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Levels of Gram-Negative Bacteria, *Aspergillus fumigatus*, Dust, and Endotoxin at Compost Plants

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Airborne gram-negative bacteria, endotoxins, dust, and *Aspergillus fumigatus* were measured in four compost plants in Sweden. At sites where material was processed, the number of airborne *A. fumigatus* exceeded $10^6/m^3$, whereas the number of gram-negative bacteria was usually lower. Dust levels were moderate, and endotoxin levels were well below 0.5 $\mu g/m^3$. Medical studies to evaluate the effects of this type of microbial exposure are recommended.

Increasing amounts of solid waste being produced and more restrictive environmental regulations being applied to traditional methods of wastewater sludge disposal have led to the expanded use of alternative treatment and disposal methods. Particularly attractive are methods that result in a productive end use of the waste either as a fuel or, after composting, as a soil conditioner.

Processes used to produce refuse-derived fuel and compost pose potential health risks to the waste processing workers. Municipal solid waste contains viruses, bacteria, and parasites excreted by humans, and the use of disposable diapers in many countries may have increased this contamination. Other microorganisms from plant and soil materials are also present.

In recent years, public policy has encouraged the composting of processed solid wastes in Sweden. As a result, a number of waste composting facilities employing a variety of techniques have been put into operation. Most of these facilities incorporate about 10 to 20% municipal wastewater treatment plant sludge into the solid waste material before composting.

During the processing of solid waste, microorganisms may become airborne. Since the processing generally occurs within enclosed buildings, insufficient ventilation and reduced die-off rates from the lack of solar radiation can contribute to a high number of organisms in the air. An epidemiological study of solid waste recovery systems has been recommended as the result of a study of bacterial aerosols at a solid waste processing system producing refuse-derived fuel (5). Adverse health effects attributable to exposure to endotoxins from gram-negative bacteria have been observed in a Swedish plant processing solid waste before composting (6). Composting of processed solid waste creates an environment conducive to the proliferation of thermophilic fungi such as *Aspergillus fumigatus* (2, 7, 8).

The purpose of this investigation was to measure air concentrations of gram-negative bacteria, endotoxin (lipopolysaccharide [LPS]), dust, and *A. fumigatus* at different waste composting plants. In addition, selected solid waste samples from the plants were analyzed for these parameters, and the gram-negative bacteria that were found were identified.

MATERIALS AND METHODS

Four compost plants were selected for the study. Three utilized a mixture of solid waste and sludge from wastewater treatment, and the fourth used a mixture of wastewater treatment plant sludge and wood chips. Brief descriptions of the facilities are found in Table 1. Except for the enclosed bioreactor at Landskrona and the Dano drum at Borlange, all composting operations were conducted outdoors. Solid waste was processed indoors before composting.

Airborne bacteria and fungi were collected with six-stage Andersen samplers operating for 30 s to 4 min and located about 1.5 m above the ground. The Andersen samplers were equipped with plastic petri dishes containing 35 ml of the appropriate media and were calibrated with plates in place. Drigalski agar modified after Conradi-Drigalski agar (M. Lundholm and R. Rylander, Br. J. Ind. Med., in press) was used as a selective medium for gram-negative bacteria. A modified Czapek-Dox agar (2) was used for *A. fumigatus*. Bacterial plates were incubated at 35°C for 48 h, and *A. fumigatus* plates were incubated at 45°C for 48 h. The colonies were then counted. Only colonies in the hole pattern were counted, and the positive hole correction was applied (1). For an assessment of the respirable fraction of the airborne colony-forming particles, the number of CFU on plates 3 through 6 was expressed as a percentage of the total CFU.

TABLE 1. Description of waste processing and compost facilities

Community	Vol of waste treated	Processing methods			
		Transferring waste from tipping pit	Treatment before composting	Composting	Transferring material between composting stages
Stromstad	Variable (resort community)	Clamshell bucket	Milling, magnetic separation, screening; paper, plastics, and metal separation	Intensive composting (2–3 wk) on aerated pad, maturing (12 wk) at a lowered forced aeration rate, and screening	Front-end loader
Landskrona	24,000 tons of household refuse, 8,000 tons of sludge (20% dry matter), per yr	Conveyor	Magnetic separation, primary and secondary milling, strainer, sorter, cyclone; paper, plastics, glass, and metal separation	Enclosed biorector (14 days) followed by maturing on aerated pad (3–4 mo) after size separation	Conveyor to and from biorector, front-end loader thereafter
Borlange	60,000 tons of household refuse, 18,000 tons of sludge (15% dry matter), per yr	Conveyor	Milling, magnetic separation, screening; metal, plastic, and paper separation (after initial composting in Dano drum)	Modified Dano drum (48 h) followed by additional composting on an aerated pad (6 wk) before maturing (14 wk) at a lowered forced aeration rate and eventual screening	Enclosed conduit to Dano drum, conveyor to initial compost aeration pile, front-end loader thereafter
Gothenburg	16,000 m^3 of sludge (25% solids) and wood bark at a 3:1 ratio (by vol)	Does not apply	Sludge concentration and filter pressing	Natural aeration for 15 wk in periodically turned piles followed by screening	Truck to sludge-chip mixing area, front-end loader thereafter

TABLE 2. Airborne concentrations of microorganisms at composting plants

Sample location	Gram-negative bacteria			*A. fumigatus*		
	No. of samples	Concn (10^3 CFU/m^3)		No. of samples	Concn (10^3 CFU/m^3)	
		Median	Range		Median	Range
Stromstad						
Inside						
Control room	5	1.9	0.3–5.1	6	12	4.2–>630
Refuse hopper	4	43	15–260	6	>640	380–>1,900
Separator drum	5	11.4	1.4–19.1	8	>220	16–>1,200
Outside						
Processed refuse	2		2.8–6.0	2	>1,250	
Short-term compost	6	2.2	0.3–6.3	7	>1,250	14–>3,200
Long-term compost	2		3.3–14	4	>1,500	670–>6,000
Landskrona						
Inside						
Control room	4	1.2	1.1–1.5	4	9.8	2.8–490
Tipping floor	4	2.0	0.29–2.6	4	1.6	0.59–2.9
Waste processing building	8	94	76–370	8	49	19–>1,500
Bioreactor building						
Not operating	2		0.2–0.29	2		7.7–8.8
Operating	2		1.8–14	2		20–>2,600
Roller room	1	2.6		3	21	12–46
Outside						
Refuse-sludge mixer	4	1.4	0.88–3.2	4	6.2	1.5–8.8
Long-term compost	4	34	9.8–56	4	126	19–>3,700
Borlange						
Inside						
Control room	3	1.1	0.85–1.3	3	0.46	0.43–0.65
Tipping floor	3	1.8	0.04–3.4	3	0.1	0.04–0.18
Waste processing building	6	0.6	0.22–1.1	6	1.5	0.58–3.5
Fine compost screening area	3	20	14–28	3	85	51–94
Outside						
Fine compost screening area	4	96	59–110	2	54	36–73
Short-term compost	3	1.8	0.94–2.8	1	80	
Gothenburg						
Inside						
Lunch room and office	3	0.07	0–0.1	3	0.3	0.1–4
Outside						
Screener-hopper	2		1.5–4.9	3	>67	19–>3,500
Bark outlet	2		0.2–0.6	3	>25	22–42
Control panel	4	0.08	0–0.2	4	1.4	0.8–2
Screened compost pile				2		2.2–21

[a] The Gothenburg plant treated a mixture of wastewater sludge and wood chips; the other three plants used a mixture of solid waste and wastewater sludge.

Airborne dust samples were collected about 1.5 m above the ground on preweighed Millipore cellulose acetate filters (diameter, 37 mm; pore size, 0.8 μm) supplied with a metal grid (mesh size, 2 mm) to exclude large particles. The airflow was 12 liters/min for about 1 h. The amount of dust was determined by weighing the filters after sampling.

For LPS determinations, the filters used for dust

TABLE 3. Respirable fractions (plates 3–6) of CFU of microorganisms in inside and outside air at compost plants

Sample location	Gram-negative bacteria		A. fumigatus	
	No. of samples	% Respirable (±SD)	No. of samples	% Respirable (±SD)
Stromstad				
Inside	13	84 ± 9	6	91 ± 6
Outside	8	45 ± 13	3	47 ± 22
Landskrona				
Inside	14	65 ± 26	18	77 ± 17
Outside	6	62 ± 26	5	81 ± 10
Borlange				
Inside	14	67 ± 29	12	80 ± 10
Outside	7	20 ± 22	3	49 ± 24
Gothenburg				
Outside	6	60 ± 23	11	79 ± 19

exposure determinations were shaken in 10 ml of pyrogen-free water, and serial dilutions were prepared. Samples of Limulus amebocyte lysate (0.1 ml; Cape Cod, Inc.) and of dilutions of the filter water extract (0.1 ml) were mixed in glass tubes and incubated in a water bath at 37°C for 30 min. The tubes were inverted, and the concentration in the lowest dilution forming a stable clot was compared with the lowest dilution forming a clot in a dilution series of known amounts of *Escherichia coli* LPS (Difco Laboratories).

Taking the amount of air drawn through the filter into consideration, the results could be expressed as micrograms of LPS per cubic meter. The sensitivity of the test allowed the determination of amounts down to 0.05 ng/ml. All glassware used in the analysis was rendered LPS-free by heating it at 180°C for 3 h. Control tests with LPS-free water, as well as positive controls with *E. coli* LPS dilutions, were regularly included to assess the accuracy of the determinations.

Sampling sites in the plants were selected to be representative of worker locations for both waste processing and compost manipulation. For plants processing solid wastes, typical sampling sites were the control room, a location near the entry of wastes into the processing stream, interior process locations where emissions were possible, and the exit of wastes from the processing operations. Sampling sites in the compost area of the plants were downwind of the front-end loaders manipulating compost piles and at other locations where compost was being agitated or transported by other methods. Selected bulk samples of sludge, solid waste, and compost were collected for enumeration and identification of bacteria.

RESULTS

Airborne concentrations of gram-negative bacteria and *A. fumigatus* at the plants at Stromstad, Landskrona, Borlange, and Gothenburg are presented in Table 2. A considerable range in microbial contamination was found in all plants. Control rooms, the lunch room and office in the Gothenburg plant, and sites where no handling of material was done showed low values both for gram-negative bacteria and *A. fumigatus*. Inside sites where material was handled and processed were highly contaminated. The refuse hopper, separator drum, and waste processing rooms, including the bioreactor building, had from 19×10^3 to 370×10^3 gram-negative bacteria per m^3 and over 10^6 *A. fumigatus* per m^3. Outside sites where material was handled, such as sites near compost piles that were being moved and sites near compost screening areas, showed equally high values. Sites where material was not treated generally had low values, equal to those in control rooms.

The ratio of *A. fumigatus* to gram-negative bacteria varied between 22 and 1,000 in areas where material was handled. The same ratio in waste-wastewater treatment plants where the number of airborne *A. fumigatus* was low (data not shown) ranged from 0.005 to 0.1.

A comparison of the respirable and nonrespirable fractions of the CFU detected in inside and outside samples (Table 3) revealed that on the average more than 50% were in the respirable size range. An exception was at the fine compost screening area at Borlange, where most of the gram-negative bacterial CFU were of nonrespirable size at both inside (80%) and outside (95%) sampling sites. At Stromstad, an even higher portion in the inside samples (80 to 90%) was

TABLE 4. Average airborne dust and endotoxin concentrations

Sample location	No. of samples	Total dust (mg/m^3)	Endotoxin (μg/m^3)
Stromstad			
Refuse hopper			
Active	4	0.92	0.014
Inactive	2	0.16	0.010
Separator drum	2	0.24	0.010
Control room	3	0.26	0.001
Other locations	2	0.14	0.010
Landskrona			
Tipping floor	2	0.42	0.001
Waste processing room	2	5.10	0.038
Control room	1	0.56	0.002
Borlange			
Tipping floor	2	0.58	0.015
Waste processing room	2	0.19	0.0026
Control room	1	0.28	0.001
Screening area	2	10.6	0.042
Gothenburg			
Mechanics area	3	0.23	0.009
Compost piles	3	0.18	0.002

respirable; the proportion of respirable CFU-forming particles was higher at the inside sites than outside (Table 3). No difference was found at the Landskrona plant. The most commonly found gram-negative bacteria were in the genera *Klebsiella, Enterobacter, Serratia*, and *Pseudomonas*.

The average amounts of airborne dust and endotoxin are reported in Table 4. The dust levels were below 1 mg/mg^3 at all sites, except at the fine compost screening area at Borlange and in the waste processing room in Landskrona. Personal samplers carried by two workers in different parts of the Landskrona plant yielded an average value of 1.52 mg/m^3.

Endotoxin values ranged from 0.001 to 0.014 μg/m^3. The amount of endotoxin in airborne dust ranged from 0.007 to 0.87 μg/mg.

DISCUSSION

The measurements reported here are based on studies in a small number of plants and must be evaluated with caution until more data are available. It is possible, however, to make several observations in relation to data from similar studies in other environments contaminated with organic dust. The number of *A. fumigatus* were generally lower at the plant utilizing the sludge-wood bark mixture than at the plants using a mixture of domestic refuse and sewage sludge. Concentrations of gram-negative bacteria were frequently higher inside solid waste processing buildings than at either indoor or outdoor sites at wastewater treatment plants (3; Lundholm and Rylander, in press). Concentrations inside the waste processing buildings were similar to those reported in swine confinements (4).

The results obtained in the present study suggest that the waste processing environment is different from other environments where organic dusts are present regarding the relative numbers of gram-negative bacteria and fungi. In cotton processing plants, very high numbers of gram-negative bacteria are present, and the amount of airborne endotoxin is also high (9). The number of *A. fumigatus* in cotton processing plants is low.

The medical consequences of a long-term exposure to high numbers of airborne *A. fumigatus* as found in the plants studied here are not known (2). Infections with *A. fumigatus* may appear in susceptible individuals whose resistance to microbes has already been lowered. Persons with a history of asthma are also subject to additional respiratory problems resulting from exposure to *A. fumigatus*. In view of the risk for development of lung disease caused by inhalation of fungi (farmer's lung), it is desirable to conduct medical studies of employees at composting plants of the type examined in the present study.

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