Suspended particulates and bioaerosols emitted from an agricultural non-point source

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Suspended particulate and bioaerosol levels were measured at three sites downwind of an agricultural non-point source during the wheat harvesting season. Suspended particulates were detected at mean values ranging from 10000 to 2420 μg m^{-3} at distances of from 20 to 60 m downwind of the source, respectively. Airborne viable bacterial counts were recorded at mean values ranging between 10^4 and 10^6 colony forming units (cfu) m^{-3}, whereas, Gram negative (Gram −ve) bacteria varied between 10^3 and 10^5 cfu m^{-3}. Fungi levels were detected at mean values varying between 10^5 and 10^6 cfu m^{-3}. However, streptomycetes were found at lower counts than those recorded for viable bacteria and fungi. Total viable bacteria, Gram −ve bacteria, fungi and streptomycetes associated hay fragments were determined at mean values of 1.5×10^6, 1.6×10^3, 2.2×10^4 and 6×10^3 cfu g^{-1} of hay, respectively. *Cladosporium*, white and red yeasts as well as *Alternaria* were the predominant airborne fungi, whereas, *Alternaria* was the dominant species associated with hay fragments. *Pseudomonas*, *Acinetobacter* and *Enterobacteriaceae* were the dominant Gram −ve bacteria. The most common fungal genera, such as *Cladosporium* and *Fusarium* (minor short axis), as well as *Streptomyces* species have an aerodynamic diameter (dae) of less than 5 μm, which can penetrate and deposit in the alveoli. Farmers and nearby residents are exposed to high levels of organic dust and bioaerosols during the wheat harvesting season. This may cause health problems in exposed persons based on toxic or allergic reactions.

Introduction

Particulate matter is emitted into the air from various pollution sources such as industrial activities, vehicles and agricultural processes. Agricultural operations are one of the most important sources of airborne organic dust and bioaerosols in rural areas. Bioaerosols of different and complex composition are produced from soil and crops during different agricultural operations. Exposure to bioaerosols and organic dust occurs in diverse environments. Workers are exposed to microorganisms from laden aerosols emitted from sewage and sewage treatment plants,[1] grains,[2] cotton, hay, jute and tobacco.[3] Outdoor bioaerosol sampling is conducted in occupational environments such as sewage treatment plants and agricultural settings.[4] Large amounts of organic dust (particles of biological origin such as plant fragments, fungi, actinomycetes and bacteria) are produced during hay making and harvesting, and such dusts are more hazardous than natural dust.[5] The fungus *Coccidioides immitis* can cause infection (coccidioidomycosis) and workers can be exposed during harvesting or subsequent handling, such as storage of hay, animal feed and cereal grains.[6]

Fine particulates stay suspended in the air for longer than coarse particulates and they are important in relation to human health, plant damage and water contamination. Farmer's lung disease is attributed to inhalation of dust from mouldy hay.[7] Harvest drivers complain of irritation, asthma and allergic alveolitis.[8] Allergic alveolitis was reported in up to 8.6% of Scottish farm workers[9] and in 9.6% of those in Devon.[10] Organic dust toxic syndrome was reported in between 6 and 19% of Swedish farmers[11] and in 13.6% of Finnish farmers.[12] Marthi *et al.*[13] also suggest that inhalation of microbial components of agricultural dust contributes to pulmonary diseases. Terho and Lacey[14] reported that the chief sources of the antigens in hay that cause farmer's lung are thermophilic actinomycetes and *Aspergillus rubrobrunneus*.

The present study aims to examine the levels of suspended particulates and bioaerosols at three distances downwind of an agricultural non-point source during the wheat harvesting season. It also aims to identify the dominant fungal and bacterial genera, as well as to measure the aerodynamic diameter (dae) of the fungal flora and *Streptomyces*, and to assess the respiratory exposure to these organisms.

Materials and methods

Air samples were collected at three sites, 20, 40 and 60 m downwind of an agricultural non-point source (Fig. 1) during the wheat harvesting season (May, 1999). The sampling sites were located near to a residential area, at Kafer El Akram village, Menofia governerate, Egypt. Upwind samples were also collected as a control.

Air samples for suspended particulate were collected on preconditioned preweighed cellulose nitrate membrane filter (pore size 0.45 μm, diameter 25 mm). The dust samples were

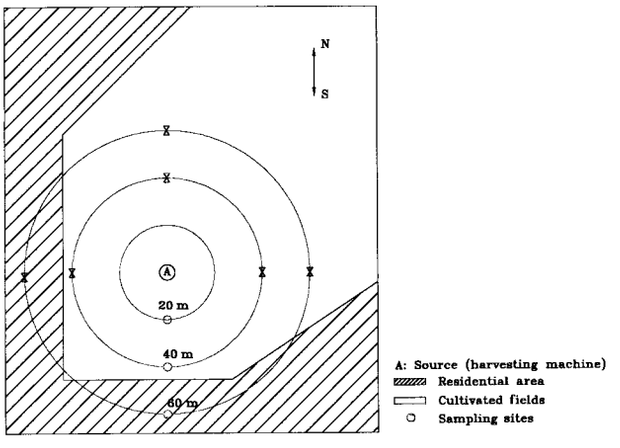

Fig. 1 Diagram of the sampling sites.

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collected using an open face filter holder, and a sampling pump calibrated to draw at a rate of 1.5 L min^{-1}.

Airborne bioaerosol samples were collected using a Liquid Impinger sampler (AGI, 30), containing 50 mL of sterile 0.1% peptone water (Difco, Detroit, MI), in the breathing zone, at a flow rate of 3 L min^{-1}. The sampling periods were between 15 and 20 min. A surface plate technique using selective media, MacConkey agar, 3% malt extract agar and casein starch agar (Difco, MI), was used for counting Gram −ve bacteria, total fungi and actinomycetes, respectively. A poured plate technique using standard plate count agar (Difco, Detroit, MI) was used to measure the total viable bacterial counts (TVBCs). The bacterial plates were incubated at 37 °C for 48 h, whereas the fungal and actinomycetes plates were incubated at 28 °C for 7 and 14 days, respectively. The resultant colonies were expressed in colony forming units per cubic metre of air (cfu m^{-3}). Five 0.1 g amounts of hay fragments were dissolved in 100 mL 0.1% peptone water and several dilutions were prepared. The microbial associated hay fragments were examined as mentioned previously and the resultant colonies were expressed in cfu per gram (cfu g^{-1}) of hay. Fungi isolates were identified microscopically, whereas bacterial isolates were identified according to Bausum *et al.*[15]

Results and discussion

The present work shows that the highest level of suspended particulates was recorded 20 m downwind of the harvesting machine, with a mean value of 10000 μg m^{-3}. The lowest mean value of 2420 μg m^{-3} was recorded 60 m downwind (Table 1). High suspended particulate levels are produced during the hay making and harvesting processes. Consequently, it may be concluded that, agricultural workers and the nearby residents can be exposed to suspended particulate of level more than 2420 μg m^{-3} during harvesting periods (Fig. 1). The results of the present study confirmed that, the particulate matter can be transported for long distances downwind of such non-point local sources. However, the transport of suspended particulates depends on several factors, such as local meteorological conditions, particle size and rates of deposition. Because of the low particle density and their fine size, plant debris can remain airborne for a long time and travel long distances downwind of their sources.[16] Moreover, this inhalable dust is associated with many microbial agents. The analysis of the microbial fraction associated hay fragment are shown in Table 1. TVBC, fungi, streptomycetes and Gram −ve bacteria were recorded at mean counts of 10^6, 10^4, 10^3 and 10^3 cfu g^{-1}, respectively. Moreover, *Alternaria* (48.5%) was the abundant fungus genera associated with the hay (Table 2). *Aspergillus*, *Penicillium*, *Cladosporium* and *Verticillium* were also recorded in the hay fragments but in low percentages. Many species of fungi found in hay and grain are implicated in asthma and allergic alveolitis.[17]

Airborne viable bacterial counts (incubated at 37 °C) ranged from 10^4 to 10^6 cfu m^{-3} with mean values of 4×10^6, 2.3×10^6 and 4.2×10^4 cfu m^{-3} at distances of 20, 40 and 60 m downwind, respectively. However, Gram −ve bacteria were recorded in the range 10^3–10^5 cfu m^{-3}. The highest mean value was recorded at a distance of 20 m (3.9×10^5 cfu m^{-3}), whereas the lowest value (2.7×10^4 cfu m^{-3}) was found at 60 m downwind. The results of the present study, are in agreement with Dutkiewicz[18,19] who found levels of viable bacteria of 2×10^4 and 1.2×10^6 cfu m^{-3} at grain stores and mills, respectively, in Poland.

The identification of bacterial isolates is summarized in Table 3. Gram +ve bacteria (cocci and bacilli) constitute 82.7% and Gram −ve bacteria constitutes 13.7% of the total bacterial isolates. *Bacillus* constitutes 36%, *Micrococcus* 26%, *Diplococcus* 21% and *Staphylococcus* 4% of the total bacterial isolates. *Pseudomonas* was the dominant Gram −ve bacteria (6.5%). *Enterobacteriaceae* and *Acinetobacter* were recorded at levels of 3.3% and 2.17%, respectively. *Achromobacter*, *Alcaligenes*, *Flavobacterium*, *Aeromonas* and *Morexella* were detected but at lower percentages. *Bacillus* species are often numerous in organic dust. It should be noted that *Bacillus* species (*B. subtilis* and *B licheniforms*) have been found to be associated with allergic alveolitis.[20] Corynebacteria and bacteria like cocci occur in large numbers in dusts of plant origin.[21] *Enterobacter agglomerans* occurs at high levels in plants and plant products especially cereal grains and cotton bracts.[22] *Alcaligenes faecalis* is common in bioaerosols from animal farms and at herbage processing plants.[23,24] Gram −ve bacteria of plant origin are a potential health risk for exposed workers and residents because they contain endotoxins in their outer cell wall.[21,25] *Acinetobacter*, *Alcaligenes*, *Pseudomonas* and *Flavobacterium* are present in wood working shops.[26] Consequently, the opportunity for infection by Gram −ve bacteria, as presented by bioaerosols and organic dust during the wheat harvesting season, is possible.

Fungi levels ranged from 10^5 to 10^6 cfu m^{-3} at distances of between 20 and 60 m (Table 1). They were detected at mean values of 1.1×10^6, 7.7×10^5, and 5.1×10^5 cfu m^{-3} at distances of 20, 40, and 60 m downwind, respectively. *Cladosporium*, yeasts (white and red types), *Alternaria*, *Monosporium* and *Verticillium* were the dominant types of airborne fungus (Table 2). *Fusarium*, *Mucor* (weak parasite in plants) and *Sporothrix* (dimorphic mold) were detected at low levels. The results of the present study are in agreement with Dutkiewicz[18,19] who also found viable fungi, at mean values of 2.4×10^3 and 4.5×10^6 cfu m^{-3} , respectively, at grain stores and mills. Darke *et al.*[8] found fungi spore concentrations of 2×10^8 cfu m^{-3} in dust from both the cutter bar and at the rear

Table 1 The concentrations of suspended particulates and bioaerosols in the air (upwind and downwind) and in microbial laden hay during wheat harvesting [a]

		Downwind distance/m[c]			
Indicator[b]	Upwind[c]	20	40	60	Hay fragment[d]
SPM/μg m^{-3}	(110–130)	(10000–10001)	(4200–7500)	(340–4500)	—
	[121]	[10000]	[5900]	[2420]	—
TVBC	$(1.6 \times 10^4$–$2 \times 10^4)$	$(2.6 \times 10^6$–$5.5 \times 10^6)$	$(1.75 \times 10^6$–$2.9 \times 10^6)$	$(4.7 \times 10^4$–$7.9 \times 10^5)$	$(6.2 \times 10^5$–$3 \times 10^6)$
	$[1.8 \times 10^4]$	$[4 \times 10^6]$	$[2.3 \times 10^6]$	$[4.2 \times 10^4]$	$[1.5 \times 10^6]$
Total fungi	$(1.6 \times 10^4$–$1.5 \times 10^5)$	$(1 \times 10^6$–$1.13 \times 10^6)$	$(6.4 \times 10^5$–$9.02 \times 10^5)$	$(3.08 \times 10^5$–$7.15 \times 10^5)$	$(3.3 \times 10^3$–$3.3 \times 10^4)$
	$[8.3 \times 10^4]$	$[1.1 \times 10^6]$	$[7.7 \times 10^5]$	$[5.1 \times 10^5]$	$[2.21 \times 10^4]$
Streptomycetes	0	$(2 \times 10^4$–$3 \times 10^4)$	$(1.8 \times 10^4$–$4 \times 10^4)$	$(0$–$8.3 \times 10^3)$	$(0$–$1.3 \times 10^4)$
	0	$[2.25 \times 10^4]$	$[2.9 \times 10^4]$	$[1.1 \times 10^3]$	$[6.0 \times 10^3]$
Gram −ve	0	$(3.3 \times 10^5$–$4.5 \times 10^5)$	$(2.3 \times 10^4$–$3.2 \times 10^5)$	$(5.0 \times 10^3$–$5.3 \times 10^5)$	$(1.7 \times 10^3$–$1.5 \times 10^3)$
	0	$[3.9 \times 10^5]$	$[1.2 \times 10^5]$	$[2.7 \times 10^4]$	$[1.6 \times 10^3]$

[a]Values in parentheses denote ranges. Values in square bracket denote an arithmetic mean. [b]SPM suspended particulate matter. TVBC, total viable bacterial counts. [c]Colonies expressed as cfu m^{-3}. [d]Colonies expressed as cfu g^{-1} of hay.

Table 2 The dominant genera and aerodynamic diameter (dae) of isolated fungi

	Upwind		Downwind		Hay fragment		Dae	
Genera	cfu	%	cfu	%	cfu	%	Range[a]	Mean
Cladosporium	3	33.3	283	46.5	1	3.03	2.4–4.6	3
Verticillium	1	11.1	6	1	1	3.03	1.1–2.4	1.3
Monosporium	—[b]	—	7	1.2	—	—	3.2–4.6	3
Aspergillus	—	—	—	—	2	6.06	3.2–4.6	3.9
Alternaria	3	33.3	13	2.14	16	48.5	7–10(sh.ax.)	10
Mucor	—	—	1	0.16	—	—	10–12	10
Fusarium	—	—	5	0.82	—	—	2.4–3.2 (sh.ax.)	3
Sporothrix	—	—	1	0.16	—	—	1.7–3.8	2.7
Penicillium	—	—	—	—	1	3.03	2.2–3.3	3
Non sporulating hyphae	—	—	—	—	4	12.12	—	—
Yeast	2	22.2	292	48.03	8	24.20	6–9	>5
Total	9		608		33			

[a]Sh.ax., short axis. [b]Not detected.

of combine harvesters during cereal crop harvesting, with concentrations of about 10% of this at the driving position. Lacey and Dutkiewicz[5] found that, the fungi counts reached 10^6 cfu m^{-3} during crop harvesting and crop handling. *Cladosporium* and yeasts are commonly found outdoors,[27] whereas *Aspergillus* and *Penicillium* are of indoor origin[28] and are rarely found outdoors.

The aerodynamic diameter of the fungi isolates is summarized in Table 2. The aerodynamic particle size is a critical factor in evaluating the respiratory exposure to fungal particles. *Cladosporium*, *Verticillium*, *Sporothrix*, and *Fusarium* have aerodynamic sizes <5 μm (Table 2), which can penetrate into and deposit in the lung tissue. In contrast, *Mucor* and *Alternaria* have aerodynamic sizes >5 μm and deposit in the upper respiratory tract. Reponen *et al.*[29] found that the maximum alveolar deposition for particles larger than 0.4 μm occurs at 2 μm during nose breathing and at 2.9 μm during mouth breathing. Allergic rhinitis and asthma result from exposure to particles >5 μm, such as *Aspergillus spp*.[30] Lacey and Crook[17] found that, fungi species associated with hay fragments and grain are implicated in asthma and allergenic alveolitis.

Streptomycetes were found at lower levels than the other microbial indicators. Levels ranged from 0 to 10^4 cfu m^{-3} with mean values of 2.25×10^4, 2.9×10^4 and 4.1×10^3 cfu m^{-3} at distances of 20, 40 and 60 m downwind, respectively (Table 1). The white series was the predominant one, and the aerodynamic diameter varied between 0.7 and 1.7 μm. *Streptomyces* species are well suited for deep penetration into lung on inhalation. Lloyd[31] found that the number of actinomycetes is highly dependent on the amounts of dust in the air. Lacey and Dutkewicz[5] stated that *Streptomyces spp.* form an important part of bioaerosols that originate from soil and from some plants. Actinomycetes from mouldy hay are important in the etiology of farmer's lung disease[32] and are implicated in the respiratory diseases of livestock.[33] Several types of actinomycetes are related to the incidence of allergic alveolitis.[34] *Streptomyces* species stimulate lung macrophage reactions and lead to inflammation and tissue injury.[35]

Table 3 Identification of bacterial isolates

Type	Number	%
Gram +ve—		
Diplococci	20	21.74
Micrococci	24	26.09
Staphylococci	4	4.34
Bacilli	28	30.43
Gram −ve—		
Pseudomonas	6	6.52
Alcaligenes	1	1.09
Acinetobacter	2	2.17
Achromobacter	1	1.09
Flavobacterium	1	1.09
Enterobacteriaceae	3	3.26
Aeromonas	1	1.09
Morexella	1	1.09
Total	92	

Conclusion

Airborne organic dust and bioaerosols consist of particles of biological origin. It is important to measure the levels of suspended dust and bioaerosols and to identify the taxa distributions of fungi and bacteria in order to evaluate the outdoor occupational agricultural environment. Farmers and residents are exposed to high levels of organic dusts during harvesting and post-harvesting processes and this may lead to adverse health effects. Pulmonary disease, allergenic rhinitis and asthma are common among farmers during the wheat harvesting season. The hazardous agents should be controlled to protect farmers and nearby rural residents against the organic dust emitted from these sources of air pollution. Further clinical and epidemiological tests should be carried out because of the presence of these etiological agents of toxicity and allergy. Another recommendation would be the determination of a buffer zone between the agricultural area source and the residential areas.

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