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Waste Workers' Exposure to Airborne Fungal and Bacterial Species in the Truck Cab and During Waste Collection

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ABSTRACT

A large number of people work with garbage collection, and exposure to microorganisms is considered an occupational health problem. However, knowledge on microbial exposure at species level is limited. The aim of the study was to achieve knowledge on waste collectors' exposure to airborne inhalable fungal and bacterial species during waste collection with focus on the transport of airborne microorganisms into the truck cab. Airborne microorganisms were collected with samplers mounted in the truck cab, on the workers' clothes, and outdoors. Fungal and bacterial species were quantified and identified. The study showed that the workers were exposed to between 112 and 4.8×10^4 bacteria m^{-3} air and 326 and 4.6×10^4 fungi m^{-3} air. The personal exposures to bacteria and fungi were significantly higher than the concentrations measured in the truck cabs and in the outdoor references. On average, the fungal and bacterial concentrations in truck cabs were 111 and 7.7 times higher than outdoor reference measurements. In total, 23 fungal and 38 bacterial species were found and identified. Most fungal species belonged to the genus *Penicillium* and in total 11 *Penicillium* species were found. Identical fungal species were often found both in a personal sample and in the same person's truck cab, but concentrations were on average 27 times higher in personal samples. Concentrations of fungal and bacterial species found only in the personal samples were lower than concentrations of species also found in truck cabs. Skin-related bacteria constituted a large fraction of bacterial isolates found in personal and truck cab samples. In total, six *Staphylococcus* species were found. In outdoor samples, no skin-related bacteria were found. On average, concentrations of bacterial species found both in the truck cab and personal samples were 77 times higher in personal samples than in truck cab samples. In conclusion, high concentrations of fungi were found in truck cabs, but the highest concentrations were found in personal samples; fungal and bacterial species found in high concentrations in personal samples were also found in truck cabs, but in lower concentrations indicating that both fungi and bacteria are transported by the workers into the truck cab, and are subsequently aerosolized in the truck cab.

KEYWORDS: bioaerosol; exposure in cars; fungal species; MALDI-TOF; microbial transport; occupational exposure; *Penicillium* species; skin bacteria; waste collection workers

INTRODUCTION

Globally, a large number of people work with waste collection, and exposure to microorganisms is considered an occupational health problem for people handling solid waste (Binion and Gutberlet, 2012). Waste handling has been associated with symptoms of the airways, diarrhea, and ODTS (Poulsen *et al.*, 1995b), and waste workers with allergy have lower self-reported health status and health-related life quality than those without allergy (Garrido *et al.*, 2015). Exposure to bioaerosols during waste handling has been investigated in several countries (Nielsen *et al.*, 1995; Poulsen *et al.*, 1995a; Wouters *et al.*, 2006; Lavoie *et al.*, 2006; Park *et al.*, 2011) in regards to exposure to endotoxin, β-glucan, fungi, or bacteria and type of waste handled. *Penicillium* has been reported to be the dominating fungal genus present in aerosols generated during waste handling (Malta-Vacas *et al.*, 2012; Lehtinen *et al.*, 2013; Pinto *et al.*, 2015), but *Penicillium* species are difficult to identify by microscopy and PCR-based methods (Knutsen *et al.*, 2012; Madsen *et al.*, 2015), and no information is available on occupational exposure at species level. *Staphylococcus*, *Bacillus*, and *Micrococcus* have previously been found to be predominant bacterial genera present in the air at a waste packaging glass sorting plant, but the gram-negative genera *Acinetobacter*, *Shigella*, and *Klebsiella* were also present (Pinto *et al.*, 2015). To obtain knowledge on health effects of occupational exposure, it is important to acquire knowledge on exposure at species level (Wéry, 2014) because specific species may cause infections (Madsen *et al.*, 2015), while some may cause inflammation of the airways and allergy (Zhiping *et al.*, 1996; Bünger *et al.*, 2000; Douwes *et al.*, 2003).

In occupational settings sources of exposure to microorganisms can be a handled material such as moldy seeds (Madsen *et al.*, 2015), a handled product such as a biopesticide (Madsen *et al.*, 2014), or fungal growth on building materials (Sivasubramani *et al.*, 2004). For household waste collectors, the source of exposure may be very composite as Danish household waste consists of many different components, including 31% vegetable waste, 10% animal waste, 6.6% diapers, 3.3% yard waste, and 0.93% vacuum cleaner bags (Riber *et al.*, 2009). Therefore, several microbial species may be released from household waste. Endotoxin from bacteria seems to be transferred from composting plants to neighboring areas (Danneberg *et al.*, 1997), and elevated exposures to bioaerosols have been found in offices associated with work places where organic material is handled as in biofuel plants (Madsen, 2006), composting plants (Liebers *et al.*, 2012), and in homes of farmers (Normand *et al.*, 2011). Inside non-moldy buildings, the outdoor environment can be a source of exposure to fungi (Garrett *et al.*, 1998; Bush and Portnoy 2001; Gots *et al.*, 2003; Frankel *et al.*, 2012), while bacteria often seem to have indoor sources (Scheff *et al.*, 2000; Mitakakis *et al.*, 2000; Madsen *et al.*, 2012). Some workers, such as garbage collectors, drivers transporting biofuel to combusting plants, and sewage workers spend their working hours partly in a truck cab and partly in a work place where organic material is handled. In cars, the main focus has previously been on ventilation filters as sources of exposure (Li *et al.*, 2013; Angelakis *et al.*, 2014). The main hypothesis of this study is that microorganisms are transported from the outdoor work with waste collection and into the truck cab by the workers themselves.

The aim of this pilot study was to examine domestic waste collectors' exposure to airborne inhalable fungal and bacterial species during waste collection, and while seated in the truck cabs, and to acquire knowledge on transport of airborne fungi and bacteria from the outdoor work with waste collection and into the truck cab. In this study, airborne microorganisms have been collected using samplers in the truck cab and on the workers, and fungal and bacterial species have been quantified and identified using matrix-assisted-laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Previous studies have shown seasonal variations in outdoor concentrations of fungi (Frankel *et al.*, 2012) and in waste collectors' exposures to fungi (Nielsen *et al.*, 1997), with lowest concentrations in the winter. This study was performed in the winter months to minimize interference from fungi not related to the waste handling.

MATERIAL AND METHODS

The workers and trucks

Thirteen waste collectors participated in the study, and exposure was measured on each worker during one work day. Workers collected the waste in domestic areas in Copenhagen, transported waste, or waste containers to the trucks, stood next to the waste when

it was unloaded, drove the trucks between the homes, and transported the waste to the waste-to-energy incinerator plant. Most workers worked together with the same colleague every day, but did not use the same garbage truck every day. All workers participated in the waste collection. The workers sat in the truck or drove the truck for 37% of their working day. The workers did not always change clothes, nor took a shower before they left work.

In total, 10 garbage trucks participated in the study (Table 1). No under pressure was present in the truck cabs. During the night, trucks were parked outdoors where the temperature in January and February was around −5 to +1°C; two work shifts used the trucks each day. The trucks were cleaned outside and inside once a week.

Samplers

Exposures were measured using GSP inhalable samplers (Gesamtstaubprobenahme, CIS by BGI, INC Waltham, MA, USA) at a flow rate of 3.5 l min^{-1}. Air flows of the samplers were checked before and after air sampling. The samplers were mounted with polycarbonate filters (pore size 1 µm, GE Water & Process Technologies, Trevose, PA, USA).

Sampling areas

Air sampling was conducted during four Mondays in the winter 2015 (Table 1). Sampling was performed in an outdoor area where the workers met at work, and picked up their trucks, and left them again after work. These samples are called outdoor reference samples; they were taken during the first three Mondays 1.5 m above ground level; on the last Monday the sample was lost. On average, 1139 l air was sampled and the average sampling time was 325 min. Temperature and relative humidity were measured outdoors using Tinytag Plus Data Loggers (Germini Data Loggers, UK) (Table 1).

Thirteen personal samples were taken with samplers mounted on the workers clothes. At the same time, airborne dust was sampled in 10 truck cabs (Table 1). For personal samples 839 l air was on average sampled for each person, and the sampling was performed for whole working days (on average 240 min). For samples in truck cabs, 946 l air was on average sampled with an average sampling time of 270 min. In addition to the three mentioned outdoor reference samples, another 22 reference samples taken

Table 1. Dates of sampling, number of workers and weather conditions

Sampling dates	**26 January 2015**			**02 February 2015**			**09 February 2015**			**16 February 2015**		**Copenhagen references[e]**
Truck abbreviation	1a	2a	3a	1b	2b	3b	1c	2c		1d	2d	—
Number of workers[a]	1	2	2	1 (1)	1 (1)	1	1 (1)	2		1 (1)	1 (1)	—
1th or 2th work team[b]	2	2	2	1	1	1	1	1		1	2	—
Outdoor reference		Yes			Yes			Yes			No	Yes 22
Temperature[c]		3.5–3.2°C			1.5–0.7°C			5.0–8.1°C			1.0–2.1°C	Av = 4.7°C, GM = 4.0°C
RH[c]		98.2–100%			71.6–87.6%			88.1–79.1%			85%	Av = 76.2%, GM = 74.1%
Average wind speed (max)[d]		5 m s^{-1} (14 m s^{-1})			4 m s^{-1} (12 m s^{-1})			5 m s^{-1} (16 m s^{-1})			8 m s^{-1} (17 m s^{-1})	—
Sunshine hours[d]		0			1			6			0	Av = 2

[a]Workers with GSP samplers (workers without samplers).
[b]First or second work team using the truck.
[c]Morning and noon temperatures or RHs.
[d]data obtained from DMI: Danish Meteorological Institute.
[e]Twenty-two outdoor measures in January and February 2011.

outdoors in domestic areas in Copenhagen in January and February 2011 were included in this study; the aim was to get knowledge on outdoor reference levels of bacteria and fungi.

Extraction of bacteria and fungi from filters

The same day as the sampling was performed, the bacteria and fungi collected on filters were extracted in 6.0 ml sterile water solution (with 0.05% Tween 80, 0.85% NaCl) by orbital shaking for 15 min (500 rpm) at room temperature.

Quantification of bacteria and fungi

Two-fold dilution series of extracts from polycarbonate filters were prepared. An amount of 600 µl or 500 µl aliquots were plated on agar plates for quantification of bacteria or fungi. The number of fungi culturable on Dichloran Glycerol agar (DG-18 agar, Oxoid, Basingstoke, UK) at 25°C was counted after 3 and 7 days of incubation. DG-18 agar was used as it in most studies gives higher counts than other agar media (Jo and Lee 2008). The numbers of bacteria were quantified after 3 and 7 days of incubation on 100% Nutrient agar (Oxoid) with actidione (cycloheximide; 50 mg l^{-1}; Serva, Germany) at 25°C. The data are presented as time weighted average exposures (TWA) in colony forming units (CFU) per m^3 air.

Identification

Bacterial and fungal isolates were identified using the MALDI-TOF MS Biotyper System (Bruker Daltonics, Bremen, Germany). Bacterial isolates were prepared using the extended direct transfer method according to manufacturer's recommendations. Briefly, a small amount of bacterial colony was smeared onto the MALDI-TOF MS target plate (MSP 96 target polished steel BC; Bruker Daltonics) and 1 µl of 70% formic acid was added. The mixture was allowed to dry at room temperature before being overlaid with 1 µl of HCCA matrix solution (α-cyano-4-hydroxycinnamic acid; Bruker Daltonics). Bacterial isolates that failed to give a positive identification using the extended direct transfer were subsequently extracted using the ethanol extraction method as described elsewhere (Madsen *et al.*, 2015). Fungal isolates were in all cases processed using a more elaborate sample preparation method based on ethanol extraction of liquid overnight cultures in Sabouraud Growth Medium (SGM; Oxoid) as previously described (Madsen *et al.*, 2015). A Microflex LT mass spectrometer (Bruker Daltonics) was used for the analysis and spectras were analysed using Bruker Biotyper 3.1 software with the BDAL standard library and filamentous library 1.0. Extractions and MALDI-TOF analysis were repeated up to four times in attempts to obtain positive identifications of fungal or bacterial isolates. A bacterial test standard (Bruker Daltonics) was used to calibrate the instrument. Fungi and bacteria are presented as TWA exposures (CFU of the specific species or genus per m^3 air).

Treatment of data

Concentrations were log transformed to approximate normal distribution. Pearson correlation was calculated between concentrations found in truck cabs versus in personal samples. Concentrations of microorganisms outdoor, in truck cabs, and in personal samples were compared using general linear model (GLM). Ratios between concentrations of bacteria and fungi measured in each of eight truck cabs on three Mondays versus outdoor on the same days were calculated; ratios were also calculated between concentrations in the 10 truck cabs versus the average concentrations during 22 days in the winter. In addition, fungi and bacteria divided into groups of species: (i) found both in truck cabs and personal samples, (ii) found only in personal samples, and (iii) found only in truck cab samples, were compared using GLM. SAS statistical software version 9.4 (SAS Institute, Cary, NC, USA) was used for all statistical analysis. Distributions of species or genera were calculated as follows: the concentration of a species in a sample (personal or truck cab) relative to concentration of all fungi or bacteria in that sample, and then the average presence of each species or genus in all 13 personal or all 10 truck cab samples was calculated.

RESULTS

Exposure levels

The concentration of microorganisms in the personal, the truck cab, and reference samples are presented in Table 2. There was a significant effect of sampled area on concentrations of fungi ($P < 0.0001$) and bacteria ($P = 0.0001$). There was no significant correlation between personal exposures to fungi

Table 2. Concentrations (geometric mean and range) of airborne fungi and bacteria

Samples	*n*[a]	Fungi CFU m^{-3} air		Bacteria CFU m^{-3} air	
A: Personal	13	5.7×10^3	1b	1.1×10^3	1
		$326–4.6 \times 10^4$		$112–4.8 \times 10^4$	
B: Truck cab	10	1.2×10^3	2	261	1,2
		$219–1.5 \times 10^4$		$48–2.6 \times 10^3$	
C: Outdoor reference[c]	3	73	3	50	2,3
		5–349		9.5–132	
D: Outdoor in Copenhagen[d]	22	15	3	11	3
		3–88		5–160	
GM (Av) and range of truck cab: outdoor reference ratios[e]	8	17 (111)		4.1 (7.7)	
		1.01–677		0.36–20	
GM (Av) and range of truck cab:average outdoor ratios[f]	10	85 (185)		24 (46)	
		14–420		3.7–226	

AV, average; CFU, colony forming units; GM, geometric mean value.
[a]*n* = number of samples.
[b]Values with same superscript numbers are not statistically significant different.
[c]Outdoor references taken where the workers picked up their trucks.
[d]Outdoor references from residential areas in Copenhagen.
[e]Ratio B to C.
[f]Ratio B to D.

and concentrations of fungi in truck cabs ($r = 0.25$, $P = 0.41$), and similar not between personal exposure to bacteria and bacterial concentration in the truck cab ($r = -0.45$, $P = 0.14$). Indoor (truck cab): outdoor ratios for bacteria were in 7 of 8 cases above 1, and for fungi always above 1. The ratios of concentrations in truck cabs versus average outdoor concentration in Copenhagen were in all 10 cases above 1 for both fungi and bacteria (Table 2).

Exposure to fungal species

The average of distributions of fungal species (%) calculated from CFU m^{-3} data for each sample is shown in Figs 1 and 2. *Penicillium* constituted 84 and 92% of all fungi in personal and truck cab samples, respectively, with *P. digitatum* constituting 18.8 and 23.4% of fungi in personal and truck cab samples, respectively. In total, 11 *Penicillium* and 6 *Aspergillus* species were found and identified.

Identical *Penicillium* and *Aspergillus* species were found both in a personal sample and in the same person's truck cab (Table 3). For example, *P. brevicompactum* and *P. italicum* were found both in truck cab 3a and in the personal samples of the workers using that truck. On average, concentrations of species found both in the truck cab and in the personal sample were 27 times higher in personal samples (Table 3). Some species were found only in the personal (Table 4) or truck cab (Table 5) samples. The average concentration of species found only in personal samples was 1123 CFU m^{-3} air while the concentration in personal samples of species found also in truck cabs was 3512 CFU m^{-3} (Table 3 and 4)—the difference was significant ($P = 0.0004$). The concentration of fungal species found only in truck cabs (Table 5) versus concentrations in truck cabs of species also found in personal samples (Table 3) were not significantly different ($P = 0.38$).

Outdoor measurements showed presence of only few species: *A. fumigatus* (12 CFU m^{-3}, week 1), *C. herbarum* (week 1: 18 CFU m^{-3}, week 2: 51 CFU m^{-3}, week 3: 159 CFU m^{-3}), *P. brevicompactum* (week 1: 185 CFU m^{-3}), *P. commune* (week 3: 49 CFU m^{-3}), and *P. digitatum* (week 3: 141 CFU m^{-3}).

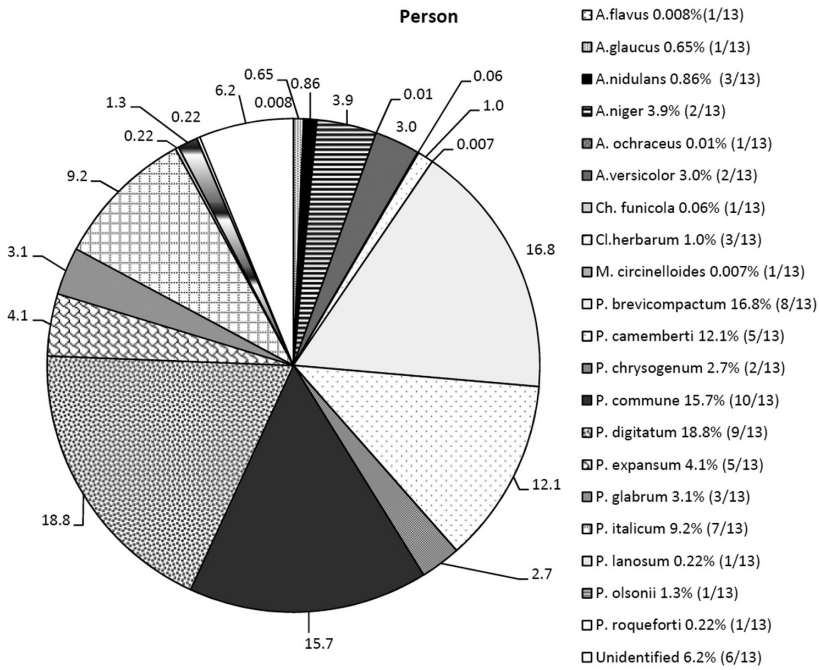

Figure 1. Average distribution (%) of concentration of fungal species in personal (n = 13) samples. A. = *Aspergillus*, Ch. = *Chaetomium*, Cl. = *Cladosporium*, M. = *Mucor*, P. = *Penicillium*. The fractions mentioned after each name are fractions of positive samples, e.g. *A. flavus* was found in 1 of 13 samples.

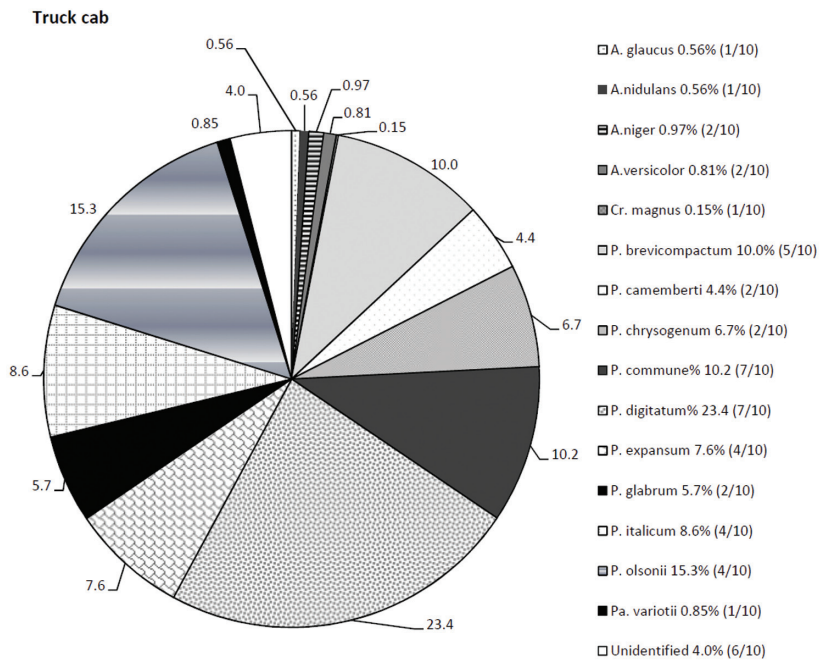

Figure 2. Average distribution (%) of concentration of fungal species in cabin (n = 10) samples. A. = *Aspergillus*, Cr. = *Cryptococcus*, P. = *Penicillium*, Pa. = *Paecilomyces*. The fractions mentioned after each name are fractions of positive samples, e.g. *A. glaucus* was found in 1 of 10 samples.

Table 3. Concentrations of airborne fungal species found both in a personal sample and in the same person's truck cab

Date	Truck[a]	Genus	Species	Personal samples CFU m^{-3} air	Truck cab samples CFU m^{-3} air	Ratio Personal: truck cab
26 January 2015	**1a**	*Penicillium*	*brevicompactum*	157	58	2.7
			commune	313	161	1.9
			digitatum	1.0×10^3	37	28
	2a	*Penicillium*	*commune*	6.9×10^{3b}	68	102
				6.0×10^3		89
			digitatum	2.1×10^3	306	6.8
				2.3×10^3		7.4
	3a	*Penicillium*	*brevicompactum*	7.9×10^3	205	38
				Bd[3]		—
			commune	66	46	1.4
				2.0×10^3		31
			digitatum	1.3×10^3	58	23
				667		12
			italicum	2.0×10^3	615	3.2
				3.3×10^3		5.4
02 February 2015	**1b**	*Penicillium*	*camemberti*	543	487	1.1
			italicum	4.9×10^3	143	34
	2b	*Penicillium*	*brevicompactum*	8.8×10^3	86	103
			commune	442	43	10
			digitatum	4.4×10^3	43	103
	3b	*Penicillium*	*brevicompactum*	309	305	1.0
			commune	254	103	2.5
09 February 2015	**1c**	*Penicillium*	*commune*	4.1×10^3	416	10
			digitatum	3.7×10^4	3.3×10^3	11
			italicum	1.1×10^3	416	2.6
	2c	*Penicillium*	*chrysogenum*	bd	154	—
				2.3×10^3		15
			olsonii	bd	154	—
				1.5×10^3		9.9

Table 3. ***Continued***

Date	Truck[a]	Genus	Species	Personal samples CFU m^{-3} air	Truck cab samples CFU m^{-3} air	Ratio Personal: truck cab
16 February 2015	**1d**	*Penicillium*	*commune*	3.2×10^3	61	53
			digitatum	3.9×10^3	1408	2.7
	2d	*Aspergillus*	*nidulans*	408	42	10
			niger	3.5×10^3	24	147
		Penicillium	*expansum*	1.2×10^3	59	20
			glabrum	589	59	10
			italicum	1.2×10^3	59	20
Average of positive samples				**3.5×10^3**	**320**	**27**
Geometric mean of positive samples				**1.5×10^3**	**132**	**11**

Bd, below detection limit in samples from one of two workers; CFU, colony forming units.
[a]Truck cabs positive of the species.
[b]Two concentrations are written as two workers were present in the same truck cab.

Exposure to bacterial species

The average of distributions of bacterial genera (%), calculated from CFU m^{-3} data, in truck cabs and personal samples are shown in Figs 3 and 4. In total, 38 different bacterial species were found. The genus *Micrococcus* followed by *Staphylococcus* constituted the largest portions of all bacteria in both truck cab and personal samples. Several skin-related bacteria as *Brevibacterium aurantiacum*, *M. luteus*, *S. capitis*, *S. epidermidis*, *S. hominis*, and *S. warneri* were found. Within the *Micrococcus* genus three different species were found, while six different *Staphylococcus* species (*S. capitis*, *S. epidermis*, *S. equorum*, *S. hominis*, *S. saprophyticus*, and *S. warneri*) were found.

Some species were found both in personal and truck cab samples (Table 6). Other species such as *Bacillus cereus*, *B. megaterium*, *B. pumilus*, *Kocuria rhizophila*, *Microbacterium phyllosphaerae*, *Pseudomonas putida*, and different *Streptomyces* species were found mainly in personal samples (Table 7).

The average concentration of bacterial species found only in personal samples was 115 CFU m^{-3} air, while the average concentration of species in personal samples also found in truck cabs was 2.7×10^3 CFU m^{-3} (Table 6 and 7)—the difference was significant ($P = 0.0055$). The concentration of bacteria in truck cabs of species also found in personal samples (Table 6) were significantly higher than the concentration of bacteria found only in truck cabs ($P = 0.028$) (Table 8).

Exposure was measured on both workers in trucks 2a, 3a, and 2c. In some cases both workers were exposed to comparable concentrations of the same species, e.g. workers in truck 2c and the bacterium *M. luteus* (Table 6), while only one of two workers was exposed to e.g. low concentrations of a *Bacillus* species (Table 7).

Only few bacterial species were found in the outdoor reference samples: *Kocuria palustris* (Week 1: 65 CFU m^{-3}), *Rhodococcus fascians* (Week 2: 103 CFU m^{-3}), and *Paenibacillus amylolyticus* (Week 3: 23 CFU m^{-3}). These species were not detected in the truck cabs.

DISCUSSION

The concentrations of airborne fungi in the truck cabs (GM = 1.2×10^3 CFU m^{-3}) were significantly elevated in comparison with levels outdoor and higher than levels previously measured in Danish homes (median =26 CFU fungi m^{-3} in the winter) (Frankel

Table 4. Concentrations of fungal species found in personal samples, but not in the same person's truck cab

Genus	Species	Concentrations CFU m^{-3} air	*n*[a]
Aspergillus	*flavus*	12	1
	glaucus	759	11
	nidulans	48; 78	2
	niger	1517	1
	ochraceus	Bd[b]; 18	1
	versicolor	20; 52	2
Chaetomium	*funicola*	11	1
Cladosporium	*herbarum*	24; 157	2
Penicillium	*brevicompactum*	1.4×10^3; 1.5×10^3; 2.9×10^3	2
	camemberti	589; 7.7×10^3	2
	commune	333	1
	digitatum	238	1
	expansum	Bd; Bd; 78;759; 3.3×10^3	3
	glabrum	Bd; 238	1
	italicum	Bd; 78; 7.6×10^3	2
	lanosum	441	1
	roqueforti	442	1
Mucor	*circinelloides*	Bd; 18	1
Average (geometric mean) of positive samples		**1.1×10^3 (233)**	—

CFU, colony forming units.
[a]*n*, numbers of truck cabs with positive workers.
[b]Bd, below detection limit on one of two workers, i.e. if only one of two workers in the truck cab was exposed to that species.

et al., 2012). Interestingly, fungal species found in the workers' inhalation zones were often also found in the same worker's truck cab, albeit in lower concentrations. Different species were found in different truck cabs during the same day, and thus most spores in the truck cabs seem to be transported into the truck cabs by the workers' clothing and/or hair. We have found no other papers describing this transport of fungal spores by workers into truck cabs and the subsequent reaerosolization of spores. However, in social rooms at composting plants (Liebers *et al.*, 2012), and in offices at biofuel plants (Madsen, 2006) elevated bioaerosol concentrations have been measured, indicating transport of bioaerosols from one environment to another. Furthermore, humans are seen to carry fungal spores on their skin, and *Cryptococcus* spp., *Cladosporium* spp. and *Penicillium* spp. have been found in high frequencies in samples from human skin (Adams *et al.*, 2013)—but re-aerosolization was not described.

Indoor (truck cab): outdoor ratios for bacteria were higher than 1. Generally, studies comparing the indoor and outdoor levels of bacteria in buildings have found that the indoor:outdoor ratios are above 1 (Moschandreas *et al.*, 2003; Bartlett *et al.*, 2004; Kalogerakis *et al.*, 2005; Adhikari *et al.*, 2010;

Table 5. Concentrations of fungal species found in the truck cabs, but not in personal samples of workers belonging to that truck cab

Genus	Species	Concentrations (CFU m^{-3} air)	Trucks[a]
Aspergillus	*glaucus*	51	3b
	niger	416	1c
	versicolor	12, 416	2a; 1c
Cryptococcus	*magnus*[b]	22	1d
Paecilomyces	*variotii*	43	2b
Penicillium	*brevicompactum*	1248	1c
	camemberti	153	2a
	chrysogenum	86	2b
	digitatum	354	2d
	expansum	24; 305; 4.9×10^3	1b; 3b; 1d
	glabrum	689	2a
	olsonii	80; 215; 683	1a; 3a; 2b
Average (geometric mean)		**569 (177)**	—

CFU, colony forming units.
[a]Truck cabs positive for the species.
[b]Yeast.

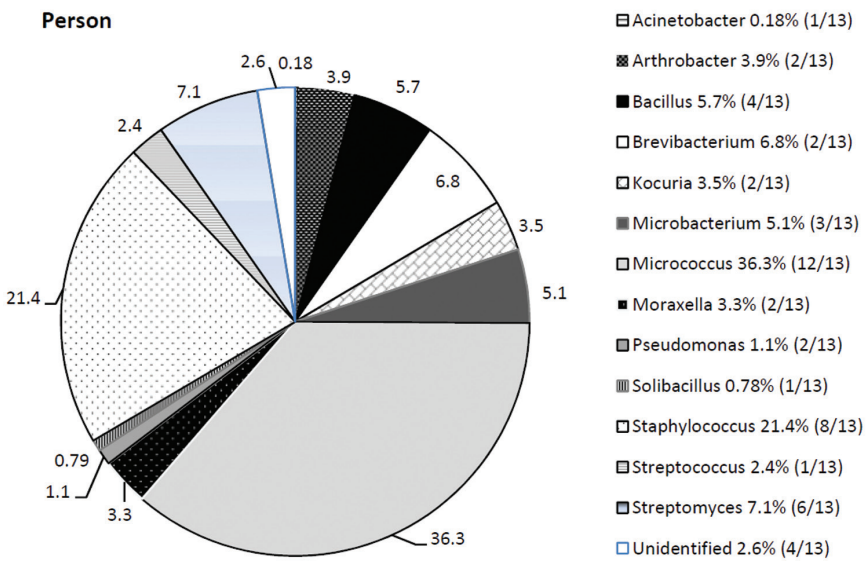

Figure 3. Average distribution (%) of concentration of bacterial genera in personal ($n = 13$) samples. The fractions mentioned after each genus are fractions of positive samples.

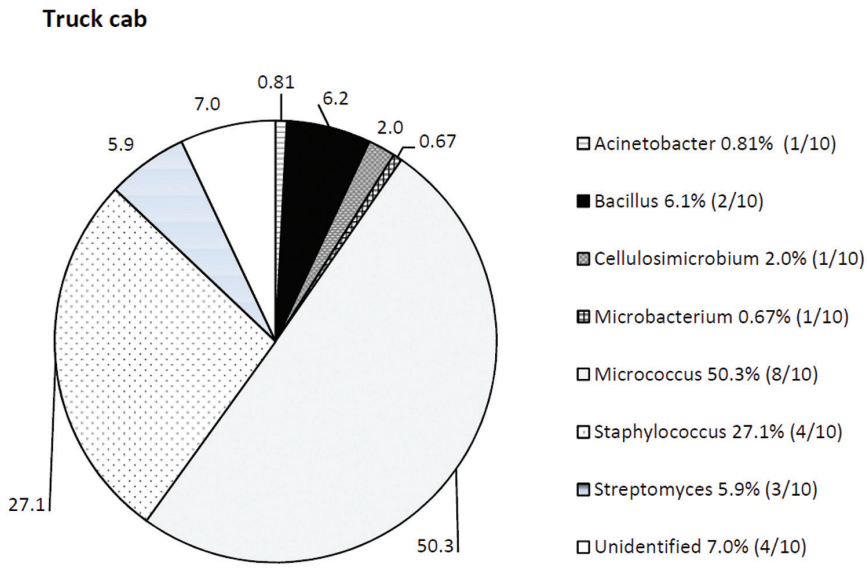

Figure 4. Average distribution (%) of concentration of bacterial genera in cabin (n = 10) samples. The fractions mentioned after each genus are fractions of positive samples.

Frankel *et al.*, 2012), while the usual indoor:outdoor ratio for fungi in buildings is below 1 (Scheff *et al.*, 2000; Bush and Portnoy 2001; Millington and Corden 2005; Frankel *et al.*, 2012). However, in temperate climates during winter, indoor fungal concentrations can become higher than outdoors (Hunter *et al.*, 1988; Frankel *et al.*, 2012). In the truck cabs, the indoor:outdoor ratio for fungi was 17 (GM value). In large scale enclosed and open-air composting facilities, concentrations of fungi and bacteria have been measured inside and outside the truck cabs. In these environments, concentrations were much higher outside the cab than inside (Schlosser *et al.*, 2012). At composting facilities, large amounts of organic material are present in open boxes and front-end loaders are used (Schlosser *et al.*, 2012). In contrast, waste collectors handle closed containers which are only opened when they are unloaded in the rear end of the trucks. The waste collectors went in and out of the truck cab during the whole work day, and a portion of the indoor spores may have entered the door and may derive from the unloading process. However, this contribution may be limited due to the wind and dilution in the outdoor air and the fact that unloading is in the rear end of the truck. Furthermore, for waste collectors the personal exposure to fungal species found both in personal and truck cab samples (GM = 1.5×10^3 CFU m^{-3}) was significantly higher than the exposure to species found only in personal samples (GM = 233 CFU m^{-3}). This further indicates that workers carry fungi from their outdoor work with waste collection into the truck cabs. Mainly *Penicillium* species seem to be carried into the truck cabs, and this is likely related to the high concentrations of this genus. The presence of *Penicillium* is of relevance in relation to workers' health as a review study concluded that exposure to *Penicillium* can be associated with expiratory flow rate variability in people with asthma, and can induce both immediate and late asthma in sensitive persons (Knutsen *et al.*, 2012).

The workers were exposed to 11 different *Penicillium* species, and on average the genus constituted 84% of each worker's fungal exposure. In waste sorting plants (Malta-Vacas *et al.*, 2012; Pinto *et al.*, 2015) and at solid waste management plants (Lehtinen *et al.*, 2013) most airborne fungi also belonged to the genus *Penicillium*, but species were not identified. In a study with grass seed workers, four airborne *Penicillium* species (*P. brevicompactum*, *P. camemberti*, *P. chrysogenum*, and *P. commune*) were found (Madsen *et al.*, 2015), and these species were all among the species found in this study. Waste collectors were exposed to six *Aspergillus* species, and on average the genus constituted 8.4% of each workers fungal exposure. Workers were also exposed

Table 6. Concentration of bacterial species found in personal samples and in the truck cab of the same persons

Date	Truck[a]	Genus	Species	Personal samples (CFU m^{-3} air)	Truck cab (CFU m^{-3} air)	Ratio Personal: Truck cab
26 January 2015	1a	*Staphylococcus*	*epidermidis* (h)	64	32	2.0
	2a	*Micrococcus*	*luteus* (h)	21	2.6×10^3	0.008
				76		0.029
02 February 2015	1b	*Micrococcus*	*luteus* (h)	190	110	1.72
	2b	*Micrococcus*	*luteus* (h)	5.5×10^3	431	13
		Staphylococcus	*saprophyticus* (h)	44	431	0.10
	3b	*Micrococcus*	*luteus* (h)	154	203	0.76
09 February 2015	1c	*Micrococcus*	*luteus* (h)	33	42	0.79
	2c	*Bacillus*	*altitudinis* (o)	Bd[b]	32	—
				110		3.4
			pumilus (o)	Bd	12	—
				303		25
		Micrococcus	*luteus* (h)	2.3×10^3	42	54
				1.9×10^3		45
		Streptomyces	*chartreusis* (o)	88	12	7.3
				65		5.4
			violaceoruber (o)	83	11	7.5
				76		6.9
16 February 2015	1d	*Micrococcus*	*luteus* (h)	437	227	1.9
		Staphylococcus	*capitis* (h)	3.7×10^4	29	1289
			epidermidis (h)	1.0×10^4	48	215
		Streptomyces	*violaceoruber* (o)	402	32	12.6

Table 6. *Continued*

Date	Truck[a]	Genus	Species	Personal samples (CFU m^{-3} air)	Truck cab (CFU m^{-3} air)	Ratio Personal: Truck cab
	2d	*Micrococcus*	*luteus* (h)	299	118	2.5
		Streptomyces	*badius* (o)	169	28	6.0
Average (geometric mean) of all bacteria				**2.7×10^3 (274)**	**247 (68)**	**77(4.1)**
Average (geometric mean) of 'other' bacteria				**162 (130)**	**21 (19)**	**9.3 (7.8)**
Average (geometric mean) of 'human' bacteria				**4.2×10^3 (419)**	**360 (130)**	**116 (2.8)**

Human-derived bacteria (h), which may also derive from the waste. Other environmental bacteria (o) expected to derive from the waste. CFU, colony forming units.
[a]Truck cabs positive for the species.
[b]Bd is if only one of two workers in the same truck cab was exposed.

to *Cladosporium herbarum* (0.96%) and *Mucor circinelloides* (0.36%). At a waste management plant in Finland, 93% of the fungi found in a waste sorting process hall belonged to *Penicillium*, 0.2% to *Aspergillus*, 0.2% to *Cladosporium*, and 0.1% to *Mucor*; in a control room, 89% belonged to *Penicillium*, 8.9% belonged to other *Aspergillus*, 0.8% to *Cladosporium*, and 0.2% to *Mucor* (Lehtinen *et al.*, 2013). Thus, these distributions resemble the distribution at genus level for waste collectors found in this study. *Alternaria alternata* was not found in this study, although it is common in, e.g. home dust (Barberán *et al.*, 2015). In addition to environmental differences, a likely explanation for its absence could be seasonal variation in occurrence, as highest concentrations of *A. alternata* have previously been observed during summer and autumn (Corden and Millington 2001; Skjøth *et al.*, 2012).

In total, 38 different species of airborne bacteria were found. In a waste disposal area in Saudi Arabia, 22 different airborne species of bacteria were found (Angelakis *et al.*, 2014), but only 2 species (*B. cereus* and *S. equorum*) were among the species found in this study—even though the same agar medium and identification method were used in both studies. A reason for the difference may partly be different climates. *Micrococcus*, *Staphylococcus*, and *Bacillus* were the most frequent found genera in this study as well as in a study at a waste packaging glass sorting plant (Pinto *et al.*, 2015). Four different *Streptomyces* species were found in the workers' exposure. *Streptomyces* has also been found in dust in homes (Johansson *et al.*, 2011). *B. megaterium*, *B. pumilus*, *K. rhizophila*, and *P. putida* were found mainly in the personal samples of the workers. These bacteria are typically associated with soil, food, or plant materials, and *B. pumilus* has also been found in the air in waste treatment plants (Rahkonen, 1992). These bacteria, expected to have waste as the source, were also present as airborne bacteria in the truck cabs indicating that bacteria are reaerosolized in the truck cab. In a study concerning bacteria on surfaces a significant correlation between the types of bacteria deposited on surfaces outside and inside homes was found, also indicating that bacteria from outside may have an effect on bacteria inside a home (Dunn *et al.*, 2013).

Human skin-related bacteria such as *M. luteus*, *S. capitis*, *S. epidermidis*, *S. hominis*, and *S. warneri* were all found in the truck cabs. *S. hominis*, *S. warneri*, and *M. luteus* have also been found in the air in waste

Table 7. Concentrations of bacterial species found in personal samples, but not in the same person's truck cab

Genus	Species	Concentration (CFU m^{-3} air)	*n*[a]
Acinetobacter	*lwoffii* (h)	22	1
Arthrobacter	*creatinolyticus* (o)	543	1
	oxydans (o)	8	1
	sulfonivorans (o)	18	1
Bacillus	*cereus* (o)	Bd[b]; 62; 73	2
	firmus (o)	Bd; 8	1
	megaterium (o)	Bd; 59	1
	mycoides (o)	Bd; 16; 17	2
Brevibacterium	*aurantiacum* (h)	Bd; 21; 609	2
Kocuria	*rhizopila* (o)	607; 1157	1
Microbacterium	sp. (o)	76	1
	aerolatum (o)	42	1
	phyllosphaerae (o)	Bd; 76	1
Micrococcus	*flavus* (h)	515	1
	luteus (h)	17; 67	1
	terreus (h)	48	1
Moraxella	*osloensis* (o)	95; 263	2
Pseudomonas	*antarctica* (o)	379	1
	monteilii (o)	41	1
	putida (o)	24	1
Solibacillus	*silvestris* (o)	44	1
Staphylococcus	*epidermidis* (h)	Bd; 72	1
	equorum (h)	Bd; Bd; 172; 109	2
	hominis (h)	16; 173	2
	saprophyticus (h)	Bd; 76	1
Streptococcus	*salivarius* (h)	290	1
Streptomyces	*badius* (o)	Bd, Bd; 21; 35	2
	chartreusis (o)	65; 88	1
	galilaeus (o)	Bd; 18	1
	violaceoruber (o)	Bd, Bd; 21; 28	2

Table 7. ***Continued***

Genus	Species	Concentration (CFU m^{-3} air)	n^a
Average (geometric mean) of positive samples		**115 (58)**	—
Average (geometric mean) of 'other bacteria'		**88 (47)**	
Average (geometric mean) of 'human bacteria'		**168 (91)**	—

Human-derived bacteria (h), which may also derive from the waste. Other environmental bacteria (o) expected to derive from the waste.
CFU, colony forming units.
[a]*n* = numbers of truck cabs with positive workers.
[b]Bd is if only one of two workers in the same truck cab was exposed.

Table 8. Concentrations (CFU m^{-3} air) of bacterial species found in the truck cabs, but not in personal samples of workers belonging to that truck cab

Genus	Species	Concentration (CFU m^{-3} air)	Truck[a]
Acinetobacter	*lwoffii* (h)	230	2a
Bacillus	*pumilus* (o)	37	1b
Cellulosimicrobium	*cellulans* (o)	51	3b
Microbacterium	sp. (o)	41	1d
Microbacterium	*liquefaciens* (o)	12	1d
Staphylococcus	*epidermidis* (h)	137	3a
	warneri (h)	68	3a
Average (geometric mean)		**82 (57)**	—

Human-derived bacteria (h), which may also derive from the waste. Other environmental bacteria (o) expected to derive from the waste.
CFU, colony forming units.
[a]Positive trucks.

treatment plants (Rahkonen, 1992). Studies on bacteria inside buildings show a significant correlation between staphylococci and occupancy (Bartlett *et al.*, 2004), and between numbers of persons present and concentration of total airborne bacteria (Scheff *et al.*, 2000; Sessa *et al.*, 2002; Madsen *et al.*, 2012). As human skin-related bacterial genera can be found in domestic dust (Hospodsky *et al.*, 2012), domestic dust may, in addition to the workers themselves, contribute to the exposure to skin-related bacteria found during waste collection. Skin associated bacteria have also been found on laboratory coats, and with a progressive contamination as they were worn (Wilson *et al.*, 2007). In this study, six different *Staphylococcus* species were found, but no *S. aureus* was found.

Some Gram-negative species are of special interest because they can cause gastroenteritis (Jeggli *et al.*, 2004; Oppliger *et al.*, 2005). However, none of the Gram-negative bacteria found in the present study were commonly recognized gastrointestinal pathogens. One of the Gram-negative bacteria was *Acinetobacter lwoffii*. It was found both in an air sample from the truck cab and in a personal sample. *A. lwoffii* is ubiquitous in nature, can be part of the normal human skin flora (Regalado *et al.*, 2009), and has previously been found as an airborne bacterium at a composting facility (Reinthaler *et al.*, 1997), but can also cause illness e.g. septicemia, pneumonia, urinary tract and skin infections, and in rare cases gastroenteritis in immunocompromised individuals

(Regalado *et al.*, 2009). Three species of *Pseudomonas* were also found. One of them, *P. monteilii*, has previously been isolated on surfaces in homes (Remold *et al.*, 2011), while another, *P. putida*, has been found on yard soil (Remold *et al.*, 2011), in the air of waste treatment plants (Rahkonen 1992), and a wastewater treatment plant (Laitinen *et al.*, 1994). *P. putida* can be infectious mainly in hospitalized patients (Yang *et al.*, 1996). Finally, two workers were on two different days exposed to *Moraxella osloensis*. In indoor dust in homes, the genus *Moraxella* has been associated with presence of dogs (Barberán *et al.*, 2015). The presence of only very few Gram-negative bacteria in the dust samples may be because waste-related bacteria are mainly Gram-positive. It may also be because Gram-negative bacteria are more sensitive to the aerosolization and sampling than Gram-positive bacteria. In addition, some Gram-negative bacteria may be among the unidentified bacteria. In congruence with our findings, a predominance of Gram-positive bacteria has previously been found in personal aerosol samples from grass seed workers (Madsen *et al.*, 2015), and in samples from homes (Madsen *et al.*, 2012; Rintala *et al.*, 2012). Using PCR-based methods for identification, Gram-positive bacteria (*Actinobacteria* and/or *Bacilli*) were also among the dominating bacteria on surfaces in homes (Dunn *et al.*, 2013) and in soil (Lauber *et al.*, 2009).

To our knowledge, only very few studies have previously quantified fungi and bacteria in truck cabs. In one study, bacterial concentrations in cars were found to be around 50–150 CFU m^{-3}, but could reach up to 2550 CFU m^{-3} (Jo and Lee 2008), and thus were within the levels found in this study. It was not mentioned whether people were present in these cars during sampling. In the same study, the fungal concentrations were around 20–200 CFU m^{-3} (Jo and Lee, 2008), which is lower than levels measured in the truck cabs in this study. This further supports the hypothesis of transport of fungi on waste collectors into the truck cab. It also indicates that waste collection workers should change clothes after work to reduce transfer of microorganisms to their homes or other indoor environments. Outside the cars, in the study by Jo and Lee, the concentrations of bacteria were higher than outdoor measures in this study.

This study was performed in the winter, and workers were during waste collection exposed to between 112 and 4.8×10^4 CFU bacteria m^{-3} air (GM = 930 CFU m^{-3} air), and to between 326 and 4.6×10^4 CFU fungi m^{-3} air (GM = 5.7×10^3 CFU m^{-3} air). In a study in Denmark performed in the 1990s, waste collectors were exposed to between 400 and 6×10^3 CFU bacteria m^{-3} air ($n = 6$), and between 4.8×10^3 and 1.8×10^4 CFU fungi m^{-3} air ($n = 6$) in the winter (Nielsen *et al.*, 2000). At a waste management plant in Finland, 1.2×10^3 CFU bacteria m^{-3} and 3.5×10^3 CFU fungi m^{-3} were measured (average of four seasons, stationary sampling) (Lehtinen *et al.*, 2013). In an indoor composting facility in France in summer and spring, using stationary samplers, fungal concentrations between 2×10^3 and 1×10^5 CFU m^{-3} have been found (Duquenne *et al.*, 2012). At a paper selection landfill area, stationary measurements showed a concentration of 469 CFU fungi m^{-3}, and at a plant recycling glass and plastic, 944 CFU fungi m^{-3} was found in the winter (Carducci *et al.*, 2013). Thus, exposure levels, measured in this study, resemble exposure levels in similar environments.

In conclusion, fungal and bacterial species, found in high concentrations in personal samples, were also found in truck cabs, but in lower concentrations indicating that fungi and bacteria are transported by the workers into the truck cab. *Penicillium* species dominated the fungal exposure, and workers were exposed to 11 different *Penicillium* species and 12 other fungal species. Furthermore, workers were exposed to 38 bacterial species including skin-related bacteria and bacteria expected to origin from the waste. To reduce exposure inside the truck cab focus should be on interventions related to the unloading of waste and on good hygiene in the truck cab.

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