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ORIGINAL PAPER

Airborne dust, bacteria, actinomycetes and fungi at a flourmill

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Abstract A study was carried out on suspended dust, bacterial and fungal aerosols in a four-storey flourmill building located in Giza, Egypt. Airborne microorganisms were quantitatively isolated using liquid impinger and gravimetric samplers during the period from March 2004 to February 2005. Suspended dust varied from 1.96 to 16.3 mg m^{-3} and 0.69 to 1.8 mg m^{-3} in the indoor and outdoor environments, respectively. Suspended dust was significantly greater ($P < 0.05$) at bran package, double roller, purifiers and flour storage units in comparison to the outdoor reference site. The dust levels exceed the occupational exposure limit (OEL) of 0.5 mg m^{-3} for flour dust. Airborne microbial counts were found at median values, between sampling locations, ranged from 0 to $>10^4$ CFU m^{-3}. Gram-negative bacteria were found in small numbers (0–10^2 CFU m^{-3}). The highest concentration of actinomycetes ($>10^3$ CFU m^{-3}) was detected in the storage unit. Airborne fungal counts were found at the median values, between sampling locations, varied from 10^3 to 10^4 CFU m^{-3}. The counts of airborne bacteria and fungi were significantly greater ($P < 0.05$) at the purifiers and double roller mill units in comparison to the outdoor reference site using the liquid impinger sampler. Microbial levels associated with bulk deposited dust averaged between 10^5 and 10^6 CFU g^{-1}. *Alcaligenes* (5.4%) *Pseudomonas* (3.87%) and *Enterobacter* (3.1%) were the predominant Gram-negative species while *Bacillus* (29.4%) and *Micrococci* (13.9%) were the major components of Gram-positive bacteria. *Aspergillus* and *Penicillium* were the predominant fungal types indoor whereas *Cladosporium* (35.2%) and *Aspergillus* species (22.2%) were the predominant fungal types outdoor. A number of allergenic and toxigenic bioaerosols were found in the flourmill workplace.

Keywords Flourmill · Air quality · Suspended dust · Bacteria · Actinomycetes · Fungi

Abbreviations

CFU/p/h	Colony forming unit per plate per hour
CFU m^{-3}	Colony forming unit per cubic meter
OEL	Occupational exposure limit
dae	Aerodynamic diameter
AGI	All-glass impinger

1 Introduction

In recent years the awareness of bioaerosols within indoor environments has grown.

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Bioaerosols have been studied in different occupational and nonoccupational settings: markets (Narayn, Ravichandran, & Sullia, 1982), poultry farms (Surekha, Krishnareddi, & Reddi, 1996), animal feed industry (Abdel Hameed, Shakour, & Yasser, 2003), rice mill (Desai & Ghosh 2003), saw mill (Jothish & Nayar, 2004), schools (Aydogdu, Asan, Otkum, & Ture, 2005), bakeries and flourmills (Musk et al., 1989; Singh & Singh, 1994).

Grain dust harbors both viable and nonviable microorganisms and their toxins (Szponar & Larsson, 2001). In a study of a British bakery up to 1.2×10^3 CFU m^{-3} of air composition mostly *Penicillium* species were found where flour was weighed (Crook, Venables, Lacey, Musk, & Newman Taylor, 1988). Swan and Crook (1998) found that airborne fungal spores reached 6.7×10^4 CFU m^3 in wheat for humans and 1.6×10^6 CFU m^{-3} in animal feed wheat; bacteria reached 1.6×10^5 CFU m^{-3} and 2.3×10^6 CFU m^{-3}, respectively, whereas thermophilic bacteria and actinomycetes reached 8×10^2 CFU m^{-3} and 3.9×10^3 CFU m^{-3}, respectively. High concentrations of microorganisms have been found in airborne and settled dust samples in European and North American grain industries (DeLucca, Godshall, & Palmgren, 1984; Eduard 1997). Moreover, Karpinski (2003) reported that employees in a Canadian flourmill were exposed to dust level ranging from 0.5 to 20 mg m^{-3}.

Inhalation of grain dusts can decrease lung function and induce the development of immunological respiratory symptoms, which may include allergic and immunotoxic effects (Rylander, 1994). The etiology of toxic pneumonitis is unknown but it has also been speculated that one causative factor could be the inhalation of fungi, bacteria, actinomycetes and endotoxins that are present in flour and saw mills (Chan-Yeung, Enarson, & Kennedy, 1992; Malmberg, Rask-Andersen, Lundholm, & Palmgren, 1990). Allergenic and toxic effects may be the health hazardous associated with exposure to Gram-negative bacteria (Olenchock, 1990), actinomycetes (Lacey & Crook, 1988) and fungi (Sumi, Takeuchi, Miyakawa, & Nagura, 1994). Many of the microorganisms found in grain dust are well-known respiratory sensitizers, e.g., *Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium* (Dutkiewicz, Kus, Dutkiewicz, & Warren, 1985; Dutkiewicz et al., 1989) while *Enterobacter agglomerans* may be a source of endotoxins (Dutkiewicz, 1978). Moreover, workers exposed to the dust in flourmills have developed chronic bronchial irritation and asthma (Meo, 2004; Taytard et al., 1988).

The state of knowledge about biologically originated indoor air pollution in Egypt has been narrow and inadequate. A qualitative and quantitative knowledge of airborne bacteria, fungi and actinomycetes in Egyptian flourmills is essential for the effective diagnosis of associated health problems. The present study aims to evaluate the concentrations of suspended dust, bacteria, fungi and actinomycetes at different locations within a flourmill in Giza, Egypt.

2 Materials and methods

2.1 Description of flour mill

A four-storey flourmill building located in Giza, Egypt was chosen for this study. The wheat grain passes through the plan sifters at two stages in the milling process (break and reduction systems). The stock flour is sifted into various fractions (semolina, middling and bran) for further processed after each break roller mill. A series of rollers interspersed with plan sifters are used to roll and reduce semolina into fine flour. Semolina stock and finer endosperm particles are separated in the purifiers and sent to the reduction rollers. The purifiers use a combination of sieve sizes to remove the remaining fine bran particles. Finally, flour is packed into 50-kg bags and stored.

2.2 Air sampling locations

Air samples were collected at the following sites: (1) the bran package unit, (2) the double roller mill and wheat scourers, (3) the flour packing machine and storage unit, (4) the purifiers, horizontal bran finishers, flour collecting and screw conveyors, and (5) an outdoor reference site. The air samples were taken between 11 AM and 3 PM during the normal working hours. Eight sampling trials were taken from March 2004 to February 2005. The air

samples were taken from fixed sites at the center of the each unit, whereas the outdoor samples were collected 40 m from the flourmill, near a management office building. Indoor and outdoor air samples were collected at a height of 1.5 m, the breathing zone, above ground level.

2.3 Biological air sampling

Air samples were collected by liquid impinger (AGI-30) sampler, containing 25 ml phosphate buffer solution (KH_2PO_4 0.4%, K_2HPO_4 1.36%). The air samples were collected at a flow rate of 12.5 l min^{-1} for 15 min. Aliquots (0.2 ml) of the original sample and its serial dilutions (up to 10^4) were spread-plated in duplicate onto the surface of malt extract agar (MEA), trypticase soy agar (TSA), MacConkey agar (Mac) and starch casein agar (SC), (Difco, Detroit, Mi) for counting of fungi, total viable bacteria, Gram-negative bacteria and actinomycetes, respectively. Moreover, two plates for each microbial indicator, containing the previously mentioned media, were exposed to the air for 10 min using gravitational methodology (Pelczar, Chan, & Krieg, 1993).

2.4 Suspended dust and its microbial contents (Aerosol monitor)

Suspended dust was collected on sterilized preweighed cellulose nitrate membrane filters (0.45 μm, pore size and 25 mm, diameter). One-hour samples were obtained using an open face holder (25 mm, diameter, Casella, London) and sampling pump calibrated to draw 8 l min^{-1}. Suspended dust concentrations were determined gravimetrically and expressed in mg m^{-3}. The filters were washed with 10 ml phosphate buffer solution with 0.1 ml Tween 80. Aliquots (0.1 ml) of the original suspension and its dilutions (up to 10^5) were spread-plated onto the surface of the previously mentioned media.

2.5 Deposited dust

Bulk deposited dust samples were only collected indoors, using a sterilized spatula and plastic bag, from the centre of the different flourmill locations and were immediately sealed. Ten grams of the dust was mixed with 100 ml phosphate buffer, shaken vigorously for ~1 h and serially diluted tenfold to 10^6. Aliquots (0.1 ml) of the final two dilutions were spread-plated in duplicate onto the previously mentioned media.

2.6 The microbial analysis

The bacterial plates were incubated at 25°C (trypticase soy agar) and 37°C (MacConkey agar) for 48 h for the environmental bacteria and Gram-negative bacteria, respectively. Fungal and actinomycete plates were incubated at 28°C for 5 and 7–14 days, respectively. The resultant colonies were reported as colony-forming unit (CFU m^{-3}) for air and aerosol monitor and deposited dust were reported as colony forming unit per gram (CFU g^{-1}).

Representative bacterial colonies of the most prevalent from each site were selected and isolated into pure culture. They were identified using colony morphology, Gram staining and biochemical reactions (Vanderzent & Nickelson, 1969). Actinomycetes could not be identified at the species level and only numerical results are presented. Macroscopic and microscopic investigation of actinomycetes was carried out according to Waksman (1967). Fungal isolates were mainly identified by the direct observation on the basis of micro-and macro-morphological features using various literatures (Barnett & Hunter, 1999; St-Germain & Summerbell, 1996). Aerodynamic diameter (dae) of fungal spores was calculated from the physical diameter assuming a density of 1 g cm^{-3} and sphere shape.

Student's *t*-test ($P < 0.05$) was used to estimate the significant difference between the mean concentrations of suspended dust and airborne microorganisms using different samplers at all sampling locations (Gregory, 1963).

3 Results

Suspended dust ranged from 1.96 to 16.3 mg m^{-3} in the work environment with median values between sampling locations ranging from 2.7 to 6.46 mg m^{-3}, with the highest degree of dust pollution (16.3 mg m^{-3}) found in the flour storage

unit. Suspended dust concentrations were significantly greater ($P < 0.05$) at the bran package; double roller, and storage units in comparison to the outdoor reference samples (Table 1).

Tables 1 and 2 summarize the concentrations of the microbial indicators using gravimetric and liquid impinger samplers. The median microbial counts between sampling locations ranged from 7.14×10^3 to 7.9×10^4 CFU m^{-3} for environment bacteria, 0 to 2.82×10^2 CFU m^{-3} for Gram-negative bacteria, 4.23×10^2 to 2.15×10^3 - CFU m^{-3} for actinomycetes and 1.06×10^3 to 3.1×10^4 CFU m^{-3} for fungi using gravimetric and AGI samplers. Outdoor reference samples were 1–2-fold lower than those indoors. Gram-negative bacteria were only detected by using liquid impinger sampler (Table 2).

Significant differences ($P < 0.05$) were observed between the counts of total bacteria and fungi at the purifiers and double roller units in comparison to the outdoor reference samples using the liquid impinger sampler (Table 2). Actinomycetes constituted 0.6–4% and 2.6–40% of the total bacterial counts using the liquid impinger and gravimetric samplers, respectively. The highest concentration of actinomycetes was found in the flour packing unit using gravimetric sampler.

Deposited bulk dust contained higher levels of microbial indicators $>10^5$ CFU g^{-1}. Total Bacteria, Gram-negative bacteria, actinomycetes and fungi were found at median values of 4.67×10^5, 9.5×10^4, 2×10^4 and 5×10^5 CFU g^{-1}, respectively (Table 3).

Airborne Gram-positive and negative bacteria constituted 83.7 and 16.3% of the total bacterial isolates, respectively. *Bacillus* (29.4%) and *Micrococcus* spp. (13.9%) were the major components of the Gram-positive bacteria, whereas *Alcaligenes* (5.4%), *Pseudomonas* (3.87%) and *Enterobacter* spp. (3.1%) were the predominant Gram-negative bacteria (Table 4).

A total of 640 airborne fungal isolates belonging to 24 fungal genera were identified (Table 5). *Aspergillus* (26.78–78.3%), *Penicillium* (7.4–11.8%) and yeasts (4.96–27.7%) were the predominant species identified indoor areas, whereas *Cladosporium* (35.2%), and *Aspergillus* species (22.2%) were the predominant outdoor species. Among the *Aspergillus* species, *A. candidus*, *A. niger* and *A. ochraceus* were found at all sites while *A. fumigatus* and *A. flavus* were only detected indoors. The highest percentage of *Aspergillus flavus* was found in the storage unit. Zygomycete was found at low levels and represented by *Rhizopus*, *Absidia* and *Mucor*. Field

Table 1 The concentrations of suspended dust and the microbial indicators at various flourmill operation units using gravimetric sampler

Site	Suspended dust (mg m^{-3})	CFU $\times 10^3$ m^{-3}			
		Total bacteria	Gram-negative bacteria	Actinomycetes	Fungi
Bran package	[4.3–6.7] (5.358*** ± 0.87) {5.4}	[12–19] (15 ± 2.9) {14}	0	[0–0.67] (0.4 ± 0.29) {0.54}	[2.4–28] (14 ± 10) {12}
Double roller mill, wheat scourers	[1.96–3.23] (2.63** ± 0.52) {2.7}	[17–42] (32 ± 10.6) {35.7}	0	[0.48–2.2] (1.3 ± 0.7) {1.2}	[1–6.4] (2.9 ± 2.50) {1.2}
Flour packing and store	[2 –16.3] (7.8* ± 5.2) {6.46}	[19–37.6] (29.8 ± 7.9) {33}	0	[1.4–4.7] (2.7 ± 1.4) {2.15}	[1–13.1] (2.5 ± 2.7) {2.2}
Purifiers, horizontal bran finishers	[1.96–8.1] (4.43* ± 2.64) {3.23}	[7.1–9.7] (7.9 ± 1.2) {7.14}	0	[0.54–2.4] (3.2 ± 1.6) {1.8}	[1.1–3.6] (2.2 ± 1) {1.8}
Outdoor (reference location)	[0.69–1.8] (1.15 ± 0.47) {1.39}	[7.6–29] (15.2 ± 9.7) {7.6}	0	[1.1–1.4] (1.2 ± 0.12) {1.2}	[1.3–1.8] (1.5 ± 0.2) {1.2}

[Range], (mean ± standard deviation), {median}, ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$, in comparison to the control

Table 2 The concentrations of microbial indicators at various flourmill operation units using liquid impinger sampler

Site	CFU × 10^3 m^{-3}			
	Total bacteria	Gram-negative bacteria	Actinomycetes	Total fungi
Bran package	[12.5–68] (32.8 ± 24.9) {11.8}	0	[0.0–0.8] (0.36 ± 0.29) {0.54}	[0.67–4] (1.91 ± 1.48) {1.06}
Double roller mill, wheat scourers	[7.9–180] (86.7 ± 73.6) {79}	[0.0–0.21] (0.117 ± 0.09) {0.14}	[0.42–0.67] (0.54 ± 0.01) {0.54}	[31.4–119.4] (89.9* ± 41) {31.4}
Flour packing and store	[2.97–101.5] (39 ± 34) {25}	[0.0–0.67] (0.17 ± 0.29) {0}	[0.4–5.3] (1.7 ± 1.8) {0.423}	[2.3–18.4] (7.8 ± 6.5) {5.2}
Purifiers, horizontal bran finishers	[22–68] (37.2* ± 18.8) {22.5}	[0.21–0.5] (0.33 ± 0.12) {0.28}	[0.57–1.75] (0.87 ± 0.63) {0.565}	[1.98–2] (1.97* ± 0.46) {1.97}
Outdoor (reference location)	[6.93–9] (7.7 ± 0.76) {7.9}	0	[0.33–0.5] (0.41 ± 0.06) {0.4}	[2.7–4] (3.2 ± 0.57) {3}

[Range], (mean ± standard deviation), {median}, *$P < 0.05$, in comparison to the control

Table 3 The concentrations of microbial indicators associated with bulk deposited dust samples (grain, flour and bran)

	CFU × 10^5 g^{-1}			
	Bacteria	Gram-negative bacteria	Actinomycetes	Fungi
Range	2.6–12	0.0–4	0.15–0.2	0.1–44
Mean ± standard deviation	5.9 ± 3.7	1.47 ± 1.5	0.18 ± 0.023	10.4 ± 15.2
Median	4.67	0.95	0.2	5

Table 4 Identification of airborne bacterial isolates

Gram-positive bacteria	Number	Percent	Gram-negative bacteria	Number	Percent
Rods	**49**	**37.9**	**Rods**	**21**	**6.3**
Bacillus	38	29.4	*Acinetobacter* spp.	2	1.6
Corynebacteria	6	4.6	*Alcaligenes* spp.	7	5.4
Spore former	5	3.8	*Enterobacter* spp.	4	3.1
Cocci	**59**	**45.7**	*Klebsiella* spp.	3	2.3
Diplococci	11	8.5	*Pseudomonas* spp.	5	3.8
Micrococci	18	13.9			
Sarcina	6	4.6			
Staphylococci	16	12.4			
Streptococci	2	1.5			
Tetrads	6	4.6			
Total isolates	**129**				

fungi (*Fusarium*), prestorage (*A. flavus* and *A. parasiticus*), postharvest (*A. ochraceus*), stored xerophilic (*Eurotium*), less xerophilic (*A. candidus*) and high moisture content (*Stachybotrys*, *Ulocladium* and *A. fumigatus*) were detected in the flourmill units.

A total of six fungal genera were associated with suspended dust. *Aspergillus*, *Cladosporium* and *Penicillium* were frequently recovered at all flourmill locations (Table 6). Among *Aspergillus* species, only *A. parasiticus* was found in all sampling locations.

Table 5 shows the aerodynamic diameter (dae) of fungal spores. Neither fungal diameter nor shape are constant, however most fungal spores have different shapes ranging from globose to

Table 5 Identification of fungal types isolated from different mill units and their aerodynamic diameter (dae)

Types	Bran package		Double roller		Stores		Purifiers		Outdoor		dae (μm)
	No.	%	No.	%	No.	%	No.	%	No.	%	
Aspergillus	159	78.3	67	47.5	30	26.78	32	42	24	22.2	
A. candidus	141	69.45	40	28.4	8	7.14	4	5.3	2	1.85	2.5–3.5
A. flavus	2	0.98	11	7.8	12	10.7	6	7.9	–	–	3–5
A. fumigatus	1	0.5	2	1.42	4	3.57	3	3.9			2
A. niger	3	1.48	9	6.4	2	1.8	10	13.2	5	4.6	3.8
A. ochraceus	2	0.98	2	1.42	1	0.9	2	2.6	1	0.9	3.5–4
A. parasiticus	2	0.98	2	1.42			2	2.6	4	3.7	3.5–5
A. terreus	–	–	–	–	2	1.8	–	–	–	–	2.1
Aspergillus spp.	8	3.9	1	0.71	1	0.9	5	6.6	3	2.8	2.5–4
Absidia	–	–	2	1.42	–	–	–	–	–	–	5–6
Alternaria	2	0.98	4	2.8	9	8.0	7	9.2	9	8.3	10–15*
Ascomycetes	–	–	–	–	2	1.8	–	–	–	–	–
Botrytis	–	–	–	–	1	0.9	–	–	–	–	2.5
Cladosporium	6	2.95	29	20.6	11	9.8	13	17.1	38	35.2	2.5–4.5*
Chrysonilia	–	–	1	0.71	–	–	–	–	–	–	–
Chaetomium	–	–	1	0.71	1	0.9	–	–	–	–	12
Bipolaris	–	–	1	0.71	–	–	–	–	1	0.9	>35
Emericella	–	–	1	0.71	1	0.9	–	–	–		–
Epicoccum	–	–	–	–	–	–	–	–	1	0.9	17
Eurotium	–	–	2	1.42	–	–	2	2.6	–	–	4.9–5.8
Fusarium	–	–	3	2.12	2	1.8	–	–	–	–	2–3.1
Geotrichum	–	–	–	–	3	2.67	–	–	–	–	4*
Mucor	2	0.98	3	2.12	3	2.67	1	1.3	1	0.9	5.5
Nigrospora	–	–	–	–	1	0.9	–	–	–	–	10–13.5
Penicillium	15	7.4	11	7.8	12	10.7	9	11.8	13	12	2–3.5
Rhizopus	2	0.98	1	0.71	1	0.9	1	1.3	–		5–8
Scopularopsis	–	–	1	0.71	1	0.9	2	2.6	–	–	5.3
Spicaria	–	–	–	–	–	–	2	2.6	–	–	2.4–4.2
Stachybotrys	–	–	2	1.42	–	–	–	–	–	–	4–5
Sterile hyphae	1	0.5	3	2.12	3	2.67	3	3.9	6	5.6	–
Ulocladium	–	–	2	1.42	–	–	–	–	–	–	15
Yeasts	16	7.9	7	4.96	31	27.7	4	5.2	15	13.9	3.1–5
Total	**203**		**141**		**112**		**76**		**108**		

No.: number, *: short axis, –: not found

fiber-like. *Alternaria*, *Bipolaris*, *Rhizopus*, *Ulocladium* and *Epicoccum* have dae > 5 μm, whereas *Aspergillus*, *Penicillium*, *Spicaria* and *Fusarium* (short axis) have dae < 5 μm.

4 Discussion

The suspended dust levels exceeded the OEL of 0.5 mg m^{-3} for flour dust as suggested by the American Conference of Governmental Industrial Hygienists (ACGIH, Karpinski, 2003). This is attributed to the milling activities, lack of control measures, insufficient ventilation and floor cleaning. The results of the present study correspond with Nieuwenhuijsen et al. (1994) who found suspended dust in the range of 0.5–16.9 mg m^{-3} in a flourmill in England. Desai and Ghosh (2003) found suspended dust concentrations significantly greater ($P < 0.01$) at the workplace site compared to the control site.

The high microbial content associated with deposited dust indicates that microorganisms may be associated with grains both during harvesting and after storage. Therefore, resuspension of settled dust into the air may be a plausible explanation for increasing airborne microbial contents in dusty units. Microorganism associated dust may be inhaled by workers. The results of the present study correspond with Dutkiewicz

Table 6 Identification of fungal isolates associated with suspended dust samples

Types	Bran package		Double roller		Packing and store		Purifiers		Outdoor	
	No.	%	No.	%	No.	%	No.	%	No.	%
Aspergillus	32	51.6	11	52	5	19.2	9	56.2	6	19.4
A. candidus	27	43.54	4	19	–	–	1	6.25	–	–
A. flavus	–	–	–	–	–	–	2	12.5	1	3.2
A. fumigatus	–	–	2	9.5	1	3.8	–	–	–	–
A. niger	3	4.84	–	–	–	–	3	18.7	4	12.9
A. parasiticus	2	3.22	2	9.5	4	15.4	1	6.25	1	3.2
A. terreus	–	–	1	4.7	–	–	–	–	–	–
A. versicolor	–	–	2	9.5	–	–	2	12.5	–	–
Alternaria	1	1.6	1	4.7	2	7.7	2	12.5	10	32.2
Cladosporium	9	14.5	2	9.5	5	19.2	1	6.25	12	38.7
Eurotium	4	6.4	1	4.7	1	3.8	1	6.25	–	–
Penicillium	16	25.8	6	28.6	12	46.2	3	18.75	2	6.5
Ulocladium	–	–	–	–	1	3.8	–	–	1	3.2
Total	**62**		**21**		**26**		**16**		**31**	

No: number, –: not found

(1986) and Dutkiewicz et al.(1989) who found high microbial concentrations in airborne and settled dust during grain handling.

Different sampling methods were used to maximize recovery of the various microorganisms present in the flourmill environment. The AGI and gravimetric samplers were chosen for air sampling because they can be used in highly contaminated areas without overloading (Swan & Crook, 1998). Additionally, the cut diameter (d_{50}) of AGI is 0.3 μm (Cown, Kethley, & Fincher, 1957), therefore it is useful for studying the respiratory infection potential of bioaerosols (Jensen, Lighthart, Mohr, & Shaffer, 1994). In the present study the AGI sampler gave significant higher airborne microbial counts at indoor locations in comparison to the outdoor location. Because flour milling processes generate high concentrations of bioaerosols in the workplace environment (indoor). In addition, AGI provides protection for vegetative organisms and improves recovery of viable, culturable, organisms (Macher, Chatigny, & Burge, 1995). In contrast the gravimetric sampler yielded higher numbers of recovered actinmoycetes. Quantitatively, concentrations of fungi, actinomycetes and bacteria differed little in suspended dust from different locations within the flourmill. The absence of significant variations in the microbial aerosols (associated with suspended dust) may be due to the same composition and nature of dust particles at all locations, and may confirm that AGI provides some protection during the sampling process for air microorganisms.

In the present study, Gram-negative bacteria were found in low numbers, and were not recorded through the gravimetric method. These results could be attributed to their sensitivity to environmental stresses, and confirmed that the liquid impinger provided protection for vegetative bacteria from physical stresses and sunlight. The gravimetric method does not seem suitable for evaluating vegetative cells due to desiccation of bioaerosols by airflow (Wang, Reponen, Grinshpun, Gorny, & Willeke, 2001).

The dominance of Gram-positive bacterial isolates is an indication of inadequate ventilation and overcrowding in the evaluated flourmill (Morey et al., 1986). This finding corresponds with Dutkiewicz et al. (2000) and Abdel Hameed and Khoder (2001) who found bacteria (*Bacillus*, Corynebacteria, Cocci) and Streptomycetes in airborne organic dust. Moreover, Swan and Crook (1998) found Gram-positive spore-forming *Bacillus* and Cocci (*Curtobacterium*, *Micrococcus* and *Staphylococcus*) and Gram-negative bacteria including *Pseudomonas* and *Enterobacter* spp. in grain dust.

Airborne microbial counts exceeded the proposed value of OEL 5–10 $\times$ 10^3 CFU m^{-3} of total

microorganisms for occupational environments (Malmros, Sigsgaard, & Bach, 1992). In the present study, airborne bacteria and fungi recovered using the AGI sampler at the double roller mill location were found in the range exceeding the proposed value of OEL 1×10^5 CFU m^{-3} (Gòrny & Dutkiewicz, 2002) for industrial settings contaminated by organic dust. In contrast, Gram-negative bacterial levels did not exceed the proposed values of OEL 1×10^3 CFU m^{-3} (Clark, 1986). Otherwise the presence of Gram-negative bacteria has occupational hazards. Gram-negative bacteria possess strong allergenic and endotoxinic properties. Endotoxins can cause acute toxic effects, fever, malaise and decreased pulmonary function (Rylander & Jacobs, 1994).

In the present study, the highest degree of airborne actinomycetes pollution was detected in the location with high amounts of dust because they are a group of soil bacteria and are commonly found in fodder, soil and agricultural areas. Actinomycetes are important air biocontaminants in agricultural and waste composting plants (Lacey, 1989). Their presence in the indoor environment is an indicator of contamination (ACGIH, 1989). Swan and Crook (1998) found actinomycetes in the range of 0–1.2×10^3 CFU m^{-3} during grain handling, which corresponds with the results in the present study. Actinomycete spores are an important risk factor for adverse respiratory health effects because their aerodynamic diameter ranges from 0.7–1.5 μm, which may penetrate deep into the lungs causing allergic alveolitis (Lacey & Crook, 1988) and stimulate lung macrophage reactions, which could lead to inflammation and tissue injury (Hirvonen, Nevalainen, Makkonen, Mönkkönen, & Savolainen, 1997).

Flour milling processes add different allergenic and toxigenic fungal types into the air. A rich diversity of *Aspergillus* species with varying incidence and distribution were found in the flourmill workplace, *A. flavus* and *A. niger* were majority species at all locations. *Aspergillus* is widespread in the environment where they may colonize grains, leaves and soil. The predominance of *Aspergillus* and *Penicillium* is in agreement with Pandit, Singh and Singh (1995) who reported that *Aspergillus*, *Penicillium*, *Cladosporium* and smut were the dominant species in bakeries. Lugauskas, Krikstaponis, & Sveistyte (2004) found a variety of fungal types including: *Cladosporium*, *Alternaria*, *Geotrichum*, *Oidiodendron*, *Rhizopus*, *Torula*, *Spegazzinia*, *Acremonium* and *Cochliobolus* in grain mills. Additionally, *Alternaria* and *Fusarium* were dominant in the freshly harvested grain but not the stored ones (Abdel Hameed, 2005).

Alternaria and *Fusarium* have been shown to be the dominant fungal organisms recovered with grain in the field (Hocking, 2003). However in the different flourmill locations evaluated in the present study *Aspergillus*, *Penicillium* and *Eurotium* were the dominant recovered fungal organisms. This corresponds with Lacey (1980) who reported that, if the water content of the grain is higher, spores of storage fungi *Aspergillus*, *Penicillium* and *Eurotium* species may germinate and grow. The dominance of *Aspergillus candidus* and *A. flavus* are an indication of inadequate storage and high water content of grains. The presence of *Stachybotrys* and *Eurotium* in the double roller and purifiers is an indication of dampness and high moisture content.

The workers in a flourmill may be exposed to mycotoxin-producing species such as *Aspergillus flavus*, A. *ochraceus* and *A. versicolor*. Therefore workers are at high risk of inhalation of the spores and fungal fragments. Krysinska-Traczyk (1992) reported that the inhalation of large counts of *Aspergillus candidus* spores caused the organic dust toxic syndrome. Moreover, *Aspergillus candidus*, *A. fumigatus*, *A. flavus*, *A. terreus*, *A. parasiticus*, *A. clavatus*, *Eurotium*, *Penicillium* and *Trichoderma* have been identified as a cause of allergic alveolitis (Lacey & Dutkiewicz, 1994).

Aerodynamic diameter is a critical factor for evaluating respiratory exposure. The size distribution causes difference in the airborne behavior of fungal particles. Allergic rhinitis and asthma can result from exposure to relatively large (>5 μm) fungal spores such as *Alternaria* species deposited in nasal or thoracic regions of the respiratory system. Allergic alveolitis requires smaller fungal spores (<5 μm) such as *Aspergillus* and *Pernicillium* species that can penetrate deep into the alveoli (Burge, 1989; Lacey & Crook, 1988). Fungal spores smaller than 5 μm in diameter can penetrate into the

lungs so the likelihood of particle penetration into the workers lungs is high in the flourmill environment.

5 Conclusion

The flourmill is a potential source of organic dust. Data on bioaerosols allow the identification of the critical sites where workers are exposed in the flourmill. Suspended dust exceeded the OEL at all flourmill locations. The storage and double roller units were the worst locations for occupational exposure. Deposited dust contained high microbial levels that could be resuspended into the air adding another human health risk. Allergenic and toxigenic airborne fungi and bacteria were found in the workplace environment. Flourmill workers are consistently exposed to elevated levels and types of bioaerosols that may trigger respiratory disorders. Further health evaluations should be carried out and suitable countermeasures taken to avoid excess biocontamination.

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