Microorganisms in the Air over a Bio-solid Waste Landfill in Egypt

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Abstract: The aim of the present investigation was to evaluate microbial air quality (bacteria, actinomycetes and fungi) in adjacent and at different downwind distances at a municipal biosolid waste landfill (Shoubramant landfill). Airborne microbial composition was studied using a liquid impinger sampler during the period from June 2006 to June 2007. Air quality was evaluated using two microbiological contamination indices: the global index of microbiological contamination per m^3 (GIMC per m^3) and the amplification index (AI). Airborne microbial concentrations were usually higher downwind than upwind. The maximum downwind concentrations were 8.554×10^5 colony forming unit per cubic meter of air (CFU/m^3) for bacteria, 7.36×10^5 CFU/m^3 for actinomycetes and 1.088×10^4 for fungi. AI demonstrates that concentrations at downwind distances always superior to those of the upwind. There were no distinct correlation patterns found between air-microorganisms and weather conditions; the correlations differed according to the type of organism. The downwind microbial concentrations did not reach to the background ones, which raise the question about health risk. Human activity, type of organisms and meteorological factors were the main criteria controlling the temporal variations of microorganisms in the air. It is important to monitor microbial air quality near potential sources of bioaerosol emissions. In Egypt, detailed and systematic data is lacking on airborne microorganisms associated with waste application facilities.

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1. Introduction

Biological treatment of solid waste falls into three general categories; sanitary landfills, composting and soil stabilization. Landfills cause short-term but characteristic emission peaks of biological pollutants when turned or screened (Wüst *et al*., 1999). Rahkonen and Ettala (1990) concluded that the concentrations of fungi and mesophilic bacteria at the landfills were 2-30 times higher than the background concentrations. At landfills, the concentrations of bioaerosols can be hundred to thousands times higher than that of clean environments (Shusterman, 1992). Compost windrows can cause occupational hazards when turned, the concentrations of bacteria and fungi exceeded $10^2 – 10^3$ CFU/m^3 during turning (Häminen *et al.,* 1993).

Atmospheric air is less favorable for microbial survival as airborne microorganisms are subjected to certain conditions which can inhibit their survivability. Many parameters are being potentially damaging and lethal for microbial organisms such as solar radiation, changing temperatures, relative humidity, atmospheric pollution and free radicals (Sinha *et al*., 2000; Maier *et al*., 2000; Levetin and Dorsey, 2006, Karra and Katsivella, 2007).

Bioaerosols in indoor and outdoor environments have been found to cause adverse health effects (Douwes *et al*., 2000; Den Boer *et al*., 2002). Airborne bacteria and fungi can be toxigenic, allergenic and/or infectious (Burrell, 1991). Many of the microorganisms found in dust generated during composting are known respiratory sensitizers. Inhalation of organic dust can cause a range of immunological respiratory symptoms (Lacey, 1990; Chan-Yeung *et al*., 1992; Rylander, 1994). Different studies have shown that workers at waste handling and composting plants may have more gastrointestinal symptoms, irritation of the skin, eye and throat and respiratory disorders than in other occupations (Poulsen *et al*., 1995).

Expansion of existing waste application facilities (landfill and wastewater treatment plants) is necessary, however due to economic, environmental and political constraints many of waste applications have been constructed in places near to the residential areas without buffer zones. The impact of waste application facilities on the background concentrations of bio-aerosols are varied depending on wind direction and speed, concentrations of various microorganisms at the source and the type of activity at site (Reinthaler *et al*., 1999).

Nowadays, environmental regulators require operators of waste processing plants to submit risk assessment in support of environmental permits and licenses or exemption from these forms of regulatory control (Pollard *et al*., 2006). Risk assessments provide operators with the basis for operational controls on site and allow them to target controls, where exposures to significant risk are of greater concern. Therefore the aim of the present study was to evaluate air microbiological agents, bacteria, fungi and actinomycetes, upwind and downwind distances of a solid waste landfill in addition to determine the relationships between microbial concentrations and meteorological factors.

2. Materials and Methods

Description of the landfill

The landfill site is an area of ~ 1714 acres located in Shoubrament desert at Giza governorate. It is capable of holding at least 20-30 years production of solid wastes. It accepts domestic, commercial, soil and small amounts of special refuse such as slaughter refuse, animal manure and industrial refuse (e.g. paper, wood and rubber). The amounts of waste handled are 60,000 – 70,000 tons/ year. It is bounded by a desert area from the north and south and it shares two village's boarders namely Abu-Sir and Zawiet–Musalam from the east and west, respectively.

The refuse is spread and compacted to its maximum concentration by bulldozers. The compaction and burial processes for the solid wastes refuse is contained until the landfill filled and the site reached its capacity. Consequently, a new site must be designed and developed before the old landfill site is filled. Composting of the refuse is apart of waste management processing in Shoubrament's landfill. Solid waste undergoes an initial separation to remove all of non- biological biodegradable materials and ~ 66% of domestic solid waste is suitable for composting.

Sampling strategy and procedures

Air samples were collected at source point, 150 and 300 m downwind of fresh solid waste pills, Background samples were also collected 200 - 300 m upwind of the landfill. The samples were collected by using liquid impinger sampler (AGI-30), containing 40 ml sterilized phosphate buffer solution. The air was aspirated into the sampler by using vacuum pump calibrated to draw 12.5 L/min, as manufacture recommended rate, for 15 min, because of the suspected effect of sampling time on the concentrations.

Two consecutive samples were collected at each sampling point, two times per month, because of the short sampling time. The samples were collected at 1.5 - 2 m height above the ground level (breathing zone). The sampling was carried out during the period from June 2006 to June 2007, and sampling time varied between 9 am - 2 pm. The collected samples were maintained in ice box during sampling and transporting. The samples were subjected to the microbiological examination within 3 - 4 hours of the collection.

Microbial analysis

Aliquots of the original sample and its serial dilutions were spread plated, in duplicate, onto the surface of Trypticase soya agar, Starch casein agar and Rose-bengal streptomycin agar for counting the culturable airborne bacteria, actinomycetes and fungi, respectively. The bacterial plates were incubated at 28ºC for 48 hrs. Actinomycetes and fungi plates were incubated at 28ºC for 5 – 7 and 7-15 days, respectively. The growing colonies were counted and the mean count from replicate plates was calculated. The airborne microbial concentrations were calculated and expressed as colony forming unit per cubic meter of air (CFU/m^3).

Microbial contamination index

Contamination levels of microorganisms were analyzed using two different microbial contamination indexes: 1) The global index of microbial contamination (GIMC/ m^3) representing the sum of the values of bacteria, actinomycetes and fungi, and 2) the amplification index (AI) determined by calculating the ratio between GIMC / m^3 downwind distances and those measured upwind according to Dacarro *et al.* (2005).

Meteorological conditions

During every sampling event, temperature and relative humidity were measured with a portable Psychrometer (SATO; PC-5000 TRH-II Sampler, China). Wind speed records during the period of study were obtained form the Egyptian Meteorological Authority. In the present study, temperature ranged between between 22 – 37.5 °C, relative humidity 25.5 - 62% and wind speed between 1.85 – 5.75 m/s.

Statistical analysis

The linear correlation coefficient (r) and student t-test ($P \leq 0.05$) were used to examine the relationships between microbial agents and the meteorological parameters. Different student *t*- test was used to determine the degrees of the significance of difference between airborne microorganisms in the different sampling sites.

3. Results

The monthly concentrations of airborne culturable bacteria are presented graphically in figure 1. Upwind concentrations ranged between 1.05×10^3 and 1.6×10^4 CFU/m^3 and downwind 10^3 and 10^5 CFU/m^3. The bacterial concentrations were found in the mean values of 1.657×10^5 CFU/m^3; 1.478×10^5 CFU/m^3 and 7.98×10^4 CFU/m^3, respectively (Table 1).

Significant differences ($P < 0.0001$) were found between airborne bacterial concentrations detected at 150 m with zero point (t=4.309) and 300 m (t =8.455). A significant difference was only found between upwind and 300 m downwind distance concentrations.

Table 1. The range and mean concentrations of airborne bacteria, actinomycetes and fungi at the landfill site

Bio-pollutant	CFU/m^3 × 10^3			
	Downwind			Upwind
	Zero point	150 m	300 m	
Bacteria	(7.14 – 832.5) [165.7±274.78]	(4.35- 855.4) [147.8±260]	(1.066-304) [79.8±101.3]	(1.05-16) [6.937±4.794
Actinomycetes	(0.0 – 7.36) [0.985±2.047]	(0.0 – 2.986) [0.4607±0.8128]	(0.0 – 1.28) [0.34±0.359]	(0.0 – 0.107) [0.0537±0.029]
Fungi	(0.4266 - 10.88) [3.089±3.0027]	(0.213 - 5.12) 1.983±1.398]	(0.213 - 5.12) [1.539±1.371]	(0.09 - 0.46) [0.18±0.1018]

(range), [mean±SD]

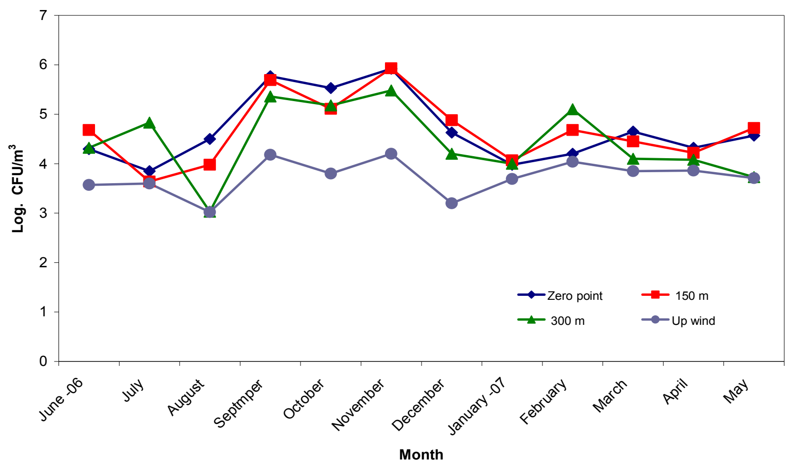

Figure 1. Monthly concentrations of airborne bacteria at upwind and downwind distances

The monthly concentrations of airborne actinomycetes are in presented figure (2). The concentrations ranged between 0.0 and 7.36×10^3 CFU/m^3 downwind and 0-1.07x10^4 CFU/m^3 upwind. The concentrations decreased gradually as the distance increased (Table 1). There were no significant differences (P > 0.05) found between the monthly actinomycete concentrations at the downwind sampling points. However a significant difference (t=2.7, P < 0.05) was only found between concentrations detected at upwind and 300 m downwind.

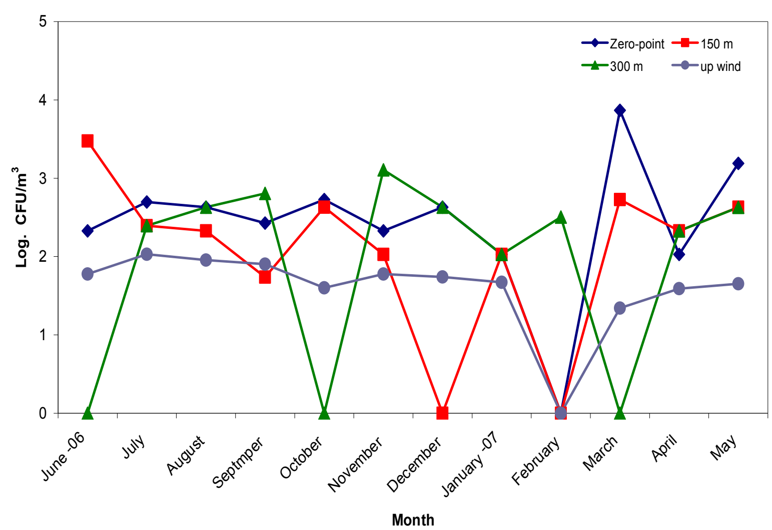

Figure 2. Monthly concentrations of airborne actinomycete at upwind and downwind distances

Figure 3 shows the mean monthly concentrations of airborne fungi. Fungal concentrations varied between 2.13×10^2 CFU/m^3 and 1.088×10^4 CFU/m^3 downwind. The upwind concentrations ranged between 90 and 460 CFU/m^3 (Table 1). Non significant differences ($P > 0.05$) were found between monthly fungal concentrations at all sampling points. On the other hand significant differences ($P < 0.05$) were found between the upwind concentrations and all the downwind concentrations.

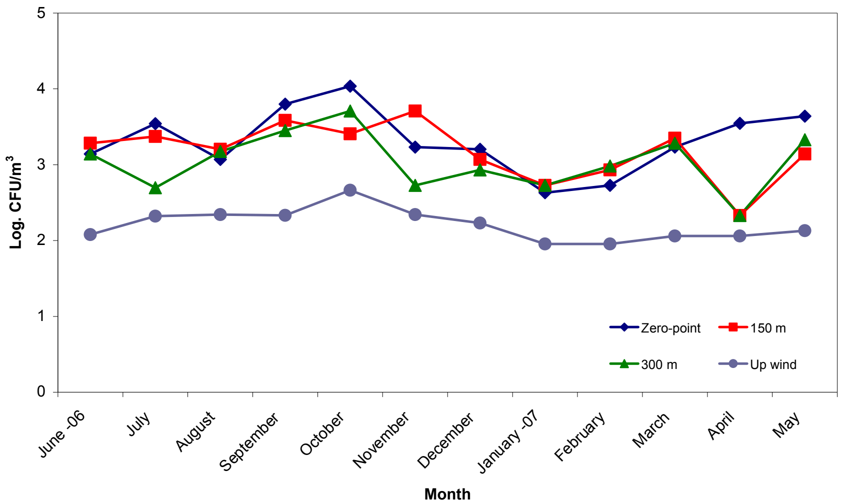

Figure 3. Monthly concentrations of airborne fungi at upwind and downwind distances

Table 2 shows the results of assessment of the microbial contamination levels based GIMC/m^3 and AI. The mean values of GIMC/m^3 were high; in some cases the microbial counts exceeded 10^6 CFU/m^3. The mean values of AI generally reduced with increasing distances. AI demonstrates that microbial concentrations at the downwind distances always superior to those of the upwind.

Table 2: Global index of microbiological concentrations (GIMC/m^3) and amplification index (AI) measured in different distances at the landfill site

Distance	GIMC		AI	
	Range	Mean ± SD	Range	mean
Zero -point	10773 - 836160	173135 ±275879	7.4 – 50.9	23.8
150 m	7703 - 863040	152450 ± 261543	5.35 – 52.5	21.03
300 m	5013 - 306986	83106 ± 101807	3.48 – 18.6	11.46
Upwind	1439 - 16423	7248 ± 4807	-	-

SD: Standard deviation.

There were no significant patterns found between airborne microbial concentrations and weather conditions (Table 3). However, actinomycetes showed significant correlations with temperature at 150 m downwind and wind speed at zero point. Airborne bacteria and fungi were positively and negatively correlated with wind speed at all sampling points, respectively (Table 3).

Table 3: Correlation coefficient between meteorological factors and airborne microorganisms

Indicator	Temperature				Humidity				Wind speed			
	Zero point	150 m	300 m	upwind	Zero point	150 m	300 m	upwind	Zero point	150 m	300m	upwind
Bacteria	-0.25	-0.30	-0.37	-0.54	0.00013	0.21	0.36	0.27	-0.18	-0.22	-0.24	0.0007
Actinomycetes	-0.19	0.73*	-0.29	0.49	-0.14	-0.38	0.39	0.63*	0.83*	-0.04	-0.25	-0.2
Fungi	-0.01	-0.04	0.32	0.41	0.19	0.33	-0.27	0.13	0.07	0.01	0.19	-0.12

*Significant ($P < 0.05$).

4. Discussion

Waste application facilities are recognized as being important sources of microbial aerosols that may constitute a health risk for workers and the surrounding population (Fracchia *et al*., 2006, Grisoli *et al*., 2009). Therefore it is required to evaluate and identify of hazardous resulting from such working facilities or being in contact with microorganisms.

These results in the present study agree with Malecka – Adamowicz *et al*., (2007) who found that, the air at landfill site in Warsow - Poland, was highly contaminated by both heterotrophic bacteria and actinomycetes. Actually, the high bacterial concentration at the landfill site is attributed to the composting processes; waste handling and pile shredding. The concentrations of mesophilic bacteria varied from 3.5×10^2 to 10^5 CFU/m^3 at two sanitary landfills in Finland (Rahkonm and Ettala, 1990). Nikaeen *et al*. (2009) found bacteria in the range of 120 - 2×10^4 CFU/m^3 and 400 – 2×10^4 CFU/m^3 with mean values of 2.887×10^3 and 6.727×10^3 CFU/m^3 for background and compost application facility, with significant differences were found between background and compost facility for bacterial concentrations. Actinomycetes are well known components of the microflora of composts and biosolids (Lacey, 1997) as composts have long been known as important sources of actinomycetes.

Significant differences were found between microbial indicator concentrations upwind with those downwind as the landfill is considered continuous source of airborne microorganisms. However, the microbial concentrations decreased as the distance downwind increased, with the furthest at 300 m away, as microbial contamination reached different levels. In many cases, the concentrations at 300m downwind were higher than those at the closed points (zero point and 150 m), depended on the quantity and quality of traffic operation near sampling point, local turbulences and human activities. In the present study, the results were highly variable due to a number of influencing factors such as operations and practices, waste composition and meteorological conditions.

Bioaerosols dispersion is affected by a number of factors, e.g. individual bioaerosol properties, geographical and meteorological conditions (Recer *et al*., 2001; Taha *et al*., 2006). The effect of the environmental conditions on bioaerosol dispersion can explain the discrepancy between the microbial concentrations at downwind samples, i.e. there is a pattern of decreasing concentrations with distance from the source point and higher downwind versus upwind (Nikaeen *et al*., 2009).

In the present study, the downwind microbial concentrations did not reach to the background ones, which raise the question about health risk, which also proved that the landfill contributed in the highest degree of microbial emissions and the concentrations depended mainly on the sampling location.

The input flux to the atmosphere is primarily from landfill, while the output flux from the air depends on primarily upon damage and decay of the microbes, transport through the wind and gravitational strategy (Gregory, 1973). On the other hand, the presence of agitation devices, mechanical activities, temperature, humidity, air turbulence and interference of the sources are some of the parameters that have potential inputs of the microbial fluxes.

Sofuoglu and Moschandreas (2003) highlights the need to identify indexes associated with occupational symptoms which can be easily understood and estimated in order to establish efficient protocols of occupational air quality monitoring. The index "GIMC" overestimates the real culturable microbial concentrations in the air and must be viewed as a practical to all for the evaluation of biological risk in the environment and for monitoring of potential sources of aerobiological contamination (Decarro *et al*., 2005). However, AI is of fundamental importance as it is an indicative of microbial accumulation and proliferation in downwind environment. Microbiological indexes are useful tool for evaluating and describing occupational air quality and may be used as reference values in the assessment and classification of the healthiness of air. In the present study, microbiological indexes showed that the downwind air quality at onsite and offsite of the landfill was bad and need policy makers to take action for reducing degradation of the air quality.

The unclear (direct) correlations between meteorological factors and microorganisms are attributed to the influence of traffic density, disturbances and re-disturbances of dust and human activity on the site that may be have more influence than the climatic factors. When the distance downwind increased, the effect of TºC and wind speed become more sever, however rh % may give some protection. These conditions vary according to the type of microorganisms. There are three forces that could influence the composition of airborne microbial community: 1) considering the atmosphere as an extreme environment, microbial cells are likely to employ various strategies to survive in atmospheric conditions (spores, pigmentation), (Tong and Lighthart, 1997); 2) if microbial community can grow in the atmosphere, this could possibly underline the changed community composition compared with that of possible sources, air is generally considered a nutrient – poor environment and active growth in aerosols is difficult to demonstrate and 3) it is speculated that, surface change, particle size, local and interference sources may determine the microbial composition in the aerosols. Collectively all of these factors may determine the type of airborne microorganism in a specific site and time under different conditions (Fahlgren *et al*., 2010).

There are no established guidelines, regulations or standards regarding bioaerosols assessment. However there are some limit values have been suggested by some researchers and agencies to help assess the significance of the bioaerosol exposure. Sigsgaard *et al*. (1990) proposed that the amount of total bacteria that site workers may be exposed to should not be over 5×10^3 or 10^4 CFU/m^3 for an 8 hours working day. Airborne microbial concentrations were recommended by Polish Standard / PN-89/2-04111/03 (1989) as following:

For bacteria: - > 3000 CFU/m^3 - strongly contaminated air, 1000 – 3000 CFU/m^3 – moderately contaminated air and < 1000 CFU/m^3 – uncontaminated air.

For fungi: - > 10000 CFU/m^3 polluted air, posing a hazard for human environment, 5000 - 10,000 CFU/m^3 - polluted air- with a potential negative effect on human environment and 3000 – 5000 CFU/m^3 - approximately clean atmospheric air.

For actinomycetes: - > 100 CFU/m^3 - strongly contaminated air, 10– 100 CFU/m^3 - CFU/m^3 moderately contaminated air and < 10 CFU/m^3 - uncontaminated air.

For *Pseudomonas*: - > 50 CFU/m^3 - strongly contaminated air, ≤ 50 CFU/m^3 - moderately contaminated air and ND – uncontaminated air.

In addition, the scientific community agrees that, the comparison of concentrations and species of bioaerosols found indoor and outdoor, upwind and downwind is a useful indication in determining the contaminated air. Guidelines of 10,000 CFU/m^3 for total bacteria and 1000 CFU/m^3 for Gram negative are proposed by the Scandinavian countries for 8 hour exposure in environment – related activities (Malmros, 1990; Poulsen *et al*., 1995).

By comparing the results in the present study with the previous suggested limit values, it is clear that the air quality over the landfill is classified as moderate to heavy contaminated. However it has to be mentioned that, increase numbers of microorganisms in the air do not have to cause an increased risk for people staying in a given ones. On the other hand, in some cases, people, spending much time in microbiologically contaminated environments become resistant (Pollard *et al*., 2006). The potential health hazard caused by aerosols depend not only the conditions of environment but also on the individual conditions especially the depositions and susceptibility persons (Emmerling, 1995), pathogenicity of specific microorganisms, immunologic response and place of landing on human body (Mohr, 2002).

Conclusion

1. Measuring air- bio-contamination in the waste application facilities has important implications for evaluating the potential risk of people exposed.
2. Downwind concentrations were always superior to upwind,
3. The comparative data is important tool in the profound understanding of the role of microorganisms in air quality.
4. There were no distinct correlation patterns between air-microorganisms and weather conditions.
5. Accidental activities and secondary microbial sources play an important role in increasing microorganisms at the farthest downwind distances.
6. Microbial indexes are useful tools for evaluating and describing air quality.
7. It is necessary to involve microbial particles monitoring and risk assessment in air quality reports.

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