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Assessing Exposures to Compost Workers from Airborne Biohazards

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Assessing exposures to compost workers from airborne biohazards

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Main Research Findings

- Workers at composting facilities are exposed to airborne, microscopic particulate of biologic origin.
- The compost facility located in the interior of BC in a desert climate (Site 2) had the highest concentration of dust (GM 2.69 mg/m^3) and endotoxin (GM 502 EU/m^3).
- The compost facility that only processed biosolid feedstock (Site 1) had the highest maximum endotoxin exposure (62,140 EU/m^3).
- Exposures to dust and endotoxin were on average 10-fold reduced inside air-conditioned cabs on front end loaders.
- Personal sample concentrations for dust and endotoxin were intermediate in mass between the 10-fold reduction inside the cab and the concentration outside the cab which indicates workers do not spend their entire working day inside their cabs, or windows were open in cabs.
- The job task associated with the highest exposures to *Aspergillus fumigatus* was blending biosolid with wood chips inside a shed at Site 1.
- This research clearly identified the benefits of the engineering control afforded by enclosed cabs on equipment.
- Workers should receive training and education regarding the importance of maintaining air filters and closing windows and doors on air-conditioned cabs.
- Some tasks may require that workers wear additional respiratory protection in cases where dilution ventilation is insufficient to remove particulate matter from the air.

Executive Summary

The objective of this research was to measure exposures to workers in the municipal composting industry from selected airborne biohazards. Two municipal-scale composting methods were compared for exposures to endotoxin (i.e. lipopolysaccharide, a component of gram negative bacterial cell walls), (1-3) β D glucan (a polyglucose structural component of fungal spore walls), *Aspergillus fumigatus* (a thermotolerant fungus) and Thermoactinomycetes (a group of spore-forming thermophilic bacteria).

Composting methods examined were: (1) static aerated piles or (2) open windrow pile turned either by (a) high technology equipment (straddle turner), or (b) low technology equipment (front end loader).

The participating composting sites were: Site 1 (approximately 6 hour drive from Vancouver, located in the Okanagan Valley) which used static pile (positive and negative) technology. Site 2 (approximately 5 hour drive from Vancouver, located west of the Okanagan Valley) used open windrow composting and has both high tech and low tech turning equipment. Site 3 used open windrow composting turned by front end loaders.

The feedstock for Site 1 was dewatered sewage sludge (biosolids) mixed with wood chips. Site 2 feedstock was primarily yard waste and had recently added a smaller capacity for biosolids composting. Site 3 exclusively composted yard waste (leaves, lawn clippings and garden waste).

Approximately 5 – 10 workers were at each site, including full time, part time and seasonal employees. Job tasks at the composting facilities included blending the feed-stocks, building the piles, turning the piles (windrow only), moving the pile after the thermophilic phase (prior to the curing phase), and screening the final product. These tasks aerosolized particulate matter that contained Gram negative bacterial components (endotoxin), fungal spores (1-3) β D glucan), *Aspergillus fumigatus* (fungal) and Thermoactinomycetes (bacterial) spores.

The study examined environmental effects of season and weather including temperature, relative humidity, and precipitation on the release of bioaerosols.

Research methodology:

Exposures to dust, endotoxin and glucan were determined by full shift sampling using a battery operated pump and a 7-hole sampling head to capture airborne particulate matter ≤ 100 μm aerodynamic diameter which could be inhaled. Viable samples for *A. fumigatus* and Thermoactinomyces were collected near different job tasks using an Andersen six-stage microbial sampler.

Results:

Two different composting techniques were examined, each of which was associated with a geographic microclimate which also contributed to the outcomes. In general, the windrow composting facility located at Site 2 had the highest exposures to dust, endotoxin and glucan (GM 2.69 mg/m^3; 502 EU/m^3; and 128 μg/m^3 respectively).

However, for all sites studied, samples of dust taken inside equipment cabs were as much as 10-fold reduced in concentration than those taken outside the cab. Personal samples were intermediate in concentration, as workers did not spend their entire shift only in the cab. Exposures were greater during warm weather (spring and summer seasons).

Task analysis could not be done for full shift samples, as most workers performed numerous tasks throughout the day, or were adjacent to multiple task areas. In contrast, the fungal and bacterial short term grab samples were well suited to task analysis. The highest geometric mean concentration of *Aspergillus fumigatus* was associated with the tasks of "blending". Blending was accomplished in a shed, where biosolid slurry was mixed with wood chips using a front end loader. This task was only done at the facility located at Site 1. The highest geometric concentration of Thermoactinomycetes was associated with grinding feedstock, and at the facility located at Site 3 which only composted yard waste.

It can be concluded that working inside the cab of equipment at composting facilities significantly reduces exposure to bioaerosols. Additional controls may be necessary to reduce exposures to micro-organisms at facilities where tasks are done in spaces sheltered from dilution ventilation.

Research Context:

Rationale and significance:

All Canadian provinces, and most Canadian cities are investigating or have introduced composting as an adjunct to landfilling for solid waste disposal (Composting Council of Canada). Indeed, the Province of British Columbia has a target of achieving a 50% reduction of waste entering landfill sites (Recycling Council of British Columbia). The 50% reduction target was to have been implemented by the year 2000; this target has not been met, but the goal remains. Diversion of organic material from landfills and composting are generally acknowledged as viable options to meet solid waste reduction goals (Recycling Council of Canada). Although composting has wide public appeal, and produces marketable product from organic wastes, insufficient attention has been focused on the exposures to airborne biohazards to workers.

Compost may be processed using different methods. Two commonly employed methods used in British Columbia are static aerated piles and open windrow piles. Each composting method has variations which may impact bioaerosol exposure to workers. Static aerated piles may be built to have air drawn through the pile (negative pressure from above) or forced through the pile (positive pressure from below). Open windrows may be turned using high technology straddle-type turners, or may be turned using lower, but more affordable technology, i.e., a front end loader.

The composting industry is growing. In 1994 it was estimated there were 100 facilities across Canada composting 300,000 metric tons of organic materials annually. In 1999 there were over 300 public and private sector composting operations processing over 1,650,000 metric tons and producing over 800,000 metric tons of compost for use

(Composting Council of Canada). In 1998 there were 46 centralized composting facilities in British Columbia. The number of facilities is expected to increase (by comparison, in the same census, Alberta had 84 facilities, followed closely by Ontario with 71) (Composting Council of Canada).

The number of workers employed by the industry will continue to increase; however, the technology is not standardized, nor is the technology chosen regarding concerns for worker health and safety. In British Columbia we had the unique opportunity to study exposures to workers performing tasks specific to at least two different composting technologies. In addition, the efficacy of potential engineering controls could be assessed which are specific to equipment (e.g. enclosed cab vs. no cab) used for the different processes and variations within the processes.

Introduction to the composting industry in British Columbia

Compost may be made of any organic material, but commonly used feed-stocks are green waste from plant materials, or biosolids (dewatered sludge from sewage treatment plants), fish and food waste, animal manure, and animal carcasses (Organic Matter Recycling Regulation, 2002). During municipal-scale composting the piles of organic materials self heat, and internal temperatures potentially reach 55 - 75^{o}C, which is sufficiently high to kill pathogenic micro-organisms associated with the feed-stock (Hassen et al. 2001). The phase during which pile temperatures exceed 45^{o}C is termed the *thermophilic phase*. Eventually the organic substrate becomes limiting and microbial action slows, temperatures drop, and the pile is considered to be in the curing phase. Compost is considered to be sanitized when the pile has met the time and temperature

criteria for static aerated (≥ 55°C for a minimum of 3 days), or windrow (≥ 55°C for a minimum of 15 days) (Organic Matter Recycling Regulation 2002). The difference in time is reflective of the different processes used. The static aerated pile is constructed over pipes that blow (positive pressure) or draw in (negative pressure) air, while the open windrow pile is turned on a regular basis, with the cooler edges being turned into the centre of the pile. In both processes oxygen is introduced into the pile to encourage optimal growth of the thermophilic microflora which participate in the breakdown of the organic materials. Environmental and other conditions influence how long an individual pile will require to meet the sanitization criteria. In some areas of interior British Columbia, open windows are subject to winter snow, while in coastal British Columbia rain may prolong the time the pile must remain in place before finishing.

There are a number of tasks which may result in the aerosolization of biohazards from the raw feed-stock (e.g. sewage sludge), or from the composting process itself which depends on microbial action to break down the complex organics into soil. Finished compost is considered to be safe from pathogenic organisms found in raw sewage sludge because the internal temperature of the pile (> 55 °C) effectively pasteurizes viruses, protozoans, helminth ova and bacterial cells. However, the thermotolerant micro-organisms that participate in the composting process, which are selected for by their ability to function at temperatures > 45°C, are present in high concentrations and can be associated with diseases resulting from pulmonary exposures. Common examples of thermotolerant microbes associated with composting are *Aspergillus fumigatus*, and *Aspergillus niger* (fungi), and Thermoactinomycetes and *Micropolyspora* (spore-forming bacteria). In addition to living organisms, cell wall

constituents of heat-killed organisms present in the feed-stock remain in the organic dust (e.g. bacterial endotoxins and (1-3) β D-glucans).(Douwes et al. 2003; Rylander. and Jacobs 1997; Rylander 1998; .Douwes and Heederik 1997).

Health effects cased by inhalation exposure to micro-organisms may be due to infectious disease caused by living, pathogenic organisms (Kramer et al. 1989; Vincken and Roels 1984; Pepys and Simon 1973; Shen et al. 1991), or neoplastic disease caused by carcinogenic mycotoxins (Sorenson et al. 1984). In the literature, the most extensively reported health outcomes to organic dust exposure have been due to host responses, resulting in temporary flu-like symptoms (organic dust toxic syndrome) (Pepys and Simon 1973) or chronic lung damage (hypersensitivity pneumonitis) (Pepys and Simon 1973; Weber et al. 1993; Brown et al. 1995).

Non-allergic respiratory effects are caused by the release of pro-inflammatory cytokines by alveolar macrophages challenged with endotoxin (lipopolysaccharide) a constituent of Gram-negative bacterial cell walls (Lundholm and Rylander 1980; Pernis et al. 1961; Michel et al. 1997; Jagielo et al. 1996). A constituent of fungal spore walls, (1-3) β D-glucan, has been shown to act synergistically with endotoxin in the lungs (Fogelmark et al. 1994; Milanowski 1997).

Exposures to biologic agents have been reported in a variety of composting settings. Marsh et al. reviewed literature reporting exposures to *Aspergillus fumigatus* in sludge composting, but included other natural substrates held at temperatures between 40-50^{o}C, including grain silos, boiler rooms, sauna baths, and the cooling canals for nuclear power generators (March et al. 1979). Rautiala et al. reported high concentrations of thermotolerant fungi and thermophilic actinobacteria (up to 10^5 CFU/m^3) in swine

confinement buildings where the composting system was functioning properly (Rautiala et al. 2003). Weber et al. reported exposures to endotoxin ranging from 650 – 16,000 endotoxin units (EU)/m^3 associated with hand loading of compost (Weber et al. 1993). Douwes et al. found endotoxin exposures to be related to job tasks and the production of inflammatory markers (Douwes, et al. 2000).

Objectives:

1) To measure airborne exposures to selected biohazards in three BC compost facilities.
    a. Biohazards: Endotoxin, (1-3) β D-glucan, *Aspergillus fumigatus*, Thermophilic spore forming bacteria
    b. Composting sites: Site 1 (static aerated pile), Site 2 (open windrow) and Site 3 (open windrow)
2) To record potential determinants related to airborne concentrations of selected biohazards.
    a. Feed-stocks (e.g. sewage sludge, green waste)
    b. Work tasks (e.g. grinding feed-stock, building pile, turning piles, screening)
    c. Environmental conditions (e.g. wind speed, wind direction, temperature, relative humidity, precipitation)
    d. Controls (e.g. enclosed cabs of machinery, ventilation in sheds housing static aerated piles, or use of personal protective equipment)

Research Design:

Airborne exposures to workers at three composting facilities in British Columbia were studied. Table 1 lists the locations and compost methods.

Table 1. Location and technology of composting for three BC sites.

Location	Composting technology	Feed-stock
Site 1 *(Okanagan Valley, hot dry summer, cold dry winter)*	Static aerated (positive pressure) Static aerated (negative pressure)	Sewage sludge mixed with wood chips
Site 2 *(Thompson River Valley, hot dry summer, winter snow)*	Open windrow (straddle turner) Open windrow (front end loader)	Sewage sludge mixed with wood chips
Site 3 *(Pacific coast, mild summer, mild wet winter)*	Open windrow (front end loader)	Plant and garden waste

Table 2 lists the measurements taken for biohazardous agents.

Table 2. Biohazardous agends associated with composting processes.

Agent	Source	Measurement period	Method
Endotoxin	Gram-negative bacteria	Full work-shift	Gravimetric, non-viable
β D- glucan	Fungal spores	Full work-shift	Gravimetric, non-viable
Aspergillus fumigatus	Thermotolerant fungal species	Grab sample for 10 minutes per sample	Culturable colony forming units
Thermophilic bacteria	Thermophilic bacterial species	Grab sample for 10 minutes per sample	Culturable colony forming units

Endotoxin and β D-glucan were measured inside and outside equipment cabs (where enclosed cabs were used). Grab samples for *A. fumigatus* and thermophilic bacteria were taken upwind of the compost facility (baseline), during the work day near locations where specific job tasks were performed, and downwind of the compost facility. Compost piles were built as feed-stock came to the facility, therefore, all stages of the composting procedure were monitored from blending the feed-stock (including grinding), building the pile, turning the windrow piles (not applicable to static aerated), moving the pile for curing, and screening the finished product.

The number of workers at each site averaged between 5 and 10 workers depending on the season and requirement for moving materials. Workers were invited to participate by wearing sampling pumps for a true personal exposure. However, in the event the worker declined to wear a pump, an area sample was taken close to the worker. For example, if the worker was driving a front end loader equipped with a cab, one pump and cassette was fixed to the inside of the cab, while a second pump and cassette was fixed to the outside of the cab. It was noted whether the cab was air-conditioned or had open windows.

The effect of weather is related to the microclimate of the site, and was recorded using a portable, wireless meteorology station at each site.

Methods:

Endotoxin (sampling): Inhalable particulate was captured on 25 mm glass fibre filters that were baked (depyrogenated) to render the filters free of contaminating endotoxin. Seven-hole sampling cassettes were attached to SKC battery operated

personal sampling pumps calibrated to 2 litres per minute (lpm) flow rate (Douwes et al. 1995). Filters were shipped on cold packs to the Bioaerosol Exposure Laboratory at the University of British Columbia (UBC) for analysis. Samples were stored at 4 ^{o}C with desiccant until the analysis was performed.

Endotoxin (analysis): Endotoxin was extracted from filters into pyrogen-free water containing 0.05% Tween-20. The extracted sample was assayed using a kinetic, chromogenic *Limulus* amebocyte lysate test (Lonza Walkersville Kinetic-QCL). The rate of release of the chromogen (V_{max}) is correlated with the concentration of endotoxin in the sample. Dilutions of standard endotoxin (*E. coli* O55:B5) were used for the standard curve and the R^2 value determined by a four parameter fit (Thorne 2000).

β D-glucan (sampling): The same filters used to capture dust particulate for endotoxin were used for glucan particulate matter.

β D-glucan (analysis): Glucan was extracted from the filters by denaturing the molecule by autoclaving (121^{o}C) the filters for one hour. Extracts were stored at – 80^{o}C prior to sending them to the laboratory of Dr. Peter Thorne, Institute of Rural and Environmental Health, University of Iowa. Extracted glucan was quantified by end-point, enzyme linked immunosorbant assay (ELISA) (Blanc et al. 2005; Phipatanakul et al. 2000a; Phipatanakul et al. 2000b; Pollart et al..1991).

***Aspergillus fumigatus* (sampling)**: A six-stage Andersen biosampler was attached to a battery operated Air-Con pump calibrated to 28.3 lpm. Malt extract agar (MEA) plates were placed in each of the stages (total of six plates per sample).

***Aspergillus fumigatus* (analysis)**: Sample plates were either returned to UBC (Site 3 samples) or taken to the microbiology laboratory at Thompson Rivers University

(Sites 1 and 2 samples) for incubation at 45°C for 48 hr. Colonies were counted and identified by microscopic morphology.

Thermophilic bacteria (sampling and analysis): Samples were taken as described for *Aspergillus fumigatus* with the following exceptions: (1) tryptic soy agar (TSA) plates were used for the capture and growth media; (2) incubation temperature was 55°C for 48 hr. Colonies were counted and identified using standard techniques.

Data Analysis:

Data were analyzed using SPSS (version 15 for Windows) statistical software. Units of concentration were dust mass (mg/m^3), endotoxin units (EU)/m^3, micrograms glucan/m^3, or colony forming units (CFU)/m^3 *A. fumigatus* or CFU/m^3 thermophilic spore forming bacteria. Descriptive statistics (counts for categorical data, and means, ranges, standard deviations, and frequency distributions for continuous data) were calculated for all variables. The distributions of the exposure variables were examined for log-normality, and log-transformed (base e) prior to analysis if necessary for parametric statistical tests.

Results and Discussion:

Dust and components of organic dust:

Full shift samples for dust, endotoxin and (1-3) β D-glucan for the three composting facilities are reported in Tables 3 - 4.

Table 3. Inhalable dust by location, sample, and season at three composting facilities in British Columbia.

Dust (mg/m^3)								
Location/ condition	**n**	**AM[a]**	**SD[b]**	**GM[c]**	**GSD[d]**	**Min.**	**Max.**	**GM p-value**
Site 1	179	2.82	3.76	1.23	4.03	0.01	25.21	
Site 2	87	5.42	7.52	2.69	3.39	0.21	41.56	< 0.001
Site 3	178	2.58	5.08	0.61	5.23	0.02	28.39	
Inside cab	152	0.51	0.55	0.34	2.47	0.03	4.11	<0.001
Outside cab	214	5.30	6.35	2.51	4.18	0.02	41.56	
Personal	78	2.87	4.79	1.01	4.58	0.01	28.29	
Winter	58	0.79	1.13	0.35	3.61	0.02	4.62	<0.001
Spring	166	3.74	5.39	1.43	4.57	0.05	36.16	
Summer	170	3.88	5.60	1.45	4.42	0.01	28.29	
Autumn	50	2.15	6.04	0.56	4.89	0.03	41.56	

[a] arithmetic mean, [b]standard deviation, [c] geometric mean, [d] geometric standard deviation

Dust samples recovered from Site 2 facility were significantly higher than either Sites 1 or 3. This is due in part to the dry climate and to the use of the high energy windrow turner. At all sites the "inside cab" dust concentrations were significantly less than either "outside cab" or "personal" samples. The personal samples include exposures to workers when they are performing duties outside the equipment cab. Samples collected in the winter and autumn were significantly lower in concentration than those taken in spring or summer.

Table 4. Inhalable endotoxin by location, sample, and season at three composting facilities in BC.

Endotoxin (EU/m^3)								
Location/ condition	**n**	**AM[a]**	**SD[b]**	**GM[c]**	**GSD[d]**	**Min.**	**Max.**	**GM p-value**
Site 1	179	1983	5607	316	8.6	0.3	62140	
Site 2	87	1244	2367	502	3.7	36	18223	< 0.001
Site 3	178	804	2147	97	8.2	1.5	17805	
Inside cab	152	113	155	52	3.9	1.5	1137	<0.001
Outside cab	214	2560	5458	689	7.1	0.3	62140	
Personal	78	528	964	144	6.1	0.6	5838	
Winter	58	260	891	47	5.9	1.8	6631	<0.001
Spring	166	1442	5138	305	6.2	3.5	62140	
Summer	170	1846	3563	368	7.4	0.6	30762	
Autumn	50	761	2649	66	10.6	0.3	18223	

[a] arithmetic mean, [b]standard deviation, [c] geometric mean, [d] geometric standard deviation

The origin of endotoxin is the cell wall membrane of Gram-negative bacteria. The bioactive component of this compound is the lipid A moiety. Microscopic pieces of bacterial cell walls are attached to and become airborne with dust particles. In this study, the facility using the high energy windrow turner (Site 2) had the highest geometric mean exposure to endotoxin, but a significant reduction of exposure was found inside the cab. Endotoxin exposures were significantly lower in winter and autumn.

Table 5. Inhalable glucan by location, sample, and season at three composting facilities in BC.

β Glucan (μg/m^3)								
Location/ condition	**n**	**AM[a]**	**SD[b]**	**GM[c]**	**GSD[d]**	**Min.**	**Max.**	**GM p-value**
Site 1	179	131	245	30	7.7	< LOD[e]	2406	
Site 2	87	460	890	128	5.6	2.5	4574	< 0.001
Site 3	177	236	515	29	9.4	< LOD	3118	
Inside cab	151	28	56	11	3.7	0.3	335	< 0.001
Outside cab	214	388	648	117	7.0	< LOD	4233	
Personal	78	229	629	24	11.6	< LOD	4574	
Winter	58	53	118	11	6.9	0.2	683	
Spring	166	345	708	79	6.3	2.0	4574	< 0.001
Summer	169	240	487	43	8.6	< LOD	3118	
Autumn	50	86	264	13	9.2	< LOD	1822	
Cold season	115	73	195	14	8.1	< LOD	1823	<0.001
Hot season	328	295	614	57	7.7	< LOD	4574	

[a] arithmetic mean, [b]standard deviation, [c] geometric mean, [d] geometric standard deviation
[e] Limit of detection = 10 ng/mL

The highest exposures to inhalable glucan were at site 2. Exposure was significantly reduced inside cabs. The lowest exposures to β glucan were in the winter and autumn, or cold season.

Figure 1 illustrates GM dust concentration comparison between the three study sites. Figure 2 graphs the GM dust concentration by location within each site. Large variations can be seen between the personal samples for sites 1 and 2, while only inside and outside cab samples were available for site 3. Figure 3 illustrates GM endotoxin concentration and Figure 4 endotoxin concentration by location. Figure 5 illustrates GM glucan concentration and Figure 6 glucan concentration by location. Variation in personal samples are related to the amount of time spent outside the cab and whether the window was consistently closed during the shift.

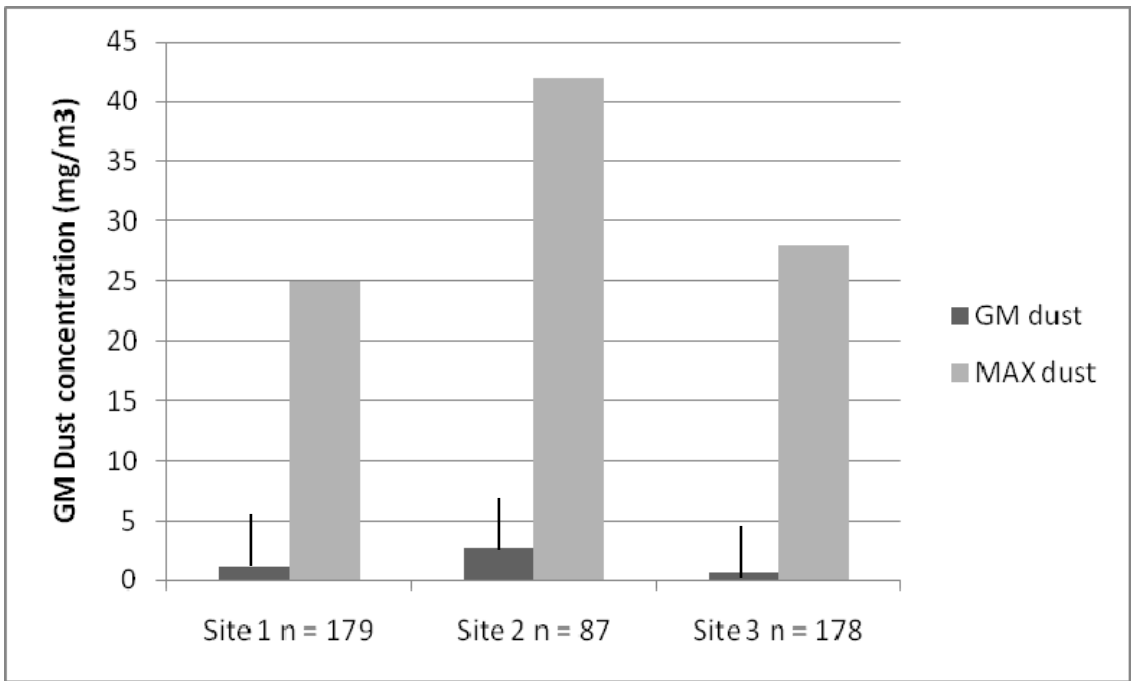

Figure 1. Comparison of geometric mean dust concentrations (mg/m^3) with maximum dust value for each site.

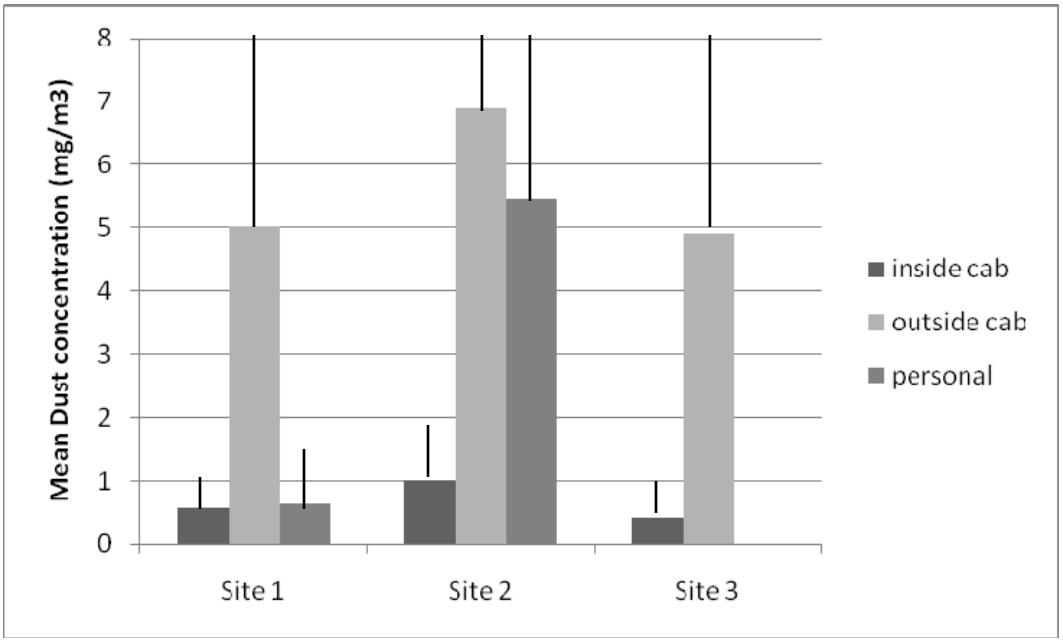

Figure 2. Comparison of AM dust concentration from area samples inside or outside cab, or full shift personal sample. Only inside and outside area samples were taken at site 3.

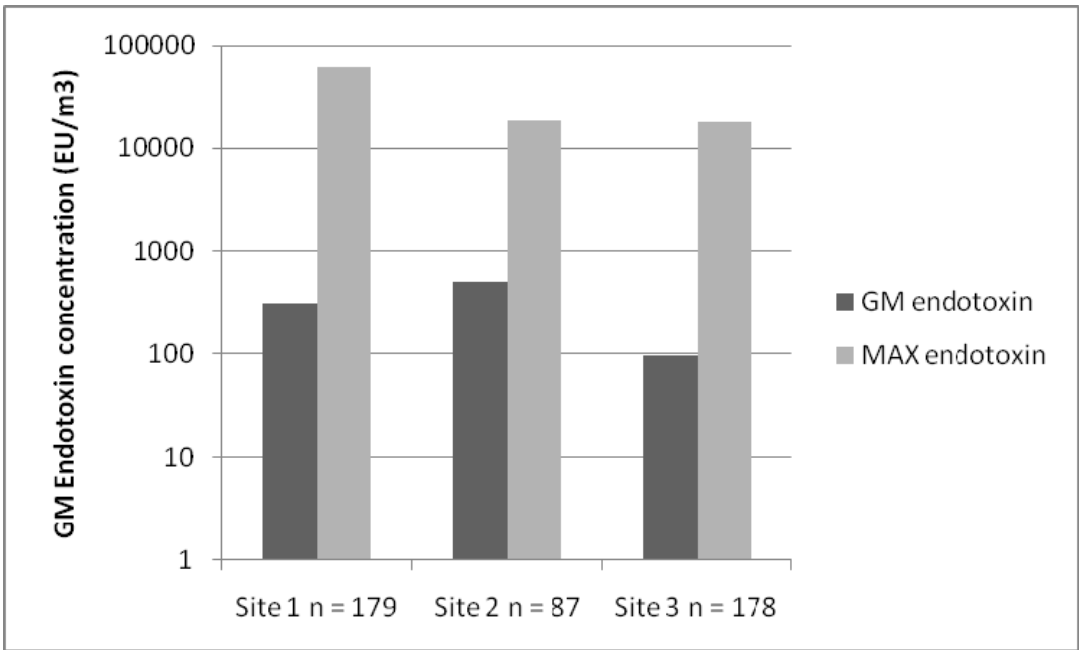

Figure 3. Comparison of geometric mean endotoxin concentration (EU/m^3) with maximum endotoxin value for each site.

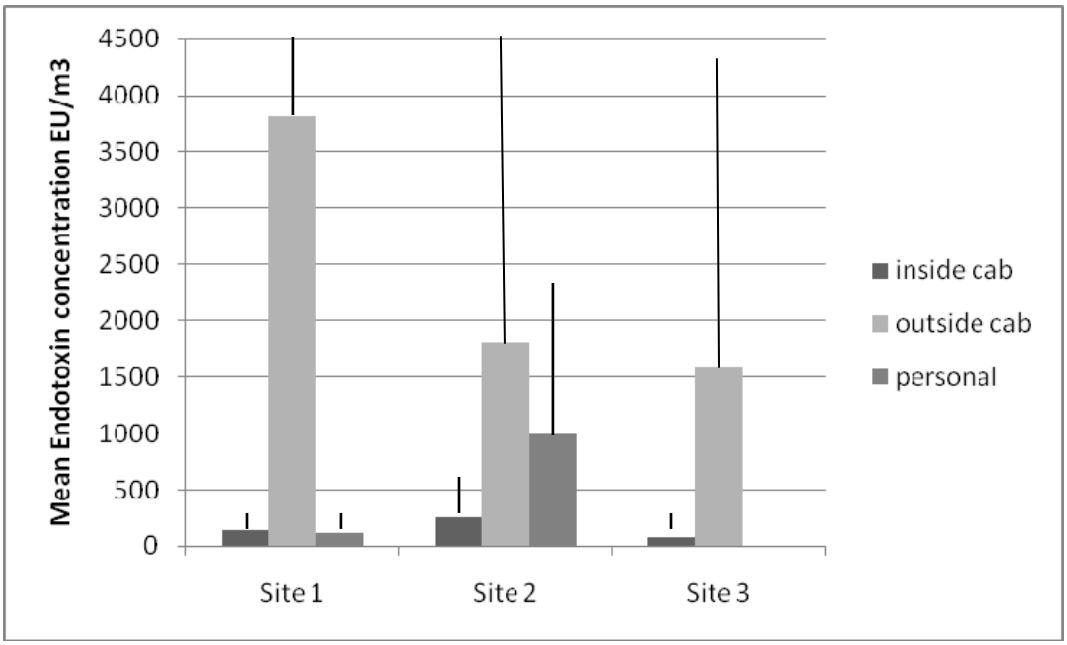

Figure 4. Comparison of AM endotoxin concentration from area samples inside or outside cab, or full shift personal sample. Only inside and outside area samples were taken at site 3.

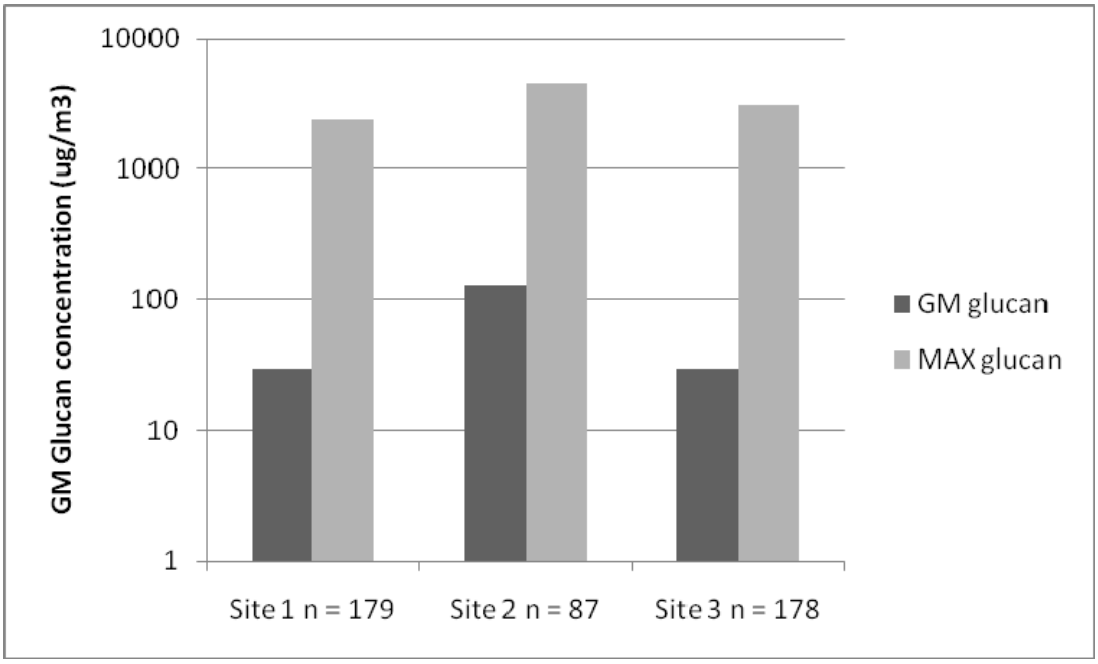

Figure 5. Comparison of geometric mean glucan concentration (µg/m^3) with maximum value for each site.

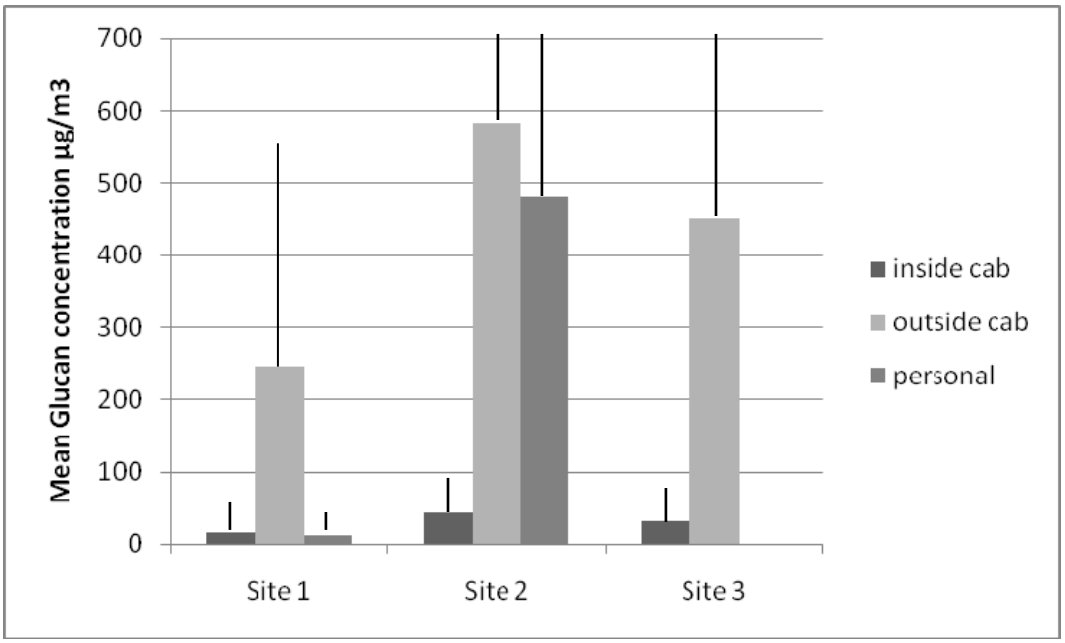

Figure 6. Comparison of AM glucan concentration from area samples inside or outside cab, or full shift personal sample. Only inside and outside area samples were taken at site 3.

Airborne fungi and bacteria

Results for the fungal bioaerosol *Aspergillus fumigatus* are reported in Tables 6 and 7.

Table 6. Airborne concentrations of *Aspergillus fumigatus* by location and associated task.

***Aspergillus fumigatus* (CFU/m^3)**								
Location/ condition	**n**	**AM[a]**	**SD[b]**	**GM[c]**	**GSD[d]**	**Min.[e]**	**Max[f].**	**GM p-value**
Site 1	233	3216	10914	153	16	< LOD	101193	
Site 2	164	1037	3033	96	10	< LOD	20786	0.003
Site 3	267	3865	12019	234	14	< LOD	111435	
At site	422	4237	12425	357	12	< LOD	111435	<0.001
Downwind	121	1223	3349	107	11	< LOD	20974	
Upwind	121	127	521	16	6	< LOD	5028	
Winter	105	3169	14792	42	14	< LOD	111435	<0.001
Spring	238	3193	9904	234	14	< LOD	101193	
Summer	231	3087	9298	217	14	< LOD	79596	
Autumn	90	1618	5110	138	9	< LOD	37429	
Tasks								
Grinding	66	4415	8534	580	11	< LOD	37900	<0.001
Building	68	1495	3668	219	8	< LOD	20655	
Blending	25	20169	27664	7029	6	39	101193	
Turning	33	9726	23741	799	15	< LOD	111435	
Moving	23	2088	4588	198	14	< LOD	18179	
Cured pile	20	1888	4626	148	12	< LOD	18710	
Screening	107	3525	10170	475	9	< LOD	92912	
Feedstock	8	310	556	98	5	14	1640	
"other"	67	1542	5910	104	11	< LOD	46282	

[a] arithmetic mean, [b]standard deviation, [c] geometric mean, [d] geometric standard deviation
[e] LOD = limit of detection = no fungal colonies on any plate.
[f] Upper limit of detection = maximum number of colonies for this sampler = 111435 CFU/m^3

Aspergillus fumigatus is a thermotolerent fungi which is amplified by the unique niche provided by the elevated temperatures present in the composting organic material. The maximum number of colonies which can be counted using an Andersen six-stage

sampler is based on colonial growth under all 400 jet impaction sites, and is calculated as 111435 CFU/m^3. The *Aspergillus* counts reported here are viable counts, and are underestimates of the actual spores present in air, as not all spores will germinate and grow on laboratory culture media. However, using this method allows definitive identification of the colonies as *A. fumigatus*. Highest overall concentrations were found at site 3, during the spring sampling season. "Blending" the feedstock (mixing feedstock with woodchips) was the task associated with the highest exposure to *A. fumigatus*.

Table 7. Airborne concentrations of *Aspergillus fumigatus* by particle size distribution.

Location/ condition	**n**	**% of particles ≥ 3 µm**	**% of particles ≤ 3 µm**
Site 1	233	48	52
Site 2	164	24	76
Site 3	267	34	66
At site	422	39	61
Downwind	121	29	71
Upwind	121	33	67
Winter	5	45	55
Spring	83	35	65
Summer	53	39	61
Autumn	21	45	55
Tasks			
Grinding	66	30	70
Building	68	11	89
Blending	25	49	51
Turning	33	40	60
Moving	23	43	57
Cured pile	20	33	67
Screening	107	39	61
Feedstock	8	19	81
"other"	67	48	52

Particles greater than 3 µm aerodynamic diameter are inhaled and deposit in the upper respiratory tract (primary bronchii and higher), while particles less than 3 µm aerodynamic diameter can enter deep into the lungs and alveoli. The split in proportions

for the smallest particles was higher at Site 2, but was not different when considering upwind or downwind samples. Building the piles and moving feedstock had the highest percentages of small particles.

Figure 7 illustrates *A. fumigatus* concentration associated with tasks broken down by site. It can be seen that tasks varied by site (blending only occurred at site 1) and concentrations of *A. fumigatus* were highest during blending (site 1) or turning (site 3).

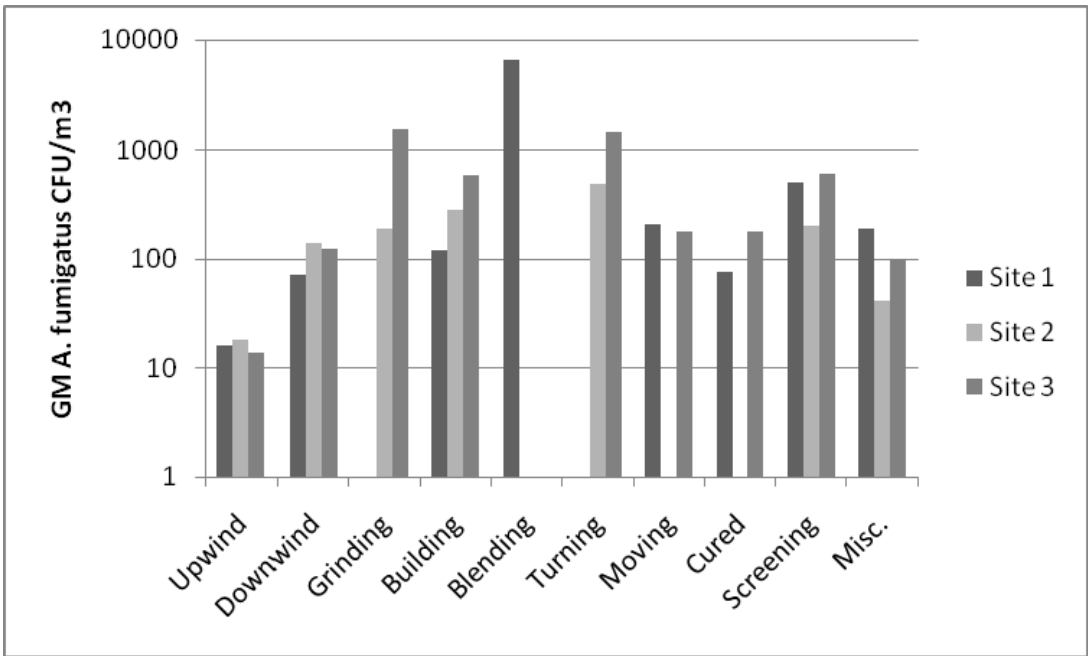

Figure 7. Geometric mean *A. fumigatus* concentrations illustrated by task and site.

Results for the bacterial group, Thermoactinomycetes are reported in Tables 8 and 9.

Table 8. Airborne concentrations of Thermoactinomycetes by location and associated task.

Thermoactinomycetes(CFU/m^3)								
Location/ condition	**n**	**AM[a]**	**SD[b]**	**GM[c]**	**GSD[d]**	**Min.[e]**	**Max.**	**GM p-value**
Site 1	237	202	980	27	7	< LOD	13282	< 0.001
Site 2	164	214	464	52	6	< LOD	3836	
Site 3	266	672	4181	92	6	< LOD	47536	
At site	424	564	3399	75	7	< LOD	47536	<0.001
Downwind	123	132	258	35	6	< LOD	1834	
Upwind	123	57	96	22	5	< LOD	847	
Winter	104	205	1304	31	5	< LOD	13282	0.020
Spring	238	218	416	54	7	< LOD	3836	
Summer	237	739	4445	63	8	< LOD	47536	
Autumn	91	152	258	52	5	< LOD	1834	
Tasks								
Grinding	68	442	564	153	6	< LOD	2323	<0.001
Building	68	155	278	37	7	< LOD	1632	
Blending	27	298	616	73	7	< LOD	2416	
Turning	31	1763	8476	58	10	< LOD	47270	
Moving	23	373	1337	41	8	< LOD	6484	
Cured pile	20	176	294	48	7	< LOD	1190	
Screening	108	1028	4881	111	9	< LOD	47536	
Feedstock	8	303	526	73	6	7	1505	
"other"	66	148	212	62	4	< LOD	1335	

[a] arithmetic mean, [b]standard deviation, [c] geometric mean, [d] geometric standard deviation
[e] LOD = limit of detection = no fungal colonies on any plate.

Thermoactinomycetes describes a group of thermophilic bacteria which produce exospores, and are often found associated with organic material. Highest overall concentrations were found at Site 3 (yard waste only, windrow), and during the summer sampling season. "Grinding" the feedstock (chopping the mixture into smaller, more homogeneous sized pieces) was the task associated with the highest exposure to Thermoactinomycetes.

Table 9. Airborne concentrations of Thermoactinomycetes by particle size distribution.

Location/ condition	n	% of particles ≥ 3 µm	% of particles ≤ 3 µm
Site 1	237	65	35
Site 2	167	60	40
Site 3	266	58	42
At site	422	59	41
Downwind	121	52	48
Upwind	121	72	28
Winter	5	69	31
Spring	83	59	41
Summer	53	59	41
Autumn	21	55	45
Tasks			
Grinding	66	53	47
Building	68	66	34
Blending	25	59	41
Turning	33	58	42
Moving	23	51	49
Cured pile	20	41	59
Screening	107	63	37
Feedstock	8	54	46
"other"	67	60	40

In general, although the spores of Thermoactinomycetes when examined under a microscope are on average smaller than 3 µm in aerodynamic diameter, the proportion of spores impacting higher or lower in the respiratory tract were fairly evenly divided, indicating that spores were often airborne in clumps or associated with particulate matter.

Figure 8 illustrates the relationship of Thermoactinomycetes concentrations with tasks performed at the compost sites. Note that tasks varied between sites, with the task "blending" only associated with site 1, and turning only associated with sites 2 and 3.

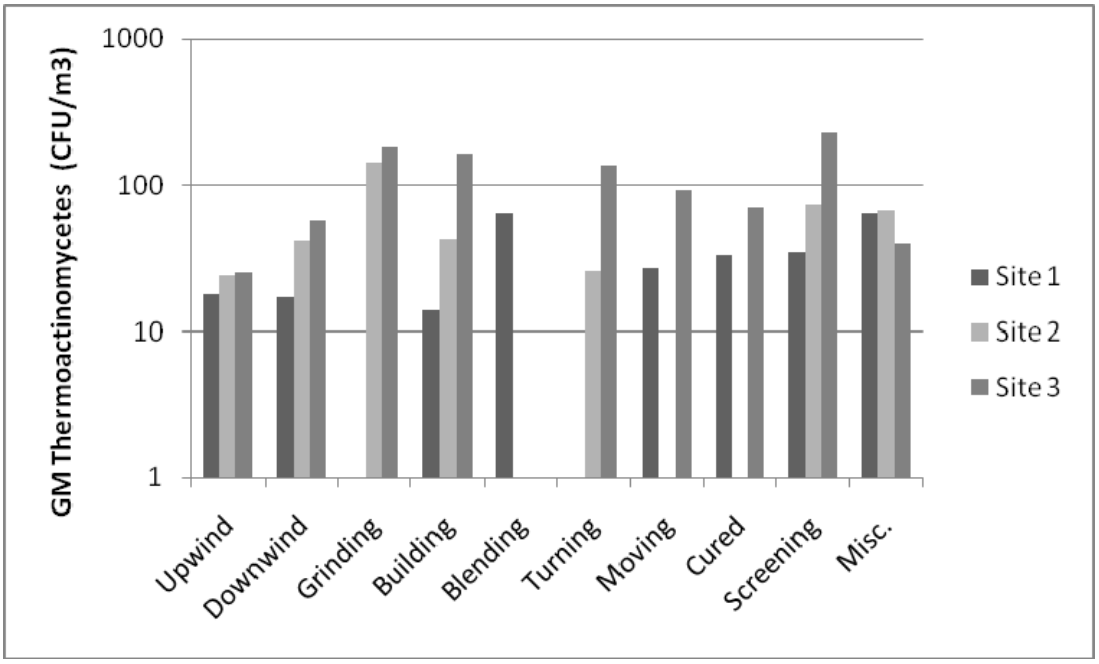

Figure 8. Geometric mean Thermoactinomycetes concentration illustrated by task and broken down by site.

Task and environment analysis

Variables that were *a priori* tested for statistical significance were: Site, Season, Weather, Precipitation, Temperature, % Relative Humidity, Wind Speed, Wind Direction, Rain on sampling day, Rain within the last week, and Task. Usable variables for the grab samples (*A. fumigatus* and Thermoactinomycetes) were: Site (upwind, downwind & at site) and Task. The Usable variables for the full shift samples (dust, endotoxin and glucan) were: Sample type (area, personal), Placement of filter (inside or outside cab) and Type of equipment (front end loader, high energy windrow turner).

The three sites chosen for the study represented three different composting technologies. Site 1 composted only biosolid waste in static piles. Site 3 used front end

loaders and only processed yard waste. Site 2 used both front end loaders to move material and high energy windrow turners to turn piles. During the first sampling season Site 2 only processed yard waste, but during the second season also began biosolids composting.

Three models are presented for the outcomes for the log transformed (base e) variables, ln-dust, ln-endotoxin, and ln-glucan concentration. Tables 10 - 12 report multiple linear regression models to examine factors which contribute to or reduce exposures to dust, endotoxin, or glucan.

Table 10. Determinants of exposure to ln dust mg/m^3.

Variables	Unstandardized		Standardized	t stat	Significance
	B	Std. error	β		
Constant	- 0.507	0.249		-2.035	0.042
City					
Site 3	- 1.082	0.172	-0.340	- 6.293	0.000
Site 1	- 0.712	0.181	- 0.226	- 3.939	0.000
Outside cab	2.811	0.275	0.904	10.231	0.000
Front end loader	0.643	0.159	0.148	4.051	0.000
Enclosed cab	- 0.936	0.203	- 0.421	- 4.613	0.000
Season					
Spring	0.945	0.176	0.294	5.374	0.000
Summer	1.012	0.181	0.319	5.593	0.000
Autumn	0.160	0.235	0.029	0.679	0.498
Rain previous 7 days	- 0.011	0.005	- 0.085	- 2.340	0.020
Final model R^2 = 0.512					

Factors which increase exposure to dust are being outside the cab during a shift, using a front end loader, during the seasons “Spring” and “Summer” and being at Site 2. Factors decreasing exposure are being in an enclosed cab, and rain within the last 7 days. These variables explain approximately 51% of the variation in dust measurements.

Table 11. Determinants of exposure to ln endotoxin EU/m^3.

Variables	Unstandardized		Standardized	t stat	Significance
	B	Std. error	β		
Constant	4.494	0.331		13.574	0.000
City					
Site 3	- 0.966	0.228	-0.228	- 4.230	0.000
Site 1	- 0.217	0.240	- 0.052	- 0.903	0.367
Outside cab	3.806	0.365	0.920	10.429	0.000
Front end loader	0.437	0.211	0.076	2.075	0.039
Enclosed cab	- 1.257	0.270	- 0.424	- 4.663	0.000
Season					
Spring	1.281	0.234	0.299	5.481	0.000
Summer	1.408	0.240	0.334	5.860	0.000
Autumn	- 0.098	0.313	- 0.014	- 0.312	0.755
Rain previous 7 days	- 0.020	0.006	- 0.119	- 3.294	0.001
Final model R^2 = 0.603					

Similarly, 60% of the variation in endotoxin measurements are explained by the same variables as were significant for dust exposure.

Table 12. Determinants of exposure to ln glucan μg/m^3.

Variables	Unstandardized		Standardized	t stat	Significance
	B	Std. error	β		
Constant	2.798	0.353		7.922	0.000
City					
Site 3	- 1.050	0.243	-0.236	- 4.319	0.000
Site 1	- 1.247	0.257	- 0.283	- 4.856	0.000
Outside cab	3.964	0.390	0.911	10.172	0.000
Front end loader	0.924	0.225	0.153	4.097	0.000
Enclosed cab	- 1.422	0.288	- 0.457	- 4.937	0.000
Season					
Spring	1.387	0.265	0.308	5.231	0.000
Summer	0.999	0.268	0.225	3.724	0.000
Autumn	- 0.631	0.336	- 0.083	- 1.877	0.061
Ave. wind speed (m/s)	0.142	0.067	0.082	2.119	0.035
Rain previous 7 days	- 0.020	0.006	- 0.119	- 3.294	0.038
Final model R^2 = 0.503					

Factors which increase exposure to glucan are being outside the cab during a shift, using a front end loader, during the season “Spring” and “Summer”, being at Site 2 and with

increasing wind speed. Factors decreasing exposure are being in an enclosed cab, and rain within the last 7 days. These variables explain approximately 50% of the variation in glucan measurements.

Exposures to airborne fungal and bacterial spores were also examined. Tables 13 and 14 report determinants of exposure to *A. fumigatus* and Thermoactinomycetes.

Table 13. Determinants of exposure to ln *A. fumigatus* CFU/m^3.

Variables	Unstandardized		Standardized	t stat	Significance
	B	Std. error	β		
Constant	3.144	0.425		7.390	0.000
City					
Site 3	1.097	0.290	0.218	- 3.786	0.000
Site 2	- 1.024	0.337	- 0.174	- 3.035	0.003
Task					
Grinding feedstock	1.405	0.385	0.204	3.649	0.000
Building pile	- 0.248	0.374	- 0.037	- 0.664	0.507
Blending pile	3.219	0.525	0.300	6.128	0.000
Turning pile	1.786	0.457	0.191	3.907	0.000
Moving pile	- 0.277	0.520	- 0.025	- 0.533	0.594
Cured pile	- 0.401	0.528	- 0.035	- 0.760	0.448
Screening	0.590	0.341	0.103	1.731	0.084
Season					
Spring	1.191	0.416	0.229	2.859	0.004
Summer	0.917	0.539	0.175	1.699	0.090
Autumn	1.623	0.378	0.227	4.294	0.000
Precipitation (y/n)	- 0.776	0.315	- 0.110	- 2.463	0.014
Average temperature	0.065	0.024	0.237	2.730	0.007
Final model R^2 = 0.314					

Table 13. Determinants of exposure to ln Thermoactinomycetess CFU/m^3.

Variables	Unstandardized		Standardized	t stat	Significance
	B	Std. error	B		
Constant	2.970	0.319		9.301	0.000
City					
Site 3	1.767	0.240	0.437	7.351	0.000
Site 2	0.781	0.281	0.166	2.776	0.006
Task					
Grinding feedstock	0.162	0.327	0.030	0.495	0.621
Building pile	- 0.937	0.319	- 0.174	- 2.941	0.003
Blending pile	0.250	0.436	0.030	0.572	0.568
Turning pile	- 0.522	0.402	- 0.067	- 1.298	0.195
Moving pile	- 0.603	0.442	- 0.069	- 1.366	0.173
Cured pile	- 0.766	0.449	- 0.083	- 1.704	0.089
Screening	- 0.078	0.288	- 0.017	- 0.270	0.787
Season					
Spring	1.065	0.286	0.255	3.723	0.000
Summer	1.731	0.293	0.414	5.907	0.000
Autumn	0.880	0.329	0.153	2.677	0.008
Average windspeed	- 0.227	0.073	- 0.145	- 3.122	0.002
Final model R^2 = 0.213					

Although different micro-organisms often are lumped together as being essentially the same thing, it is worth noting that the fungus *Aspergillus* belongs in an entirely different microbial kingdom than members of the bacterial grouping Thermoactinomycetes. Fungi prefer to metabolize carbohydrates as a primary nutrient, while bacteria prefer nitrogenous substrates. Both organisms thrive in hot temperatures (> 50^{o}C) that would disable other microbes through pasteruization. In this study, grinding feedstock, blending the pile, and turning the pile increased concentrations of airborne *Aspergillus*. The model was not as dramatic for Thermoactinomycetes, and the overall concentrations of Thermoactinomycetes were lower than for *Aspergillus*. In univariate analysis (data not shown) "Blending the pile" was a highly significant source of *Aspergillus* at Site 1. Blending is done in a large shed at Site 1, and the increased

concentration may be related to less wind driven dilution air and the association of *Aspergillus* with the feedstock or the bulking agent (wood) which is re-used because it is a source of thermophilic organisms which "jump start" the composting process. More study would be required to tease apart this effect.

Summary:

Two different composting techniques were examined, each of which was associated with a geographic microclimate which also contributed to the outcomes. In general, the windrow composting facility located at Site 2 had the highest exposures to dust, endotoxin and glucan. However, for all sites studied, samples of dust taken inside cabs were much lower in concentration (as much as 10-fold reduction) than those taken outside the cab. Personal samples were intermediate in concentration, as workers do not spend their entire shift only in the cab. Exposures were greater during warm weather (spring and summer seasons). Task analysis could not be done for full shift samples, as most workers performed numerous tasks throughout the day, or were adjacent to multiple task areas.

In contrast, the fungal and bacterial short term grab samples were well suited to task analysis. The highest geometric mean concentration of *Aspergillus fumigatus* was associated with the tasks of "blending". Blending is accomplished in a shed, where biosolid slurry is mixed with wood chips using front end loaders. This task is only done at the facility located at Site 1. The highest geometric mean concentration of Thermoactinomycetes was associated with grinding feedstock, and at the facility located at Site 3 which only composts yard waste.

It can be concluded that working inside the cab of equipment at composting facilities significantly reduces exposure to bioaerosols. Additional controls may be necessary to reduce exposures to micro-organisms at facilities where tasks are done in spaces sheltered from dilution ventilation.

Future Research:

This study examined exposures to biologic agents which in larger cohorts of workers have been shown to be associated with lung disease in some workers. The cohort of compost workers in British Columbia is too small to conduct an epidemiology study to determine whether workers here have experienced short or long term respiratory symptoms. It is probable that workers in this industry may not choose to remain in this profession if they experience on-the-job respiratory symptoms, particularly when they are new to the work or have other options. A recommendation for further study would be to administer a cross sectional respiratory health symptom questionnaire across the province or across Canada in order to increase the number of respondents (and hence the power to be able to detect detrimental health outcomes). The cross sectional design may capture workers new to the job, thus minimizing the healthy worker effect. As the compost industry is growing rapidly, new facilities and increased numbers of workers are a certainty.

Additional methods for assessing the bioactive agents in organic dust may be added to future studies. In this study we sent some samples to Dr. Rylander (BioFact Environmental Health Research Centre, Sweden) who is developing an assay using

specific enzymes to estimate fungal mass. Preliminary data showed good correlation with the β glucan assay.

Another area that should be studied is transfer stations. Transfer stations are central points where garbage trucks deposit their loads, which are then transferred to larger trucks for transportation to landfill sites. Transfer stations are often covered to prevent flooding or leachate formation from precipitation, and may have higher concentrations of biologic agents due to restricted dilution ventilation. Similarly, epidemiological studies may uncover relationships with tasks or length of employment in these facilities with detrimental health outcomes.

Policy and Prevention:

(a) This research clearly identified the benefits of the engineering control afforded by enclosed cabs on equipment used for moving organic materials in composting facilities. Provision of air conditioned cabs is essential for worker health and safety to prevent heat stress in warm weather. It should be noted that exposures to bioaerosols is common at composting facilities. Workers should receive training and education regarding the importance of closing windows and doors on cabs. Some tasks may require that workers wear additional respiratory protection in cases where dilution ventilation is insufficient to remove particulate matter from the air.

(b) The compost industry is not homogeneous. Some facilities are owned and operated by municipalities while others are contracted to independent operators. Most, if not all compost facilities produce product which is sold or given to the public and thus must comply with standards set out by the Organic Material Recycling Regulation

(2002). Operators and professionals who work in solid waste management would normally get training through schools of civil engineering. The Compost Council of Canada acts as an advocacy and educational professional association for owners and operators of composting facilities across Canada.

(c) We will be presenting our results to the participating worksites in February 2009. All managers and workers indicated a keen interest in using the study to refine workplace practices and develop health and safety training.

Knowledge Dissemination/ Transfer

Preliminary results were presented as an oral paper at the 2008 American Industrial Hygiene Conference held in Minneapolis, MN. (*Bartlett, KH, Atwater, J, Chow, Y, Zhang, X, Zhong, S, Spreeuw, A, Burgess, M. Evaluation of worker exposures to biologic particles in selected municipal composting facilities in British Columbia, Canada.* Abstract appended.) Abstracts will be submitted to the upcoming Composting Council of Canada 19th Annual National meeting (September 2009), the Northwest Occupational Health Conference (October 2009) and to the 8th Annual conference of the Canadian Rural Health Research Society (October 2009). A paper on the determinants of exposure to organic dust will be submitted to the American Thoracic Society meeting (May 2010). We have hired a web designer to create a webpage which will be part of the Centre for Health and Environment Research (CHER) network and will have links to the School of Environmental Health and Civil Engineering at the University of British Columbia. The data which we are generating is unique in the scientific community. We will submit papers to relevant occupational and environmental journals for our scientific

colleagues. As well, we will submit "interdisciplinary" papers to civil engineering journals, to increase awareness by professional engineers that worker health and safety must be considered when designing facilities.

We will make the most of other opportunities by invitation such as presentations to community groups (e.g. Master Gardeners, University of British Columbia Celebrate Research week etc.).

One of the students working on this project, Xi (Daisy) Zhang, successfully defended her research thesis and has returned to China to work in a related field. We trained 6 students who all now have a greater appreciation for composting and for workplace health and safety! (Montana Burgess, Amanda Spreeuw, Claire Jackson, Shallar Zhong, Brittany Dever, Carley Macintyre)

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Appendix A: abstracts

Presented to the American Industrial Hygiene Conference and Exhiibition, Minneapolis, MN June 2009

Evaluation of worker exposures to biologic particles in selected municipal composting facilities in British Columbia, Canada.

Karen Bartlett[1], James Atwater[2], Yat Chow[1], Xi Zhang[2], Shallar Zhong[2], Amanda Spreeuw[1], and Montana Burgess[1].

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Composting is a natural process which reduces the volume of waste that would normally go into landfill. The feed-stocks used to make compost include sewage sludge (biosolids) mixed with wood chips, plant and vegetable waste, or other organic materials. During the composting process, the organic material theoretically becomes hot enough to kill disease-causing micro-organisms present in the raw feed-stock. Even though composting produces a beneficial product, employees who work directly with the pile during the heating or curing phases may be exposed to biologic particles associated with lung disease. Examples of these biologic agents are endotoxin (from bacterial cells), β (1-3) glucan (from fungal spores), *Aspergillus fumigatus* (fungal species), and thermophilic spore-forming bacteria.

The composting industry is rapidly expanding in British Columbia, integrating different types of composting technologies are used. This allowed us to study airborne exposures to compost workers from biologic particles comparing windrow vs. static aerated pile technologies, and yard waste vs. biosolid feed-stocks. The protection offered by the enclosed cabs of the machinery used in composting was evaluated. These data will be used to formulate recommendations for best practices to protect worker health and can be incorporated into the design of composting facilities.

Workers using static aerated pile technology were significantly less exposed to endotoxin than workers using windrow technology (GM 54 EU/m^3 vs 417 EU/m^3, $p < 0.001$). Area samples for endotoxin were lower in facilities composting only yard waste (GM 94 EU/m^3) than facilities composting only biosolids (GM 763 EU/m^3) or biosolids and yard waste (GM 773 EU/m^3). Protection offered by the cab varied according to location. Exposures to *Aspergillus fumigatus* and thermoactinomycetes were estimated using Andersen six-stage impactors. At all sites, over 50% of the total *A. fumigatus* spores, and around 40% of thermoactinomycete-particulate was of a respirable size fraction (≤ 3 μm).

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Presentation to Compost Council Annual Meeting

Efficacy of controlling airborne exposures to biologic particles at selected municipal composting facilities in British Columbia, Canada.

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The purpose and objective of the research was to measure exposures to compost workers from selected biohazards. Three municipal-scale composting facilities using two composting methods were compared for exposures to endotoxin (i.e. lipopolysaccharide, a component of gram negative bacterial cell walls), β (1-3) glucan (a component of fungal spore walls), *Aspergillus fumigatus* and thermophilic spore-forming bacteria.

Composting methods examined were: static aerated piles, open windrow pile turned by high technology equipment (straddle turner), and open windrow pile turned by low technology equipment (front end loader).

The feedstock for Site 1 (static pile) was dewatered sewage sludge (biosolids) mixed with wood chips. Site 2 primarily composted yard waste in open windows turned by high energy equipment and Site 3 exclusively composted yard waste in windows turned by front end loaders.

Job tasks were classified as follows: blending the feed-stocks, building the piles, turning the piles (windrow only), moving the pile after the thermophilic phase, and screening. Viable samples for *A. fumigatus* and Thermoactinomyces were collected near these job tasks using an Andersen six-stage sampler.

Exposures to dust, endotoxin and glucan were determined by full shift sampling using a battery operated pump and a 7-hole sampling head to capture airborne particulate matter ≤ 100 μm aerodynamic diameter. Sampling equipment was worn by workers (personal samples) or suspended inside the cab near the operator. Ambient samplers were fixed to the outside of the cab.

Results: The windrow composting facility located at Site 2 had the highest exposures to dust, endotoxin and glucan (GM 2.69 mg/m^3; 502 EU/m^3; and 201 $\mu g/m^3$ respectively). However, for all sites studied, samples of inhalable dust taken inside equipment cabs were at least 10-fold reduced in concentration compared to those taken outside the cab. Personal samples were intermediate in concentration, as workers did not spend their entire shift only in the cab. The highest geometric mean concentration of *Aspergillus fumigatus* was associated with the tasks of "blending". Blending is accomplished in a shed, where biosolid slurry was mixed with wood chips using a front end loader. This task was only done at Site 1. The highest geometric concentration of Thermoactinomycetes was associated with grinding feedstock, and at the facility located at Site 3 which only composts yard waste.

It can be concluded that working inside the cab of equipment at composting facilities significantly reduced exposures to inhalable bioaerosols.

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Presentation to Compost Council Annual Meeting

Thermal variability in Windrowed Composts, its impact on microorganism variability and implications for monitoring.

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The purpose of the research was to determine the thermal regime of discrete compost quantities as they progressed through the process from pile construction, through turning and final screening. To that end, 100 miniature temperature loggers data (16 mm in diameter, 4 mm thick) inside PVC house casings were placed in a section of a windrow in three layers of approximately 33 loggers. The loggers were spaced such that there was 1 per 0.5m^3 of feedstock. Data was recorded every 8 hours. The overall objective was to determine whether all of the compost material was exposed to temperatures above 55°C for a minimum of 15 days.

The first pile was constructed in September 2007 primarily of yard trimmings. The second pile was constructed in November 2007 of yard waste with much higher percentage of leaves. Feedstock characteristics in respect to C:N ratios and moisture content were comparable. Feedstock samples were collected for microbial analysis at the time of pile construction, and subsequently with each turning. At each turning and at breakdown where loggers were exposed *in situ* they were removed along with associated compost for microbial analysis.

Temperatures went as high as 80°C in the first pile and everywhere the time/temperature requirement was met. In the second pile only about 80% of the pile material met the time/temperature however, by any conventional means of measurement the time/temperature requirement would have seemed to be met. Pile gradients were not uniform with temperature differences as much as 20°C for adjacent loggers. Contrary to expectation, the cold areas were in the interior and bottom of the pile.

In both cases, destruction of entercococci organisms was seen, whereas thermo-tolerant microorganisms including coliforms flourished with optimum growth around 55-60°C.

Acknowledgement: WorkSafe BC provided partial support for this project.

REPORT TO WORKSAFE BC

Measurement of beta-glucan in compost samples: addendum to H06-0088

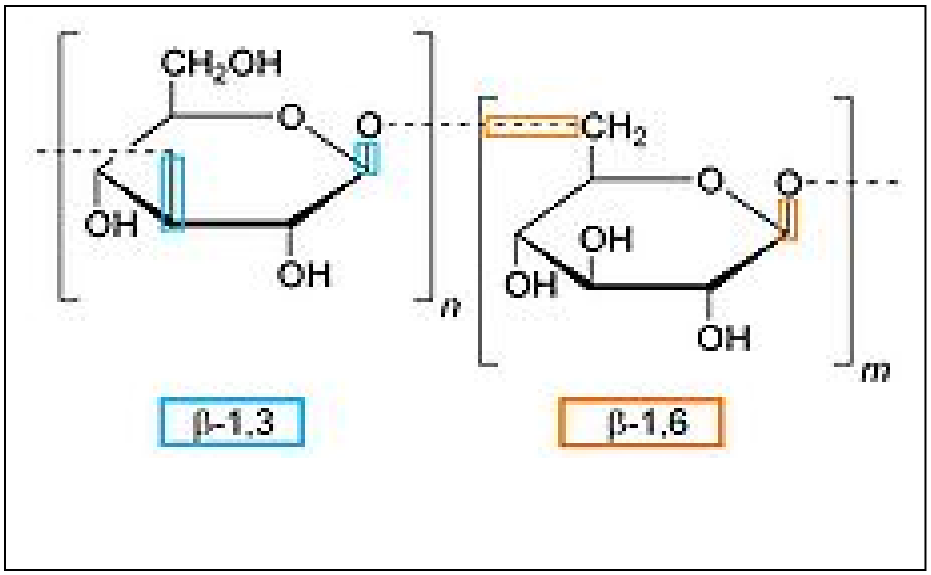

Image credit: http://en.wikipedia.org/wiki/Beta-glucan

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Main Research Findings

- Workers at composting facilities are exposed to airborne, microscopic particulate of biologic origin, including immunologically active structural components of bacterial cells (e.g. endotoxin) or fungal spores (e.g. β-glucan).
- β-glucan is a poly-glucose, structural element of all fungal spores and some bacteria and plants and is known to stimulate the human innate immune system which can result in pulmonary symptoms or disease.
- A method for the measurement of glucan in air samples has not yet been standardized, which is problematic for the study of occupational exposures.
- Compost is a complex biologic mixture, and contains inhibitory substances which interfere with the glucan-specific, highly sensitive, *Limulus*-derived lysate test, resulting in artificially low concentration compared to a specific enzyme-linked immunologic test developed by the laboratory of Dr. Peter Thorne at the University of Iowa.
- Our conclusion is that for compost (but not for all organic dusts), the glucan specific enzyme-linked immune assay (ELISA) offers the most sensitive and specific assay for airborne glucan for exposure studies.

Executive Summary

Two methods for determining the concentration of β-glucan in organic dust were compared. Some researchers have suggested that the Limulus amoebocyte lysate assay should be considered to be the gold standard for glucan detection due to its superior specificity and sensitivity. In our study, we compared an enzyme-linked immunoassay, which is also a very specific and sensitive test, with the Limulus amoebocyte lysate assay. We found that the compost samples appeared to be significantly lower in concentration when tested by Glucatell reagent than by ELISA. This difference was not reproducible for all organic dust, however, as a small series of grain dust samples collected and extracted in a similar fashion to the compost dust returned very similar results when duplicate aliquots of sample were tested by two different laboratories.

We conclude that the compost samples contained inhibiting substances that made them appear to be lower in concentration by Glucatell than by ELISA. An alternate explanation is that sample was lost due to the number of dilutions necessary to bring the samples into the range of the standard curve of the Glucatell assay. The Glucatell assay is extremely sensitive, with a working range of 5 – 40 pg/mL. In a similar fashion, the grain dust samples also required extensive dilution to bring them into the range of the standard curve, but the results of the grain dust analysis was much closer to the results of the ELISA which leads us to conclude that the compost samples behaved differently in the assay.

Our recommendation for the measurement of β-glucan in organic dust samples is to use a highly specific test such as the ELISA tests which have been developed by laboratories in the Netherlands (Douwes et al. 1996), Germany (Sander et al. 2008), and the US (Noss et al. 2010). Although these assays are available through research collaborations, it would be desirable for a β-glucan assay to be readily available in order to allow exposure studies to be conducted over potentially longer time lines, and with multiple sources of organic dust.

Research Context:

β-glucans are structural cell wall components of most species of fungi and have been suggested to play a causal role in the development of respiratory symptoms. Of particular interest are (1→3)-β-D-glucans, which are non-allergenic water-insoluble structural cell wall components of most fungi, some bacteria, and many plants. They account for up to 60% of the dry weight of fungal cell walls, and consist mainly of glucose polymers with variable weight and branching (Douwes, 2005). (1→3)- β-D-glucans are found frequently in organic dust including compost, grain and other agricultural dusts. The amount of glucan in organic dust can be correlated with the amount of fungal growth present, and can be used as a surrogate measure of the biologically active portion exposure to fungal spores (Halstensen et al., 2007).

(1→3)-β-D-glucans, though non-allergenic, are biologically active (Iossifova et al., 2008, Douwes, et al., 2003; Fogelmark et al., 1994; Milanowski et al., 1997; Rylander et al., 1998). In-vitro and animal-exposure studies have demonstrated that exposure to (1→3)-β-D-glucans can alter the function of inflammatory cells in the lungs by changing the reaction to inhaled inflammatory agents, such as endotoxin, as well as other antigens (Beijer et al., 2002). Human inhalation challenge experiments have also demonstrated that inflammatory cell function changes in response to inhalation of (1→3)-β-D-glucans, reflected by increases in some inflammatory markers and decreases in others, and also that this response is distinctive from the human response to inhaled endotoxin (Thorn et al., 2001; Beijer et al., 2002).

Epidemiological studies have indicated that exposure to (1→3)-β-D-glucans may be related to an increased prevalence in a number of symptoms of the respiratory system and other areas. A review of several studies done in a variety of environments noted that exposure to (1→3)-β-D-glucans has been associated with upper airway irritations, fatigue, and reduced lung function. Some studies have also discovered an association with increased airway inflammation (Douwes, 2005). A study done in a number of Swedish rowhouses found a relationship between exposure to (1→3)-β-D-glucans and an increased prevalence of atopy and a decrease in $FEV_{1.0}$ (Thorn and Rylander, 1998). A

British study of recycling workers also found that worker-reported dry cough and hoarse/parched throat, skin rash, unusual fatigue, and vomiting were more prevalent in individuals exposed to greater amounts of (1→3)-β-D-glucans than workers exposed to lower amounts. This difference was significant ($P = 0.008$) (Gladding and Thorn, 2003).

However, the relationship between (1→3)-β-D-glucans and adverse health effects is not yet definitive. Several other studies have failed to find a relationship between exposure to (1→3)-β-D-glucans and negative health effects in workers, and the biological inflammatory mechanism associated with (1→3)-β-D-glucan exposure in human beings is not yet well understood (Douwes, 2005). In a review of several papers, only one study was related to farming of an unspecified type, and no health effects were associated with (1→3)-β-D-glucan exposure (Douwes, 2005).

There are two primary methods of measurement of environmental (1→3)-β-D-glucan levels. One method is a modification of the limulus amebocyte lysate (LAL) assay utilized for the measurement of endotoxin. The modified version is available from the Associates of Cape Cod in Falmouth, MA, USA. Similar to the principles utilized in the endotoxin assay, glucan reacts with factor G in the lysate (endotoxin reacts with factor C). Cross-reaction with endotoxin in the modified assay is not an issue, as factor C is removed or disabled (Douwes, 2005). The glucan samples, having had factor G added to them, are then read in a spectrophotometer. The other method is the use of (1→3)-β-D-glucan specific inhibition enzyme-linked immunoassays (ELISA). In this assay, glucan is quantified by the use of rabbit anti-glucan antibodies and enzyme-linked anti-rabbit antibodies. The ELISA methods are cheaper, but not commercially available (Noss et al., 2010; Sander et al., 2008; Douwes, 2005; Douwes et al.,1996). The methods should correlate fairly well with each other, though depending on the dust source, either ELISA or LAL will detect higher glucan levels (Sander et al., 2008).

Currently, there is no exposure limitation specific to (1→3)-β-D-glucans. This is likely owing to the inconclusive nature of the relationship between (1→3)-β-D-glucans and adverse health effects.

Objectives:

Compare β-glucan-specific *Limulus* ameobcyte lysate (Glucatell, Associates of Cape Cod) with an enzyme-linked immunoassay (ELISA) (Metwali & Thorne, personal communication) for the measurement of β-glucan in compost dust.

Research Design:

Airborne dust samples were collected at three composting facilities in British Columbia. Table 1 lists the locations and compost methods.

Table 1. Location and technology of composting for three BC sites.

Location	Composting technology	Feed-stock
Site 1 *(Okanagan Valley, hot dry summer, cold dry winter)*	Static aerated (positive pressure) Static aerated (negative pressure)	Sewage sludge mixed with wood chips
Site 2 *(Thompson River Valley, hot dry summer, winter snow)*	Open windrow (straddle turner) Open windrow (front end loader)	Sewage sludge mixed with wood chips
Site 3 *(Pacific coast, mild summer, mild wet winter)*	Open windrow (front end loader)	Plant and garden waste

Table 2 lists the measurements taken for β-glucan in dust.

Table 2. β-glucan airborne dust exposure.

Agent	Source	Measurement period	Method
β D- glucan	Fungal spores	Full work-shift	Gravimetric, non-viable

β D-glucan was measured inside and outside equipment cabs (where enclosed cabs were used). Results of the overall study are reported to WorkSafe BC in "*Assessing exposures to compost workers from airborne biohazards H06-0088"* Bartlett, KH, Atwater, J and Chow, Y. 2009.

Methods:

β D-glucan (sampling): Inhalable particulate was captured on 25 mm glass fibre filters that were baked (depyrogenated) overnight at 200 ^{o}C to render the filters free of contaminating glucan or endotoxin. Seven-hole sampling cassettes were attached to SKC battery operated personal sampling pumps calibrated to 2 litres per minute (lpm) flow rate (Douwes et al., 1995). Filters were shipped on cold packs to the Bioaerosol Exposure Laboratory at the University of British Columbia (UBC) for analysis. Samples were stored at 4 ^{o}C with desiccant until the analysis was performed.

β D-glucan (analysis): Filters were first extracted for endotoxin into pyrogen-free water containing 0.05% Tween-20. After aliquots were removed for endotoxin testing, glucan was extracted from the filters by denaturing the molecule by autoclaving (121^{o}C) the filters for one hour. Extracts were split into two aliquots and stored at – 80^{o}C prior to one of two analytic techniques. The ELISA assay was performed in the laboratory of Dr. Peter Thorne, Institute of Rural and Environmental Health, University of Iowa, where the extracted glucan was quantified by end-point, enzyme linked immunosorbant assay (ELISA) (Phipatanakul et al., 2000a; Phipatanakul et al., 2000b; Platt-Mills et al..1991). The alternate analysis used the specific factor G Limulus amoebocyte lysate (Glucatell, Associates of Cape Cod, MA). The Glucatell analysis is a kinetic chromogenic assay read at 405 nm using a microtitre plate reader (SpectroMax 190, Molecular Devices, CA, and SoftMax Pro software).

ELISA protocol: Immunlon II microtiter plates were coated with mouse mono-clonal anti-(1→6), (1→3) branched β-D-glucan. Dilutions of samples to be tested were added to wells and a control curve was performed using reference Scleroglucan. The reference curve was 5 µg/mL – 2.5 ng/mL glucan.

Glucatell protocol: All work was carried out in a laminar flow hood to prevent exogenous glucan contamination of the assay. An internal control, Beta-glucan (G-6513, Sigma Chemicals, St, Louis, MO) was used to check all steps of the procedure. The

reference curve covered the range 5 pg/mL – 40 pg/mL using glucan supplied by the kit manufacturers, Associates of Cape Cod.

Comparison organic dust: A comparison dust was also tested. Grain dust (canola, wheat and barley mix) from Vancouver grain terminals was also tested by both ELISA method (Thorne laboratory, Iowa) and by Glucatell, using the same protocols as for the compost samples.

Data Analysis:

Data were analyzed using SPSS (version 15 for Windows) statistical software. Units of concentration were dust mass (mg/m^3) and micrograms glucan/m^3.

Results and Discussion:

Dust and β-glucan component of organic dusts (composting):

Full shift samples for dust are reported in Table 3. (1→3) β-D-glucan for the same composting sites are reported in Table 4 for the ELISA procedure, and in Table 5 for the Glucatell procedure.

Table 3. Inhalable dust by location at three composting facilities in British Columbia.

Dust (mg/m^3)								
Location	**n**	**AM[a]**	**SD[b]**	**GM[c]**	**GSD[d]**	**Min.**	**Max.**	**GM p-value**
Site 1	179	2.82	3.76	1.23	4.03	0.01	25.21	
Site 2	87	5.42	7.52	2.69	3.39	0.21	41.56	< 0.001
Site 3	178	2.58	5.08	0.61	5.23	0.02	28.39	

[a] arithmetic mean, [b]standard deviation, [c] geometric mean, [d] geometric standard deviation

Table 4. Inhalable glucan by location at three composting facilities in BC by enzyme-linked immunoassay procedure.

β Glucan (µg/m^3)								
Location	**n**	**AM[a]**	**SD[b]**	**GM[c]**	**GSD[d]**	**Min.**	**Max.**	**GM p-value**
Site 1	179	131	245	30	7.7	< LOD[e]	2406	
Site 2	87	460	890	128	5.6	2.5	4574	< 0.001
Site 3	177	236	515	29	9.4	< LOD	3118	

[a] arithmetic mean, [b]standard deviation, [c] geometric mean, [d] geometric standard deviation
[e] Limit of detection = 10 ng/mL

Table 5. Inhalable glucan by location at three composting facilities in BC by Glucatell factor G Limulus amoebocyte lysate assay.

β Glucan (ug/m^3)								
Location	**n**	**AM[a]**	**SD[b]**	**GM[c]**	**GSD[d]**	**Min.**	**Max.**	**GM p-value**
Site 1	175	1.51	2.63	0.38	7.0	1.0[e]	25	
Site 2	83	4.70	7.98	1.92	6.2	0.03	43	< 0.001
Site 3	172	2.94	6.17	0.28	9.1	1.0	32	

[a] arithmetic mean, [b]standard deviation, [c] geometric mean, [d] geometric standard deviation
[e] Limit of detection = 0.005 ng/mL

The concentrations of the compost samples were remarkably consistent between the ELISA assay and the Glucatell assay. However, the concentrations as determined by the Glucatell assay were consistently and significantly lower than the concentrations determined by the ELISA assay.

A comparison was made with a limited number of grain dust samples to determine if there was a systematic error in the determination of concentrations. The results are presented in Table 6.

Table 6. Comparison organic dust (grain dust) by two methods, ELISA and Glucatell.

β Glucan (µg/m^3)							
	n	**Inhalable dust**		**Glucan ELISA**		**Glucan Glucatell**	
	10	**AM[a]**	**SD[b]**	**AM**	**SD**	**AM**	**SD**
Grain dust		2631	8315	23.4	52.11	15.6	35.7

The difference between the concentrations determined by ELISA and Glucatell was not significant, although the Glucatell appeared to be slightly lower than the results returned by the ELISA assay.

Summary:

Two methods for determining the concentration of β-glucan in organic dust were compared. Some researchers have suggested that the Limulus amoebocyte lysate assay should be considered to be the gold standard for glucan detection due to its superior specificity and sensitivity. In our study, we compared an enzyme-linked immunoassay, which is also a very specific and sensitive test, with the Limulus amoebocyte lysate assay. We found that the compost samples appeared to be significantly lower in concentration when tested by Glucatell reagent than by ELISA. This difference was not reproducible for all organic dust, however, as a small series of grain dust samples collected and extracted in a similar fashion to the compost dust returned very similar results when duplicate aliquots of sample were tested by two different laboratories.

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Our recommendation for the measurement of β-glucan in organic dust samples is to use a highly specific test such as the ELISA tests which have been developed by laboratories in the Netherlands (Douwes et al. 1996), Germany (Sander et al. 2008), and the US (Noss et al. 2010). Although these assays are available through research collaborations, it would be desirable for a β-glucan assay to be readily available in order to allow exposure studies to be conducted over potentially longer time lines, and with multiple sources of organic dust.

Future Research:

Additional methods for assessing the bioactive agents in organic dust is desirable.

Policy and Prevention:

There is no exposure limit proposed for β-glucan. Standard assays would need to be made available to multiple laboratories for round robin and other studies in order to determine the best protocol to use for the quantification of β-glucan in organic dust exposure studies.

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