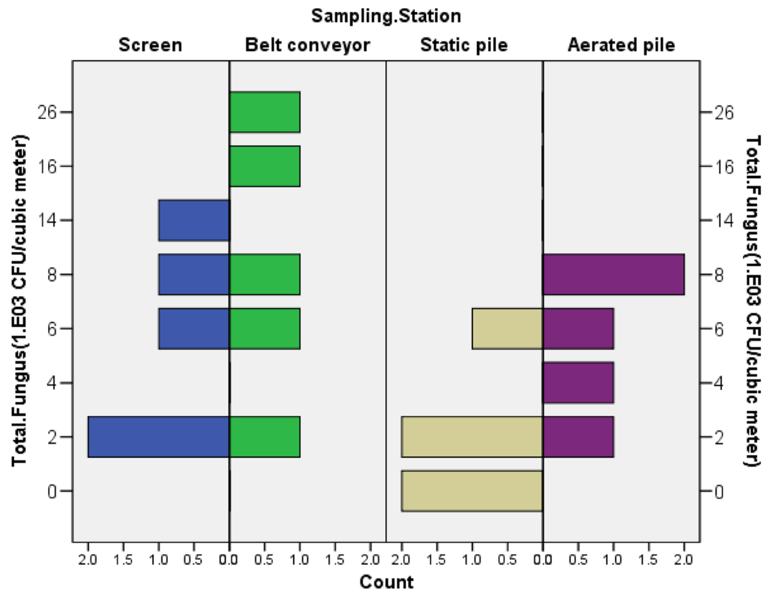

Fig. 3 Comparison of the density of total fungi at different stations

Figure 7 shows the fungi distribution in composting hall within the vicinity of the shredder, screen, and conveyor belt. The number of fungi at the sampling stations was measured in the range of 1–22. The contour lines on the map indicate that the highest contamination load of fungi occurs at around and end of the conveyor belt and screen.

Figure 8 shows the total bacteria distribution in composting hall within the vicinity of the shredder, screen, and conveyor belt. The number of total bacteria at the sampling stations was measured in the range of 1–52. The contour lines on the map indicates that the highest contamination load of fungi occurs at around and end of the conveyor belt and screen. According to the results, the mean density of bacteria, total fungi, intestine bacteria (*Klebsiella*), and *S. aureus* were $>1.7 \times 10^4$, 6.4×10^3, 4.3×10^3, and 4.1×10^3 cfu/m^3, respectively.

The highest density was observed at the waste materials processing unit and conveyor belt, where the average of the colonies formed for total bacteria and total fungi reached was 2×10^4 and $>10^4$ cfu/m^3, respectively. This can be attributed to the enclosing processing

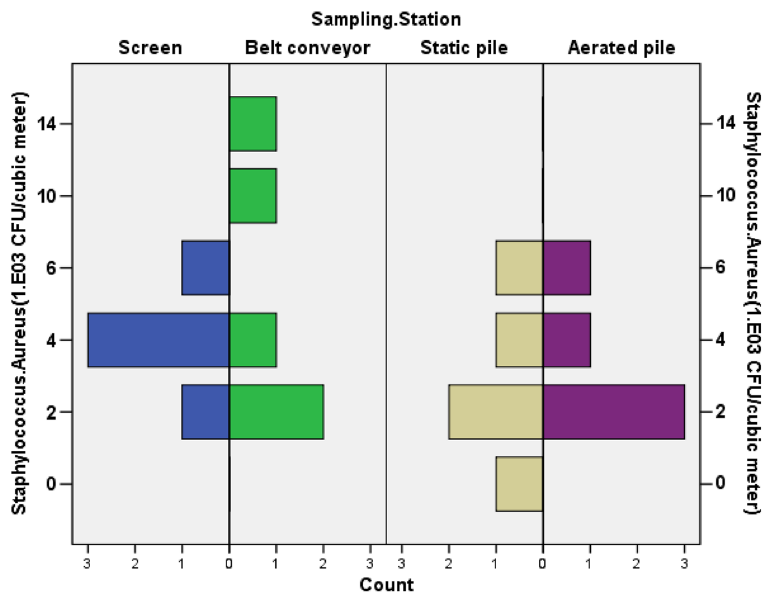

Fig. 4 Caparison of the density of *S. aureus* bacteria in different stations

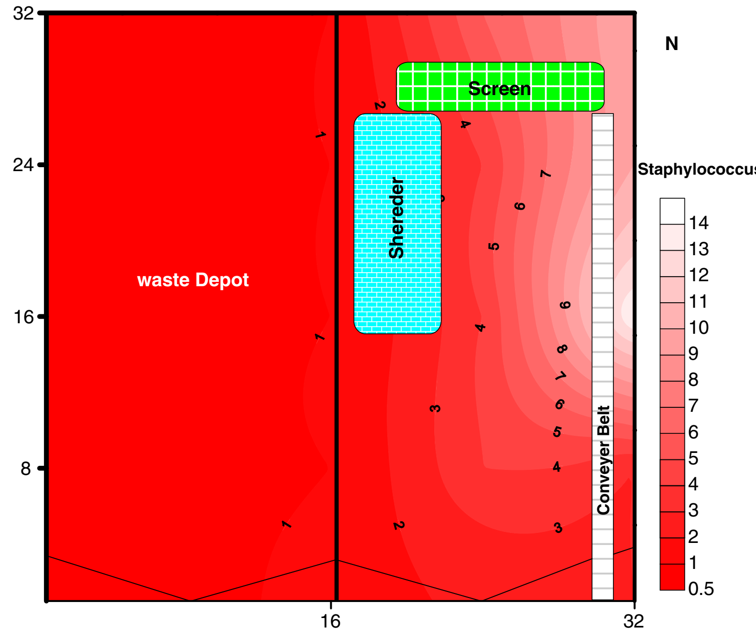

Fig. 5 Contour map of the distribution of *Staphylococcus aureus* per cubic meter of air and location of shredder, screen, and conveyor belt

unit, conveyor belt opening, and inefficiency of air conditioning system. Moreover, the accumulation of wet waste materials thrown away from the conveyor belt on the ground or other parts of the plant could cause an increase in microbial contamination in inhaled air of the workers. The lowest microbial contamination was observed in the samples collected from static compost pile, which was 1.2×10^4 and 2.0×10^3 cfu/m^3 for total bacteria and fungi, respectively. It may be attributed to low temperature and low relative humidity during sampling and pile exposure to open air. Bünger et al. (2007) affirmed that the exposure to organic dust at workplaces of composting facilities is associated with adverse acute and chronic respiratory health effects,

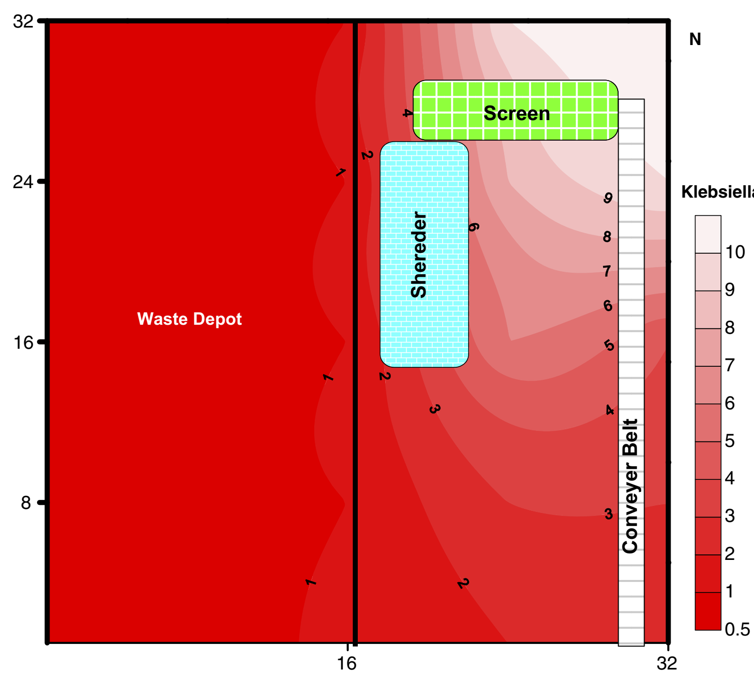

Fig. 6 Contour map of the distribution of *Klebsiella* per cubic meter of air and location of shredder, screen, and conveyor belt

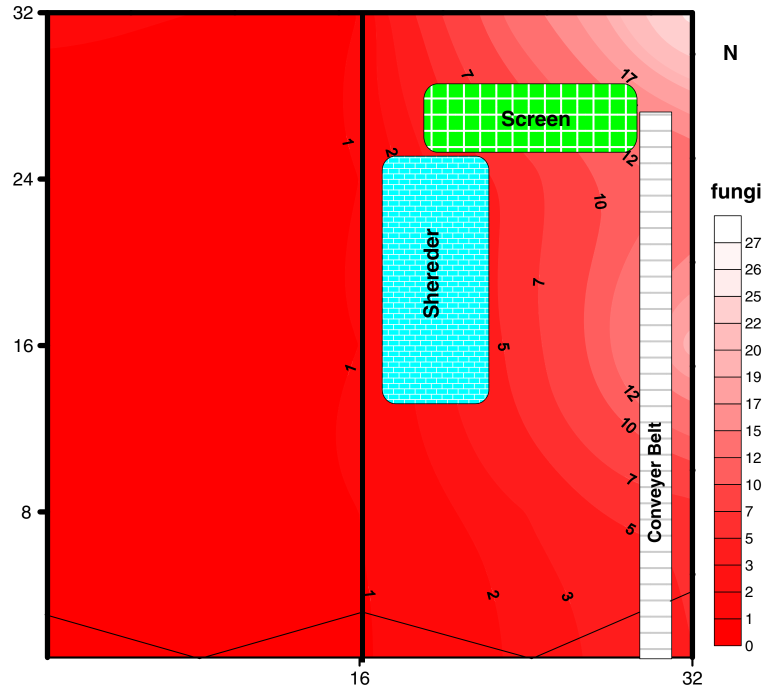

Fig. 7 Contour map of the distribution of total fungi per cubic meter of air and location of shredder, screen, and conveyor belt

including mucosal membrane irritation, chronic bronchitis, and an accelerated decline of forced vital capacity (FVC%). It has been suggested that respiratory tract inflammation increases in refuse collectors over the course of the working week (Lavoie et al. 2006).

Kiviranta et al. (1999) reported that the highest density of fungi was observed in the waste processing room. Workers in this industry (solid waste collection and composting) are often exposed to very high levels of microorganisms (Van Tongeren et al. 1997), and several

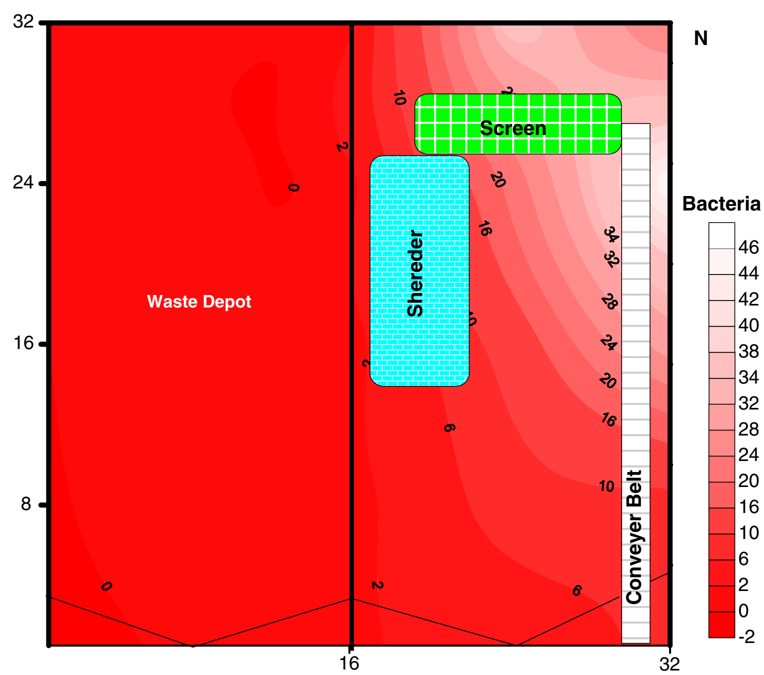

Fig. 8 Contour map of the distribution of total bacteria per cubic meter of air and location of shredder, screen, and conveyor belt

studies have indicated a high prevalence of respiratory symptoms and airway inflammation in these industries (Douwes et al. 2003). The different results from different studies may be attributed to the sampling methods and information analysis, sampling devices, operating conditions, the activity levels of composting site, type of raw material, material handling processes, climatic factors, and climatic conditions, which may affect the release of microorganisms. However, the actual amount may be more than the amount reported, because single-stage Andersen sampler can be used for the small volume of air in a short time (Hryhorczuk et al. 2001).

Although an experimental design cannot simulate a realistic microbial emission during occupational handling of compost, the relative potential was estimated at the same time when the compost workers were handling the compost (Nielsen et al. 1997a, b).

The ability of microorganisms to become airborne is dependent on several conditions including the materials where they grow and the physical characteristics of the species. The emission of airborne dust during agitation is reversely proportional with humidity, the size, and homogeneity quality of the basic material (Plinke et al. 1991). Health problems may be related to both living and non-living microorganisms, although most previous studies only reported data from cultivable microorganisms (Poulsen et al. 1995).

There are some limitations using sampling methods including assessing only culturable bacteria and fungi. Specifically, because most microorganisms were either fungal spores, spore-forming bacteria, or bacteria fixed on particles, and also because bacteria and spores from fungi and actinomycetes are much more easily released than vegetative cells, the samples did not necessarily reflect the real microflora of the waste (Nielsen et al. 1995). Indeed, Korniłłowicz-Kowalska and Rybczyńska (2015) also found that the strains isolated from the compost were mainly fungi from the genus *Aspergillus*, primarily *A. fumigatus* (up to 73% of the strains of those fungi). Furthermore, many bacteria (especially gram-negative species) are expected to die rapidly when aerosolized (ACGIH 1999; Lavoie et al. 2006). However, the obtained results are higher than standard level. The recommended limit for total bacteria and gram-negative bacteria are 10^4 and 10^3 cfu/m^3, respectively (Reinthaler et al. 1999; Fracchia et al. 2006; Domingo and Nadal 2009). Meanwhile, the mean background concentration of cultivable bacteria and fungus is 10^3 cfu/m^3 (Le Goff et al. 2012). The acceptable levels for total bacteria, gram-negative bacteria, and *Aspergillus fumigatus* are 1000, 300, and 500 cfu/m^3, respectively (Pearson et al. 2015).

It is not clear which specific component primarily accounts for the presumed health effects. There is no well-explained and available knowledge about the dose-response relationships and threshold values. Health hazards related to occupational exposure to bioaerosols are reported for different environments mostly in connection to high concentrations of organic dust. The lack of valid quantitative exposure assessment methods is the main reason for relative lack of knowledge (Douwes et al. 2002). A mixture of different agents may cause a disease, which is probably because of exposure to high concentrations of spores from fungi and thermophilic actinomycetes. Up to date, results indicated that composting facility staff is constantly affected by problems in the middle respiratory tract and inflammation of the mucous membrane (Chang et al. 2014).

To best our knowledge, this is the first report on airborne microorganisms in composting sites from Iran. Considering the importance of access to health care and workplace air monitoring, more control of occupational healthcare, and training of employees were involved in solid waste processing facilities about the potential health effects of their job. Therefore, it is crucial to establish better exposure assessment tools and validate newly developed methods.

Conclusion

This is the first study conducted in Iran on the microbial quality of the respiratory air at a composting plant. The research indicated that in the air around processing facilities of a biocomposting plant in Sanandaj City, the density of bacteria and fungi was higher than the standard level for inhaled air. Therefore, in order to reduce the exposure of staff and workers, the use of personal protective equipment, such as gloves, hats, and masks, and also actions such as frequently changing of personnel shifts, equipping the facilities with appropriate air conditions, automating the wide processing facilities, and receiving separated waste materials with good quality are recommended.

Limitations Due to the topographic conditions of solid waste processing facilities in Sanandaj City, sampling was not carried out outside facilities at 300 m in the downwind direction and 100 m in the upwind.

Suggestions

Investigation of airborne microorganisms on the spot at outside facilities in the downwind and upwind directions.

Investigation of airborne microorganisms in solid waste landfill.

Investigation of other airborne pollutants, odor, and particulate released from the processing facilities of composting plant.

Investigation of airborne microorganism using non-cultured method and direct count method.

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