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Evaluation of airborne Actinomycetes at waste application facilities

Abdel Hameed Awad [1,2], Safa A. El Gendy [1]

[1] *Air Pollution Department, National Research Centre, Dokki, Giza, Egypt*
[2] *Department of Environmental and Health Research, the Custodian of the Two Holly Mosques Institute for Hajj and Umrah Research, Umm Al Qura University, Saudi Arabia*

ABSTRACT

This study aimed to evaluate airborne meso–and–thermophilic actinomycete concentrations and their types at a wastewater treatment plant and a biosolid landfill, in Egypt. Air samples were collected at 200 m upwind, and onsite and 300 m downwind by using liquid impinger sampler, calibrated to draw 12.5 L/min, for 20 minutes. The concentrations ranged between 0.0–7 360 CFU/m^3 for mesophilic, and 106–586 CFU/m^3 for thermophilic actinomycetes. Airborne actinomycete concentrations exceeded the suggested occupational exposure limit value of 100 CFU/m^3. No significant correlations were found between actinomycete concentrations onsite and 300 m downwind distance. At the biosolid landfill, upwind thermophilic actinomycetes significantly differed (p<0.05) from onsite and 300 m downwind. A total of 40 and 69 airborne actinomycete isolates belonging to 8 genera were identified at the wastewater treatment plant and biosolid landfill. Streptomyces were the dominant actinomycete species. *Streptomyces diastaticus*, *Pseudonocardia compacta* and *Catellatospora ferruginea* were only detected at the biosolid landfill site. Meso–and–thermophilic actinomycetes positively associated with relative humidity, and wind speed positively correlated with onsite thermophilic actinomycetes (r=0.65) at the biosolid landfill. Temperature showed negative effect on survivability of mesophilic actinomycetes (r=-0.8) onsite of the wastewater treatment plant. Waste application facilities increase actinomycete concentrations onsite which may consequently deteriorate air quality in the nearby areas.

Keywords: *Air, actinomycetes species, biosolid landfill, wastewater treatment plant, meteorological conditions*

Corresponding Author:
Awad A. Abdel Hameed
☎ : *+966-56-991-3502*
🖷 : *+966-25-56-4955*
✉ : *abed196498@yahoo.com*

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1. Introduction

Sewage treatment plants and biosolid landfills have been considered as potential sources of bioaerosols (Karra and Katsivela, 2007; Heinonen–Tanski et al., 2009). Aeration tanks in wastewater treatment plants are considered the main sources for bioaerosol emissions (Pascual et al., 2003; Li et al., 2013).

Actinomycetes are important bio–pollutants in occupational environments (Nielsen et al., 1997), and the major component of bioaerosols emitted from composting facilities (Lacey, 1997; Swan et al., 2003). Thermophilic actinomycetes have been known in moldy hay, compost and self heated substrates (Unaogu et al., 1994), and have been used as indicator for bioaerosols discharged from composts (Dutkiewicz, 1997). Several studies have been published on airborne actinomycete concentrations in the urban and occupational settings (Breza–Boruta and Paluszak, 2007; Fang et al., 2008), and have indicated that actinomycetes are distributed worldwide and found in soil, decaying organic material and wastes. In Egypt, Abdel Hameed (1996) found airborne actinomycetes at mean concentrations of 10^2 CFU/m^3 at city center, and 10^3 CFU/m^3 at Zenein wastewater treatment plant. Airborne actinomycetes averaged 2.25x10^4 CFU/m^3, 2.9x10^4 CFU/m^3, and 4.1x10^3 CFU/m^3 at 20 m, 40 m, and 60 m downwind distances, respectively, of an agricultural non–point source during wheat harvesting season (Abdel Hameed and Khodr, 2001), and varied within 46–222 CFU/p/h along the main stream of the Nile river (Abdel Hameed et al., 2008). *Saccharopolyspora rectivirgula*, *Saccharomonospora spp.*, *Thermoactinomyces thalpophilus*, *Thermoactinomyces vulgaris* and *Thermomonospora* are the common thermophilic actinomycete species, and *Streptomyces* are the common mesophilic species (Swan et al., 2003).

The exposure of waste workers to airborne bacteria and fungi vary depending on site location, type of waste, treatment technology, and meteorological conditions (Nielsen et al., 1998; Thorn and Kerekes, 2001). Residents living ~200 m away of a composting plant are exposed to bioaerosols and suffered from irritations (Herr et al., 2003). Actinomycete species are considered human pathogens (Taha et al., 2007), and prolonged inhalation of actinomycetes is linked to allergic alveolitis (Herr et al., 2004), as their spores can deeply penetrate into the lungs. The present study aims to evaluate airborne meso–and thermophilic actinomycete concentrations and species upwind, onsite and 300 m downwind, and to investigate their distribution patterns in association with meteorological factors, at two waste application facilities varied in waste type and location, a wastewater treatment plant and a biosolid landfill, in Egypt.

2. Materials and Methods

2.1. Description of the waste application facilities

Zenein municipal wastewater treatment plant and Shoubrament biosolid landfill were selected for sample collection. The wastewater treatment plant is located in a suburban area of Giza governorate, Egypt ~7 km west of the Nile River. Zenein is a densely populated suburban area, characterized by various human and small industrial activities. The wastewater treatment plant capacity is 450 000 m^3/day, the system has preliminary and secondary settlement tanks, and aeration tanks. The biosolid landfill is ~1 714 acres located in the Shoubrament desert of the Giza governorate. It was constructed in 1989, and it is capable of holding at least ~30 years production of biosolid (domestic, commercial and soil refuses). The amounts of waste handled are ~70 000 tons/year. The refuse is spread and compacted to its maximum concentration by bulldozers.

2.2. Air sampling and analysis

The samples were collected 200 m upwind, onsite (zero point), and 300 m downwind. No–fixed sampling points were selected, as sampling points were chosen according to the prevailing wind direction (Table 1). Liquid impinger sampler (AGI–30, SKC) containing 40 mL sterilized phosphate buffered solution, held ~1.5–2 m above the ground level and 1–2 m far from the main source, was used to collect airborne actinomycetes. The air was aspirated at a flow rate of 12.5 L/min (manufacturer's recommended rate) for 20 min. At each sampling point, two consecutive samples were collected, two times per month, from June 2006 to 2007, between 9 am and 2 pm.

Aliquots (0.5 mL) of the original sample, and its serial dilutions (up to 10^{-2}) were spread–plated, in duplicate, onto two series of starch casein agar medium (Hi–media laboratories, Mumbai, India). The plates were incubated at 28 °C and 45 °C for 7–14 days to determine mesophilic and thermophilic actinomycetes, respectively. The resultant colonies were counted and expressed as colony forming unit per cubic meter of the air (CFU/m^3).

2.3. Biosolid and wastewater microbial analysis

One sample of biosolid and sewage water was collected once every three months, a total of 4 samples. A 100 mg of biosolid material was dissolved in 50 mL buffer phosphate solution, and serial dilutions were prepared (up to 10^{-7}). Aliquots (0.1 mL) of the original and its serial dilutions were spread plated, in duplicate, onto the surface of starch casein agar media. Aliquots (0.1 mL) of the original wastewater (collected from aeration tank), and its serial dilutions (up to 10^{-5}) were examined for the presence of meso–and–thermophilic actinomycetes. The plates were incubated at the same previously mentioned conditions. The resultant colonies were expressed as CFU/mg for the biosolid material, and CFU/mL for the wastewater.

2.4. Identification of actinomycetes

Identification of actinomycetes was carried out on the basis of morphological and biochemical features. The morphology of both spore chain and surface were examined (International Streptomyces Project, ISP) using Olympus microscopy (model CX 31, RBSF, Tokyo, Japan), and Scanning Electron Microscopy (model JEOL, JEM, Japan), respectively. Actinomycete isolates were screened on the basis of the color of colony, reverse side, and production of diffusible pigment (ISP 2, 3, 4 and 5), (Pridham and Lyons, 1961; Shirling and Gottlieb, 1966). Formation of melanin (ISP–6), (Trenser and Danga, 1958), utilization of carbon and nitrogen sources (Shirling and Gottlieb, 1966; Williams et al., 1989), producing of protease, lecithinase, and lipase enzymes (Nitsch and Kutzner, 1969), catalase and hydrogen sulfide (Kuster and Williams, 1964), hydrolysis of pectin (Hankin et al., 1971), reduction of nitrate (Kutzner et al., 1978), degradation of tyrosine, xanthine, casein, cellulose, gelatin and starch (Jones, 1949), and resistant to different antibiotics (Goodfellow and Orchard, 1974) were tested.

2.5. Meteorological conditions

Ambient temperature and relative humidity were measured with a portable psychrometer (SATO; PC–5 000 TRH–II Sampler, China) during every sampling event. Wind speed records were obtained from the Egyptian Meteorological Authority. During the study period, temperature ranged from 15.5–37.5 °C, relative humidity 25.5–69%, and wind speed 1.85–5.75 m/s. The prevailing wind direction was north–south.

2.6. Statistical analysis

Due to non normal distribution of the analyzed variables, Spearman's rank correlation coefficient test was used to examine the relationships between airborne actinomycetes, and meteorological factors. The differences between airborne actinomycete concentrations at the different sampling points were analyzed by using student t–test and Mann Whitney–U–test. A probability of less or equal to $p \leq 0.05$ was considered significant.

3. Results

3.1. Overall concentrations

Airborne actinomycete concentrations fluctuated throughout the period of study with higher concentrations onsite than both upwind and 300 m downwind. In some months, the downwind concentrations exceeded the onsite ones (Figure 1). Airborne mesophilic actinomycetes ranged from 0.0–200 CFU/m^3 upwind, and 0.0–7 360 CFU/m^3 downwind at both waste facilities (Table 2). The highest mesophilic actinomycete concentrations were found in February at the wastewater treatment plant (Figure 1a), and March at the biosolid landfill (Figure 1b). Mesophilic actinomycete concentrations did not significantly differ ($t \leq 2$, $p > 0.05$) between the all wastewater treatment plant sampling points. However mesophilic actinomycete concentrations were significantly higher at 300 m downwind than upwind (t=2.7, $p \leq 0.05$) at the biosolid landfill. Weak correlations were found between onsite and 300 m mesophilic actinomycete concentrations at the wastewater treatment plant (r=0.26) and the biosolid landfill (r=-0.12).

At both waste facilities, airborne thermophilic actinomycete concentrations ranged from 0.0–70 CFU/m^3 upwind, and 0.0–5 973 CFU/m^3 downwind sampling points (Table 2). The greatest onsite and 300 m thermophilic actinomycete concentrations were found in January and November at the wastewater treatment plant (Figure 2a) and the biosolid landfill (Figure 2b), respectively. Upwind concentrations significantly differed ($p \leq 0.05$) from onsite at the wastewater treatment plant, and with both onsite and 300 m downwind sampling points at the biosolid landfill. Positive correlation (r=0.54) was found between thermophilic actinomycetes detected onsite and 300 m downwind at the biosolid landfill.

Table 1. *Description of sampling points at both waste application facilities*

Location	Description
Zenin wastewater treatment plant	Onsite (zero point); it is located ~1–2 m adjacent to the aeration tanks of the first stage of the wastewater treatment plant, and it is considered the source of microbial emission.
	300 m downwind distance of aeration tanks, it is located at the surrounding residential areas outside the boarders of the facility.
	Background, 200 upwind of the wastewater treatment plant, and it is mainly located outside the boarders of the plant.
Shoubramant biosolid landfill	Onsite (zero point), it is located ~1–2 m adjacent to the fresh biosolid pile.
	300 m downwind distance of the main fresh biosolid pile.
	Background, 200 m upwind of the biosolid landfill according to wind direction.

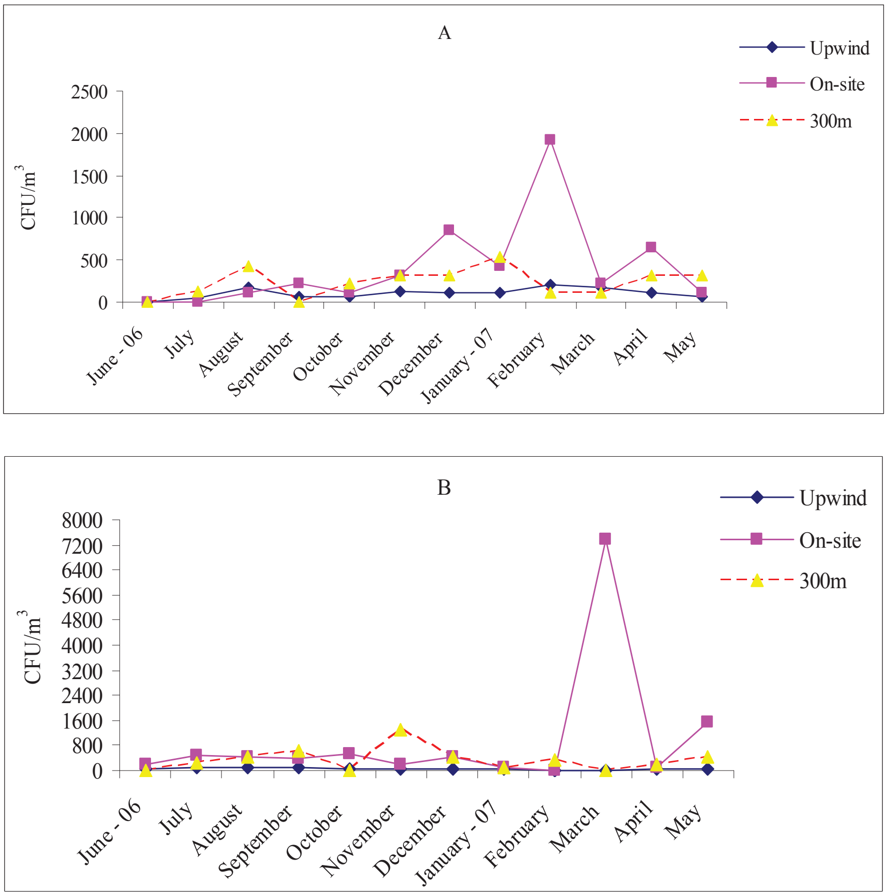

Figure 1. *Monthly concentrations of airborne mesophilic actinomycetes at the wastewater treatment plant **(A)** and the biosolid landfill **(B)**.*

Table 2. *The range and mean concentrations of airborne meso–and–thermophilic actinomycetes at the wastewater treatment plant and biosolid landfill*

Location	CFU/m^3 (range), [mean±standard deviation]			
	Wastewater treatment plant		Biosoild landfill	
	Meso	Thermo	Meso	Thermo
Upwind	0.0–200 [106±59.8]	0.0–70 [21±22.3]	0.0–107 [53.7±29]	0.0–55 [28±15.7]
Onsite	0.0-1 920 [408±541.6]	0.0–533 [178±189]	0.0–7 360 [985±2 047]	0.0–5 973 [1 697±2 117]
300 m downwind	0.0–533 [232.5±168]	0.0–1 066 [204±299.8]	0.0–1 280 [340±359]	0.0–106.6 [381±324]

The ratios of actinomycete aerosol strength in relation to waste source strength are shown in Table 3. Actinomycete concentrations averaged 3 140 CFU/mL in the wastewater, and 1 900 CFU/mg in the biosolid material. The impact of source strength to aerosol strength was higher onsite than 300 m downwind.

3.2. Correlations between airborne actinomycetes and meteorological conditions

Table 4 shows the Spearman's rank correlation coefficients between meteorological conditions and actinomycete concentrations. The correlations varied depending on type of actinomycetes, sampling location, and waste application. The relative humidity showed positive correlations with actinomycetes. Temperature significantly showed negative effect on mesophilic actinomycetes detected onsite of the wastewater treatment plant; however wind speed significantly showed positive correlation with thermophilic actinomycetes onsite of the biosolid landfill (Table 4).

3.3. Actinomycete diversity

A total of 40 and 69 airborne actinomycete isolates belonging to 8 genera were identified at the wastewater treatment plant and biosolid landfill site. Moreover, a total of 33 and 120 isolates belonging to 5 and 10 species were identified in the wastewater and biosolid waste material, respectively. Figures 3a and 3b show the types of actinomycete species in the air and source materials at the wastewater treatment plant and biosolid landfill, respectively. Airborne *Streptomyces diastaticus*, *Pseudonocardia compacta* and *Catellatospora ferruginea* were only found at the biosolid landfill site (Figure 3b). *Kineosporia aurantiaca* and *Nocardiodes albus* were the common species at the wastewater treatment plant and *Thermomonospora curvata* and *Streptomyces diastaticus* at the landfill. *Nocardia amarae* dominated in the wastewater (Figure 3a), and *Thermoactinomyces vulgaris* in the biosolid (Figure 3b). *Streptomyces albogriseolus*, *Streptomyces diastaticus*, *Streptomyces albus*, *Thermomonospora curvata*, *Nocardiodes albus*, *Nocardiopsis dassonovllei*, *Pseudonocardia compacta* and

Thermoactinomyces vulgaris were only detected in the biosolid waste, whereas *Nocardia amarae* in the wastewater. The agreement ratios between species in the air and the sources were 0.35 at the wastewater treatment plant and 0.76 at the biosolid landfill. The agreement ratios reflect the number of shared species isolated at different sites in relative to the total number of species.

4. Discussion

In the present study downwind actinomycete concentrations were higher than the background (upwind) ones. This raises the question about health risk, and proves that waste application facilities contribute high degree of microbial emissions. The communities further than 300 m away from waste application facilities are likely to be exposed to moderate actinomycete levels.

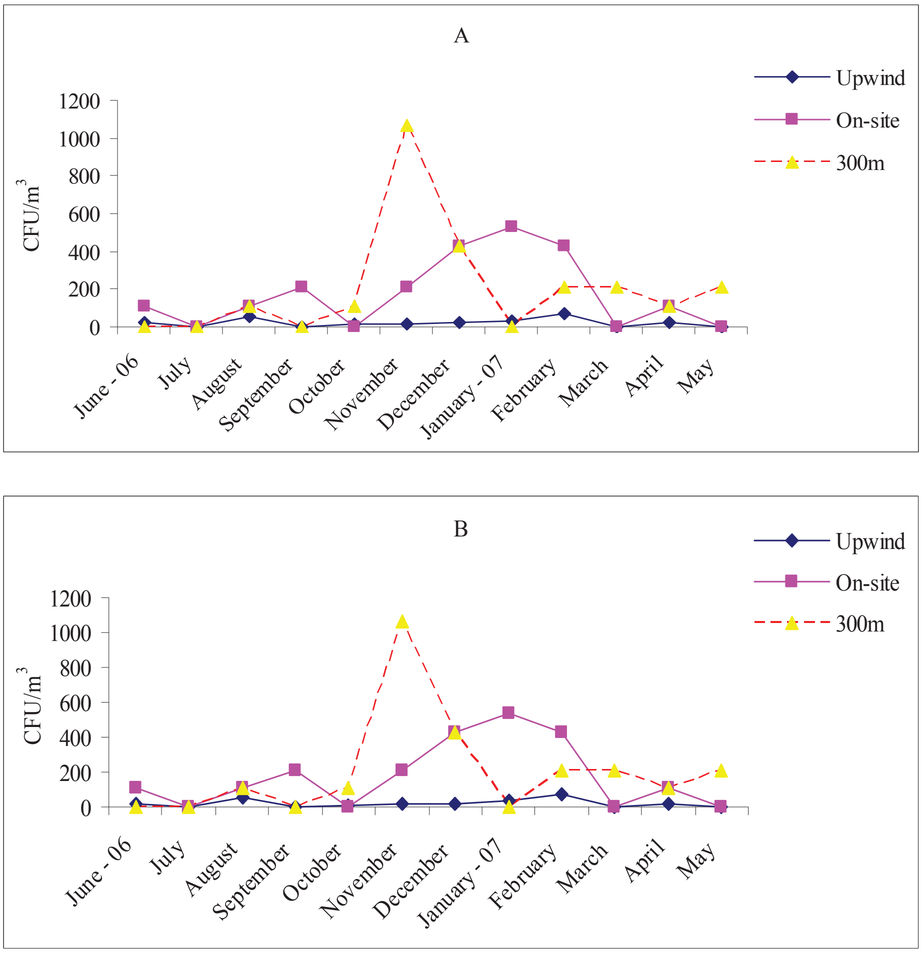

***Figure 2.** Monthly concentrations of airborne thermophilic actinomycetes at the wastewater treatment plant **(A)** and the biosolid landfill **(B)**.*

***Table 3.** Actinomycete aerosol strength in relation to source strength*

Source type	Source strength	Distance/m	Aerosol strength CFU/m^3	Aerosol strength/source strength
Aeration tank	3 140 CFU/ml	Onsite	292	0.2240
		300 m	218	0.1504
Main waste pile	1 900 CFU/mg	Onsite	1 340	0.705
		300 m	360	0.189

***Table 4.** Spearman rank correlation coefficients between airborne actinomycete concentrations and meteorological conditions*

Variable	Wastewater treatment plant				Biosoild landfill			
	Mesophilic		Thermophilic		Mesophilic		Thermophilic	
	Onsite	300 m	Onsite	300 m	Onsite	300 m	Onsite	300 m
Temperature	–0.80 [a]	–0.40	–0.60 [b]	–0.40	0.32	–0.18	0.31	–0.03
Relative humidity	0.28	0.27	0.44	0.30	0.22	0.25	0.43	0.32
Wind speed	–0.04	–0.29	0.26	–0.41	0.57	–0.17	0.65 [b]	0.56

[a] $p<0.01$
[b] $p\leq0.05$

In the present study, airborne actinomycete concentrations decreased with increasing distance from the source. The negative correlation between mesophilic actinomycete concentrations detected onsite and 300 m downwind of the landfill indicates that biosolid pile was not representing the actual main sources of actinomycetes, as operation conditions, and secondary sources are important factors controlling actinomycete emissions.

The ratios of actinomycete aerosol strength to actinomycete source strength were higher onsite than 300 m downwind (Table 3), confirms that waste facilities affect actinomycets air quality. In some months downwind concentrations exceeded those were found onsite, because the actinomycete concentratioins are associated with lateral dust dispersion, wind action, and unexpected anthropogenic activities near the sampling points. Lloyd (1969) found that the actinomycete concentration is highly dependent on the amounts of dust in the air; as dust protect them from the effects of ultra–violet, and solar radiation, this may explain the higher actinomycete counts at the landfill. Malecka–Adamowicz et al. (2007) found the highest airborne actinomycete concentrations in the central part of a landfill, and the number decreased with increasing distances downwind, as airborne microbial survivability depended on type of microbes, dispersion factors, meteorological conditions, and gravitational force (Gregory, 1973).

Thermophilic and mesophilic actinomycete concentrations were 2.5 and ~1.87 times higher at the biosolid landfill than the wastewater treatment plant, because biosolid provides favorable conditions for actinomycetes growth (Lacey, 1997). In Egypt, actinomycetes concentrations ranged from 9.02×10^2 to 9.52×10^3 CFU/m^3 with mean values of 1.3×10^3 CFU/m^3, at an animal feed manufacturing industry (Abdel Hameed et al., 2003). Actinomycete concentrations averaged 482.5 CFU/m^3 at a landfill site, in Zolwin–Wypaleviska, Poland (Malecka–Adamowicz et al., 2007). Airborne thermophilic actinomycetes averaged 5 300 CFU/m^3 at a British waste transfer station (Crook et al., 1987), and 15 000 CFU/m^3 downwind of compost windrows (Millner et al., 1980). These findings were higher than those found in the present study, because actinomycetes emitted from biosolid landfill sites should be lower than those emitted from composting facilities, which handled more biodegradable wastes.

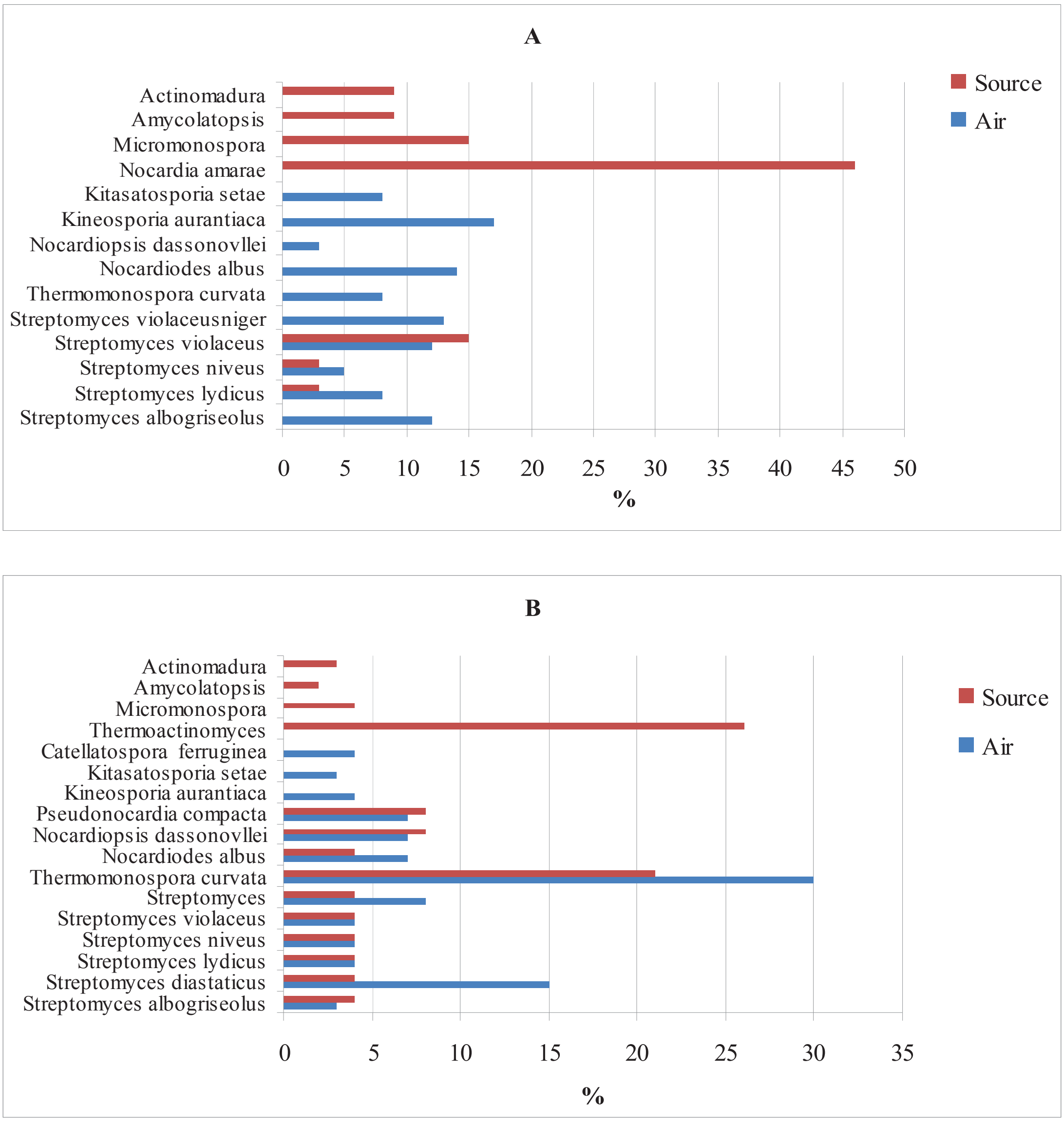

Figure 3. *Actinomycete diversity in the air and waste materials at the wastewater treatment plant **(A)** and the biosolid landfill **(B)**. Thermophilic species represented by: Strerptomyces diastaticus, Strerptomyces albogriseolus, Nocardiopsis dassonvllei, Kitasatospora setae, Thermomonospora curvata, Pseudonocardia compacta and Catellatospora ferruginea.*

There are no official standards for permissible level of airborne microorganisms. Therefore, it is difficult to evaluate the level of air biocontamination. In the present study, airborne actinomycetes exceeded the threshold value of 100 CFU/m^3 recommended by the Polish Standards (Breza–Boruta and Paluszak, 2007), such comparison indicates the heavy air microbial pollution, and actinomycetes can be used as air quality indicator at waste facilities.

Many factors affect dramatically on the survival of airborne actinomycetes. A complex respond of actinomycetes to meteorological factors is apparent, depending on type of waste, and location. Relative humidity gives protection to actinomycetes during transportation, and wind speed helps transport from the source, decreases aerosol age, and favors their survivability; at the same time, it reduces the net concentrations due to diffusion processes. The release and transfer of actinomycetes from waste piles is significantly dependent on wind speed, whereas mechanical agitation is the main factor for aerosolization from aeration tanks. Temperature negatively related with actinomycetes at almost all sampling sites, as actinomycets generation from liquid undergoes desiccation, and those generated in dust partially rehydrated (Cox and Wathes, 1995). It is worth mentioning that traffic density, rate of bubbles, disturbances and resuspension of dust particles, and human activities significantly affects actinomycete concentrations more than climatic factors at such waste facilities.

Identification of actinomycetes is a highly challenging, time consuming, and costly task. The agreement ratio indicated that biosolid pile had more effect on actinomycetes air quality than the wastewater treatment plant. *Streptomyces albus*, *Streptomyces griseus*, *Streptomyces thermoflavus*, *Streptomyces griseochromogenes* and *Streptomyces orientalis* were common in the air over sludge compost lagoon (Abdel Hameed and Kamel, 2006). *Streptomyces*, *Saccharomonospora*, *Thermoactinomyces vulgaris*, *Micropolyspora faenia* and *Thermoactinomyces sacchari* were common thermophilic actinomycetes in Cairo's air (Youssef and Karam El–Din, 1988), and *Streptomyces*, *Thermomonospora*, *Thermoactinomyces*, *Micropolyspora*, *Pseudonocardia* and *Actinobifinda*, in Kuwait's air (Diab and Al–Gounaim, 1982). Streptomyces species were abundant during composting process (Lacey, 1997).

In the present study, some of the identified species can cause human diseases. *Streptomyces albus*, *Streptomyces olivaceus* and *Streptomyces thermohygroscopicus* have been implicated in allergic alveolitis (Lacey and Dutkiewicz, 1994). Thermophilic actinomycetes are causative agents of allergic alveolitis, and pulmonary allergy in spite of their small concentrations (Prazmo et al., 2003). *Actinomyces*, *Actinomadura* and *Nocardia* can infect man and animal (Van den Bogart et al., 1993) and *Saccharopolyspora* and *Thermoactinomyces* cause occupational respiratory diseases (Millner et al., 1994). *Streptomyces* species may cause infectious diseases, inflammatory disorders, and pulmonary alveolities (Reponen et al., 2001), and stimulate lung macrophage reactions (Hirvonen et al., 1997). The high exposure to bioaerosols increased concentrations of specific antibodies against molds and actinomycetes among compost workers (Bunger et al., 2000).

The present study indicates that, persons residing or working close to sewage/landfill plants may have increased probability of contact with actinomycetes, even if they have been found in low levels, and the distance beyond which there is no risk of exposure cannot be determined precisely. Residential areas require higher standards of amenity. The width of a buffer zone in the Environmental Protection Agency guidelines is 400 m for a biological treatment plant serving up to 50 000 persons. Forgie et al. (2004) suggested that the minimum buffer zone distance from composting sites to residential area was 400–1 000 m.

5. Conclusion

Airborne actinomycete concentrations differed depending on waste type and meteorological conditions. It is difficult to determine the potential factors affecting their survivability in the air. Actinomycete concentrations increased downwind, as the effect of unsuspected human activities, and the presence of secondary sources (traffic and secondary piles). Actinomycete concentrations exceeded the threshold value of 100 CFU/m^3 recommended by the Polish Standards. Identification of actinomycetes helps determine the exposure of workers and residents to the dangerous species. The exposure to actinomycetes may lead to sensitization and development of diseases. Biosolid waste had more effect on actinomycetes air quality than the wastewater. This study emphasizes the importance of evaluating airborne microorganisms at waste facilities, and the importance of including microbial aerosols concentrations in air quality reports.

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