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Measures to reduce the exposure of waste collection workers to handborne and airborne microorganisms and inflammogenic dust

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ABSTRACT

Waste collection is associated with various health symptoms. The aims of this study were to obtain knowledge about exposure to bacteria, fungi, and endotoxin during waste collection, and to study whether it is possible to reduce the exposures and the total inflammatory potential (TIP) of those exposures through simple interventions. The study was performed with an initial baseline exposure assessment, a second assessment with intervention workers only, and a third with intervention and reference workers.

The waste collection workers were exposed to 7.8×10^3 cfu bacteria/m^3, 1.4×10^4 cfu fungi/m^3, and 92 endotoxin units/m^3 (geometric mean values). The potential exposures in the truck cabs were up to 23 times higher than outdoor reference concentrations. For the intervention trucks and workers, airborne fungi in the truck cab were reduced; fungi, bacteria, and yeasts on the steering wheels were reduced; and the concentration of fungi on the workers' hands was reduced.

Exposures were typically highest during collection of mixed household waste, in the summer, and for collection using trucks with low loading height. The TIP was highest for the reference group sampling mixed household waste, using trucks with low loading height, in the summer. Endotoxin, bacteria, and fungi contributed to the TIP of 42 personal exposure assessments.

Conclusion: Motivating workers to reduce exposure through simple interventions improved hand and truck cab hygiene, but only slightly reduced personal exposure to airborne bioaerosols. Exposure can be reduced by only using trucks with high loading height.

1. Introduction

Studies from the 1990s found that work with waste collection was associated with various symptoms related to the airways, gastrointestinal complaints, and skin irritation (Allmers et al., 2000; Bünger et al., 2000; Poulsen et al., 1995; Yang et al., 2001). Publications from 2010 and forward show that waste collection work is still associated with respiratory symptoms (Athanasiou et al., 2010; Darboe et al., 2015; Kuijer et al., 2010; Poole and Wong, 2013; Schantora et al., 2014) including e.g. reduced lung function (Athanasiou et al., 2010; Vimercati et al., 2016), chronic bronchitis (Schantora et al., 2014), symptoms of the eyes (Schantora et al., 2014), nail infections, gastrointestinal complaints, and dermatological problems (Kuijer et al., 2010). Only a few studies on exposure to bioaerosols during waste collection have been published since 2010 – even though the working environment in some countries has changed due to new and expanded waste sorting instructions in order to increase recycling, and even though this expanded sorting may cause reduced waste collection frequencies for some types of waste (Madsen et al., 2019). However, the few studies show that collection of household waste is still associated with elevated exposure to bioaerosols (Lavoie et al., 2006; Madsen et al., 2016; Ncube et al., 2017; Park et al., 2011).

For workers collecting household waste very different exposure levels have been measured in different studies, thus e.g. exposures to endotoxin ranging from below detection level (bd) to 53 endotoxin units (EU)/m^3 (medians between 3.6 and 25 EU/m^3) have been found for Danish waste collectors (Nielsen et al., 1997), between <4 and 7182 EU/m^3 (geometric mean values (GM) = 40 EU/m^3) for waste collectors in the Netherlands, and an average of 1123 EU/m^3 for workers collecting and sorting waste in South Korea (Park et al., 2011). It is not known whether these differences are related to differences in e.g. waste collection frequencies, waste types, collection systems, etc. between e.g. countries. However, studies have shown that season and humidity (Park et al., 2011),

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temperature (Madsen et al., 2019), waste type (Nielsen et al., 1997), and collection equipment (Breum et al., 1996; Nielsen et al., 1997) have an effect on the exposure level to some of the measured bioaerosol components. Furthermore, the concentrations of volatile organic compounds released from urban waste increase with decreased waste collection frequency (Statheropoulos et al., 2005).

In developed countries, the work tasks of waste collection workers typically consist of truck driving, collection and unloading of waste containers or sacks, and unloading of the waste at the waste receiving plants. The waste collection workers usually lack access to hand washing during their workday. The importance of hand hygiene is mentioned in some papers concerning waste workers (El-Wahab et al., 2014; Kiviranta et al., 1999), and Ncube et al. call for further studies on methods to provide opportunity for efficient hand washing for municipal solid waste workers (Ncube et al., 2017). For workers collecting dental solid waste, yeasts from the waste seem to contaminate the workers' hands (Vieira et al., 2018).

The aims of this study were to obtain knowledge on (a) waste collection workers' exposure levels to airborne bacteria, fungi, and endotoxin, (b) hand hygiene, and c) whether intervention in the form of a combination of attention to and knowledge of hygiene, conveniently available hand sanitizer, and adherence to a few basic, potentially exposure-reducing guidelines could reduce the exposure to airborne microorganisms and improve hand and truck cab hygiene. The study was performed with an initial baseline exposure assessment before the implementation of any intervention measures, followed by a second exposure assessment of intervention workers only, and finally a third exposure assessment of intervention and reference workers.

2. Material and methods

2.1. Study design and interventions

Two groups were formed from volunteers among waste collection workers from a waste collection company in Copenhagen, Denmark. The first group included workers who had volunteered to work according to the suggested intervention procedures (intervention workers). The other group, whose members were recruited on an ad-hoc basis the day before each exposure assessment, consisted of workers who worked according to their normal routine (reference workers). The study was performed with an initial baseline exposure assessment before any intervention measures were implemented (Day A, 7 February 2018). The second assessment of exposure included only the intervention workers (Day B, 1 March 2018), and the final, third exposure assessment included both the intervention and reference workers (Day C, 11 June 2018) (Table 1).

After the first exposure assessment, the intervention group was given a lecture (28 February 2018) on what previous studies have found regarding the impact of exposure on the health of waste collection workers and potential ways to reduce exposure. The lecture concluded with a question and answer session and a discussion of pertinent issues. The intervention measures were put forth as suggestions: Try to keep as much distance as possible to the waste when loading and unloading the truck; try to keep the inside of the truck cab clean and tidy; try to use hand sanitizer multiple times during the working day; try to use clean gloves every day, and try not to dry wet gloves in the truck cab. New instructions for the intervention workers were to collect only one container at a time and to use the built-in cart lift, which was operated from a panel on the side of the truck. To support the intervention group in integrating these measures into their daily routine, several steps were taken: The intervention teams were allocated dedicated trucks; from the interventions were implemented and until the end of the study, the intervention group also had to attend a biweekly seminar with the project group to improve their motivation to pay attention to their health in general; and finally, we made sure they were all aware that hand sanitizer was freely available. All workers already routinely used gloves during the collection of the waste, and all workers worked as both loaders and drivers during the same working day.

The municipal waste was primarily collected from residential areas with some small businesses. Inside the homes, mixed household waste is usually placed in a plastic bag, which is then disposed of in a lidded plastic container specifically for mixed household waste outside each house or apartment complex. All the trucks were compactor trucks and equipped with lifts which tipped the contents of the container into the rear end of the truck, but some trucks had high waste loading height and some low loading height. In total, 37 measurements were done on workers collecting mixed household waste (the residual household waste fraction and the biological waste fraction; in the following called household waste), 3 on workers collecting bulky waste, 1 collecting cardboard, and 1 collecting paper waste (Table 1). Bulky waste is typically collected every second month and left at the curb the night before, while cardboard and paper are each sorted into a plastic container and collected every 30 days (Madsen et al., 2019). Data on these three types are considered together as 'other waste'.

2.2. Sampling

Airborne inhalable microorganisms were sampled using personal and stationary GSP samplers (Gesamtstaubprobenahme, CIS by BGI, INC Waltham, MA, USA) at a flow rate of 3.5 l/min. The samplers were mounted with polycarbonate filters (pore size 1 μm, SUEZ – Water Technologies & Solutions, Feasterville-Trevose, PA, USA) for quantifications of colony forming units (cfu) of bacteria, fungi, and endotoxin analysis. Air flows of the samplers were checked before and after air sampling. In addition, one cassette with a filter was brought to the workplace each day. It was not connected to a pump and was used as a blank filter. Each waste worker carried a backpack with a pump inside, connected to a sampler which was attached to the shoulder strap of the backpacks, close to the breathing zone. In addition, samplers were mounted inside a total of 15 truck cabs of the garbage trucks collecting household waste and on the rear end of those trucks. However, one sampler on the back of a truck was not running at the end of the shift, and the sample was not included in the analyses (Table 1). An outdoor reference sample was taken where the workers picked up their trucks in the morning. When the waste collection shift was over, the pumps were switched off and the samplers were removed and immediately transported to the laboratory.

2.3. Extraction of dust with bacteria, fungi, and endotoxin from filters

Within 2 h after sampling, the bacteria and fungi collected on polycarbonate filters were extracted in 6.0 ml sterile solution (0.05% Tween 80 and 0.85% NaCl) by orbital shaking (500 rpm) for 15 min at room temperature. The personal samples for endotoxin analysis were centrifuged (1000*g*) for 15 min to remove particles.

2.4. Sampling from hand and steering wheel

Sampling was done using the eSwab transport system (eSwab; Copan, Brescia, Italy), consisting of a flocked nylon swab in 1 ml of modified Amies liquid transport medium. At the end of the three working days, hand samples were taken from both palms (which

Table 1
Temperatures, number of waste workers collecting different waste types, working hours, and types and numbers of samples taken.

	Day A Exposure measurement Baseline		Day B Exposure measurement		Day C Exposure measurement
Avg. temperature during the day	−3°C	–	−5°C	–	21 °C
Number of intervention workers[1)] and waste type	1 × bulk, 9 × household	Lecture on exposure	1 × bulk, 12 × household	Focus on health	1 × bulk, 1 × paper, 1 × cardboard 7 × household
Working hours[2)]	4.5 h		4.1 h		6.2 h
Number of reference workers and waste type	5 × household	–	–	–	4 × household
Working hours	3.8 h				5.4 h
Numbers and types of intervention truck samples[3)]	2 w, 3 air, 3 bt	–	3 w, 3 air, 3 bt	–	3 w, 3 air, 3 bt
Sampling time[4)]	4.8 h		3.9 h		6.8 h
Numbers and types of reference truck samples	3 w, 2 air, 2 bt	–	–	–	3 w, 3 air, 3 bt
Sampling time	3.8 h				5.1 h
Waste loading height of trucks	6 × high, 9 × low	–	4 × high, 10 × low	–	8 × high, 5 × low
Numbers of outdoor reference samples	1		1		1

[1)] The numbers of workers collecting each waste type; hand swabs and air samples were collected from each person.
[2)] Working hours during the day of sampling = sampling hours for personal air samples.
[3)] w = steering wheel sample, air = air sample inside truck cab, bt = air sample from the back of the truck.
[4)] For air samples.

counted as 1 sample), and in total 42 samples were taken. In addition, samples were taken from the surface of the steering wheels, with the sampled area in the shape of a cylinder with a length of 5 cm; in total 14 steering wheel samples were taken.

2.5. Culturing and quantification of bacteria and fungi

Two-fold and ten-fold dilution series of extracts from polycarbonate filters were prepared and these as well as the hand and steering wheel samples were plated on agar plates for quantification of bacteria or fungi. The number of fungi culturable on Dichloran Glycerol agar (DG-18 agar; Thermo Fisher Scientific Oxoid, Basingstoke, UK), and bacteria on Nutrient agar (NA; Thermo Fisher Scientific Oxoid, Basingstoke, UK) with actidione (cycloheximide; 50 mg/l; Serva, Germany), both incubated at 25 °C, were counted after 3, 5, 7, and 14 days of incubation, while gram-negative bacteria (only personal air samples) were plated on SSI Enteric medium (SSI-agar, SSI Diagnostica, Denmark) at 37 °C and counted after 24 h. The data from air samples are presented as time-weighted average exposures (TWA) in cfu/m^3 air, hand samples as cfu/hand sample, and surface samples as cfu/ml. The term 'bacteria' is used for bacteria on NA agar and the term 'fungi' is used for fungi on DG-18 agar and does not include yeasts. The term 'yeasts' is used for yeast species on DG-18 agar. Yeast and SSI-agar bacteria data are only presented sparsely as some samples had only one or no colonies. The detections limits of the air samples depends on the sampling time. The maximum detection limit was for fungi, bacteria, and bacteria on SSI-agar all 9 cfu/m^3. For hand and steering wheel samples the dl was 5 cfu/ml.

2.6. Endotoxin

The dust suspensions from GSP samplers were centrifuged (1000*g*) for 15 min, and the supernatants were analyzed (in duplicate) for endotoxin from gram-negative bacteria using the kinetic Limulus Amoebocyte Lysate test (Kinetic-QCL endotoxin kit, Lonza Pharma & Biotech, Walkersville, Maryland, USA). A standard curve obtained from an Escherichia coli O55:B5 reference endotoxin was used to determine the concentrations in terms of EU (11.0 EU ≈ 1.0 ng). The data are presented as EU/m^3 air, and the limit of detection was 0.05 EU/ml corresponding to a maximum detection limit of 0.46 EU/m^3.

2.7. Measurement of the total inflammatory potential

Measurement of the total inflammatory potential (TIP) was conducted using an assay based on granulocyte-like cells. The assay is based on the differentiated HL-60 cell line (Human Promyelocytic Leukaemia cell line) which, upon exposure to inflammogens, will react by producing reactive oxygen species (ROS), quantifiable by a luminol-dependent chemiluminometric assay. The cells were differentiated for 6–7 days by adding Tretinoin (ATRA) without changing the growth medium (RPMI 1640, Biological Industries, Cromwell, CT, USA). The cells were seeded at 3×10^5 cells/ml and incubated at 5% CO_2 at 37 °C as described previously (Frankel et al., 2014). Personal samples were diluted 20 times with sterile water with 0.001% Tween 80 to reduce the amount of Tween in the samples. A volume of 100 μl of the diluted sample suspensions were in duplicate inoculated into 50 μl of the cells. The GM concentrations of bacteria, endotoxin, and fungi in the diluted samples were 64 cfu bacteria/ml, 0.86 EU/ml, and 117 cfu fungi/ml, respectively. The chemiluminesence reaction caused by sample activity was measured by a thermostated (37 °C) ORION II Microplate luminometer (Berthold Detection Systems, Germany), which measured relative light units per second (RLU/s) for 1 s every 120 s for 180 min. For every sample, accumulated RLU/s were calculated by summing the RLU/s measurements throughout the 180 min period. As references in each run, two endotoxin concentrations (1 and 5 EU/ml), zymosan (5 μg/ml), and the extraction solution diluted 20 times were used. These references were used to see whether the cells consistently reacted to the same level each time and to test for contamination. To account for variations in sensitivities of the cells, all data were normalized to the reaction of the diluted extraction solution. The maximum detection limit was 2×10^5 AUC/m^3.

2.8. Treatment of data

Concentrations of fungi, bacteria, and endotoxin were log transformed to be normally distributed while the TIP normalized to the reaction of Tween was already normally distributed. For measurements below the detection limit 50% of the dl was used. SAS version 9.4 (SAS Institute, Cary, NC, USA) was used for statistical analysis. First, the exposures of what would become the reference and the intervention groups (the baseline data) were compared in GLM (generalised linear model). Then, the data for all three days

were treated together and the effects of interventions, person, day of measurement, waste loading height, and waste type on exposure and TIP of exposure were analyzed in a stepwise regression with backward regression. Concentrations on hands were analyzed using the GLM method, and Pearson's correlation coefficients (r) were calculated between exposures and TIP. The impacts of endotoxin, bacteria, and fungi on the TIP of the 42 personal exposure samples were studied in GLM in one model with backward transformation.

3. Results

3.1. Personal exposure to bacteria, fungi, and endotoxin

The personal exposure was between 620 and 4.7×10^5 cfu bacteria/m^3 (GM = 7.8×10^3); 1.2×10^3 and 2.2×10^5 cfu fungi/m^3 (GM = 1.4×10^4); and 9 and 3570 EU/m^3 (GM = 92 EU/m^3; Table 2). Exposure to fungi correlated significantly with exposure to bacteria (r = 0.46, $p = 0.0021$), bacteria on SSI-agar (r = 0.42, $p = 0.0052$), and endotoxin (r = 0.42, $p = 0.005$); and exposure to endotoxin correlated significantly with exposure to bacteria (r = 0.82, $p < 0.0001$) and bacteria on SSI agar (r = 0.47, $p = 0.0018$). Bacteria did not correlate significantly with bacteria on SSI-agar (r = 0.25, $p = 0.10$).

3.2. Factors affecting personal exposure

At baseline exposure assessment (Day A), no significant difference was found between the reference group and what would become the intervention group for fungi ($p = 0.14$), bacteria ($p = 0.34$), endotoxin ($p = 0.99$), SSI-agar bacteria ($p = 0.95$), and for numbers of working hours ($p = 0.074$). In the following factor analysis, the baseline exposure levels are all analyzed as reference exposure level.

Statistical analysis with all factors in one model with backward transformation showed that exposures to fungi were highest on Day C ($p = 0.0037$) and for household waste ($p = 0.0003$; Fig. 1a), and tended to be highest for low loading height ($p = 0.072$). When the factors were studied one by one only waste type had a significant effect on fungal exposure ($p = 0.0083$). As the outdoor reference measurement also showed high fungal concentrations on Day C, we re-analyzed the personal exposure data for the workers collecting household waste, but with the outdoor reference for each day subtracted. The significant effect of day on exposure was confirmed ($p = 0.028$).

The exposures to bacteria were highest for workers collecting household waste ($p = 0.0018$) and for workers having a truck with low loading height ($p = 0.038$; Fig. 1b). When the factors were studied one by one, only waste type had a significant effect ($p = 0.0061$). Exposure to the SSI-agar bacteria was highest for reference workers ($p = 0.027$) and on Day C ($p = 0.022$) (Fig. 1c). When the factors were studied one by one, no single factor had a significant effect. The household waste tended to cause a higher exposure to SSI-agar bacteria than other waste types ($p = 0.091$). The exposure to endotoxin was significantly affected by waste type ($p = 0.042$; Fig. 1d).

On Day 3 the workers collecting 'other waste' had significantly longer working days than workers collecting household waste ($p = 0.025$) (further data not shown). The whole working day exposure (the potentially inhaled dose based on numbers of working hours) has been calculated. However, this did not change the results regarding the effects of the studied factors on exposure level (further data not shown).

The waste collection workers worked in pairs, and for 15 truck teams, both workers participated in the sampling, while for an additional 12 truck teams, only one member of the team partici-

Table 2
Personal exposures[1)] to fungi, bacteria, and endotoxin, and total inflammatory potential of the exposures.

Day	Fungi (cfu/m^3)		Bacteria (cfu/m^3)		Endotoxin (EU/m^3)		TIP (AUC/m^3)	
	Intervention workers	Reference workers	Intervention workers	Reference workers	Intervention workers	Reference workers	Intervention workers	Reference workers
A[2)]	**1.5×10^4** [3.9×10^3–1.1×10^5] $n = 10$	**5.9×10^3** [1.3×10^3–1.6×10^4] $n = 5$	**6.4×10^3** [1.6×10^3–1.9×10^4] $n = 10$	**9.3×10^3** [5.8×10^3–3.3×10^4] $n = 5$	**131** [49.3–506] $n = 10$	**130** [42–630] $n = 5$	**1.3×10^7** [9.1×10^6–1.8×10^7] $n = 10$	**1.7×10^7** [1.2×10^7–2.7×10^7] $n = 5$
B	**9.9×10^3** [1.2×10^3–7.0×10^4] $n = 13$	–	**1.1×10^4** [1.5×10^3–3.3×10^5] $n = 13$	–	**100** [11.1–798] $n = 13$	–	**1.6×10^7** [8.8×10^6–3.8×10^7] $n = 13$	–
C	**1.9×10^4** [1.2×10^3–2.2×10^5] $n = 10$	**6.7×10^4** [2.7×10^4–1.8×10^5] $n = 4$	**5.6×10^3** [620–4.7×10^5] $n = 10$	**7.1×10^3** [1.7×10^3–2.6×10^4] $n = 4$	**85** [8.9–3570] $n = 10$	**95** [42–213] $n = 4$	**1.6×10^7** [1.4×10^7–2.5×10^7] $n = 10$	**2.1×10^7** [1.4×10^7–2.5×10^7] $n = 4$

[1)] For endotoxin, fungi, and bacteria the geometric mean values are presented and for TIP the averages are presented in **bold** followed by [ranges].
[2)] Baseline, before introducing the interventions. cfu = colony forming units; EU = endotoxin units; TIP = total inflammatory potential; AUC = area under curve.

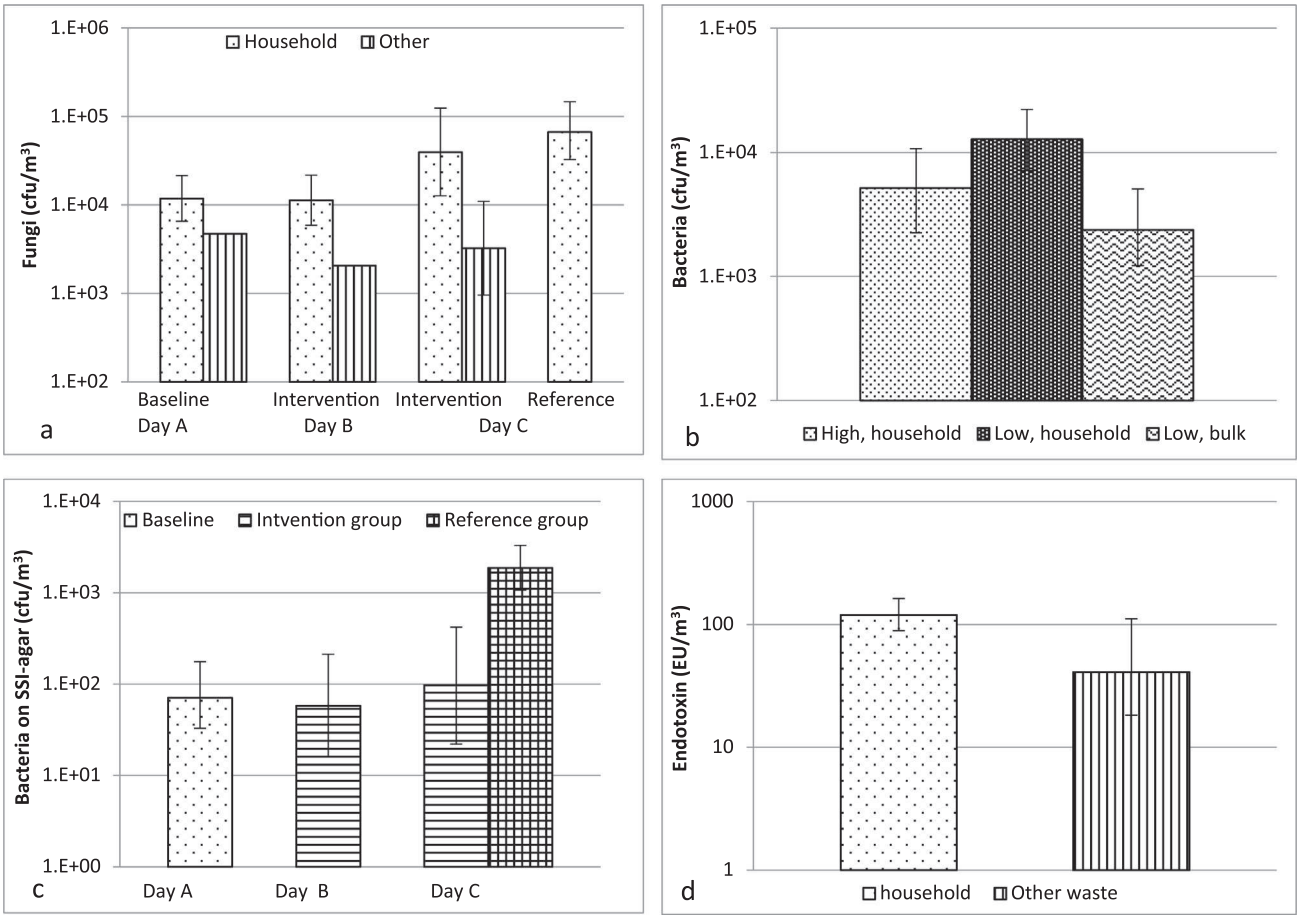

Fig. 1. Personal exposures (GM with confidence limits) to fungi (a), bacteria (b), bacteria on SSI-agar (c), and endotoxin (d) presented according to the factors affecting the exposure significantly. High = high waste loading height; low = low waste loading height; household = household waste; other (waste) = bulk, cardboard, and paper waste; for number of samples see Table 1. At baseline, the interventions were not yet implemented.

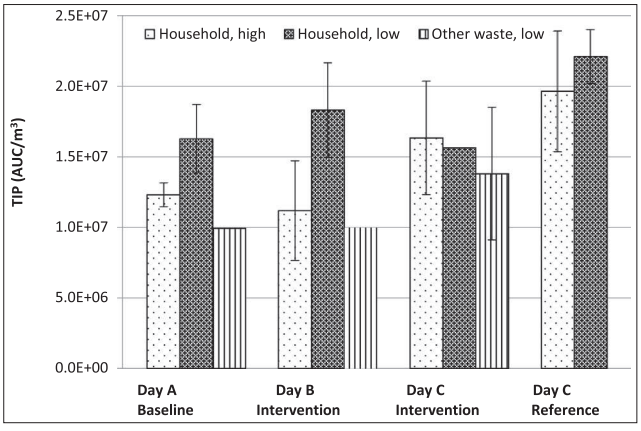

Fig. 2. The mean total inflammatory potential (TIP; expressed as the mean AUC/m^3 air, with confidence limits) of the personal air samples shown for the four factors which significantly affected the TIP value: Intervention, day of measurement, waste loading height, and type of waste. On Day A, interventions were not yet implemented. High = high waste loading height; low = low waste loading height; household = household waste; other waste = bulk, cardboard, and paper waste; ref = reference group, AUC = area under the curve.

pated. There was no significant correlation between the exposures of two workers on the same truck team: Bacteria (r = –0.10, p = 0.74), fungi (r = 0.44, p = 0.10), endotoxin (r = –0.01, p = 0.87), and TIP (r = 0.38, p = 0.17).

3.3. Inflammatory potential

On Day A, the TIP values for what would become the reference group versus the future intervention group were not significantly different (p = 0.12), and all measurements on Day A are in the factor analysis considered as reference measurements. When the data from the three days were studied together, significant effects of the intervention (p = 0.015), loading height (p = 0.0029), day (p = 0.040), and type of waste handled (p = 0.046) were found. The highest TIP was found for the reference group, low loading height, Day C, and household waste (Fig. 2). The TIP correlated significantly with exposure to endotoxin (r = 0.58, p < 0.0001), bacteria (r = 0.67, p < 0.0001), and fungi (r = 0.37, p = 0.016), but not with exposure to bacteria on SSI-agar (r = 0.24, p = 0.12). When the impacts of endotoxin, bacteria, and fungi on the TIP were studied in one model with backward transformation, bacteria (p = 0.0040) and endotoxin (p < 0.0001) had a significant effect on the TIP. When endotoxin was excluded from the model, TIP was associated significantly with both fungi (p = 0.0034) and bacteria (p < 0.0001). When bacteria were excluded from the analysis, TIP was associated significantly with both fungi (p = 0.0073) and endotoxin (p = 0.0016).

3.4. Concentrations of airborne bacteria, endotoxin, and fungi in stationary samples

Concentrations from the back end of the truck and in outdoor reference measurements are presented in Fig. 3abc. Bacterial concentrations from the truck may be underestimated due to

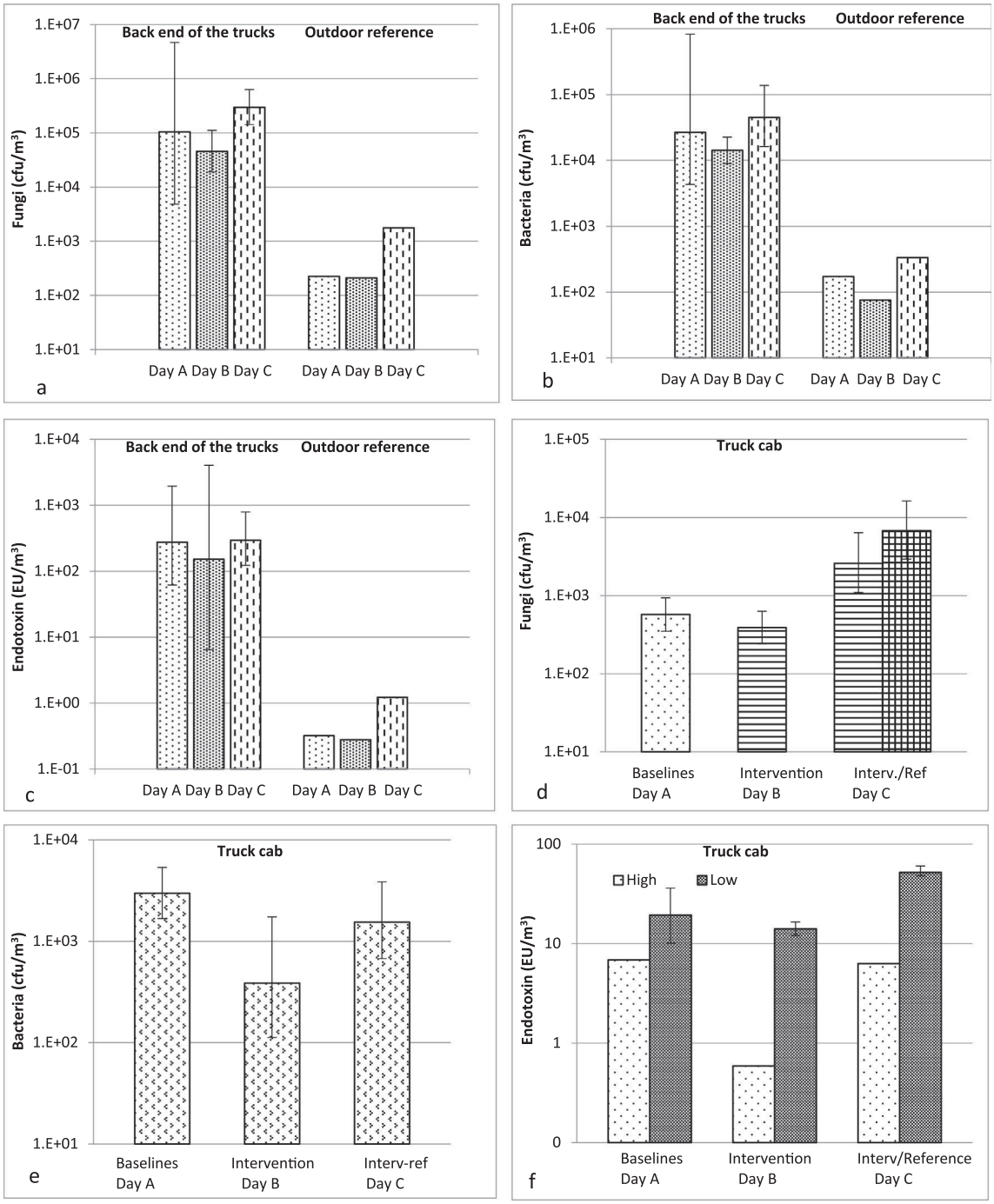

Fig. 3. Concentrations (GM with confidence limits) of airborne fungi (a), bacteria (b), and endotoxin (c) in the air measured from the back end of the trucks and in outdoor reference samples, and for the parameters resulting in significant differences inside the cabs (Intervention, Day, and Loading height; d, e, and f). At baseline, the interventions were not yet implemented.

competition from fungi on some of the plates. The bacterial concentrations on SSI-agar corresponded to 57 to 5.3×10^3 cfu/m^3 (GM = 662 cfu/m^3). None of the measured exposure parameters differed significantly between reference and intervention trucks ($ps > 0.05$). The fungal concentration ($p = 0.033$) was higher on Day C than on Day B (Fig. 3a). The concentrations of bacteria and endotoxin were not affected by the sampling day ($ps > 0.05$). The three blank samples did neither show growth of microorganisms nor contamination with endotoxin.

The bioaerosol concentrations inside the truck cabs were between 236 and 1.9×10^3 cfu fungi/m^3 (GM = 1.2×10^3 cfu/m^3), between 87 and 5.1×10^3 cfu bacteria/m^3 (GM = 1.5×10^3 cfu/m^3), and for endotoxin between 0.6 and 52 EU/m^3 (GM = 10.0 EU/m^3). At baseline, there were no significant differences between concentrations of any of the measured bioaerosols of what would become the reference and intervention groups ($ps > 0.05$). The fungal concentration was significantly higher on Day C ($p = 0.0066$), and it was higher for reference truck cabs ($p = 0.019$) than for intervention truck cabs (Fig. 3d). The concentration of airborne bacteria inside the truck cab was lower on Day B than during Days A and C ($p = 0.047$) (Fig. 3e). The endotoxin concentration was significantly associated with day ($p = 0.047$) with the lowest concentration on Day B, and with loading height ($p = 0.0033$) with the highest concentration for low loading height (Fig. 3f).

The GM ratios of the concentrations of bacteria, fungi, and endotoxin inside the truck cabins to outdoor reference concentrations were 7.6, 2.3, and 23, respectively. The ratios for bacteria were associated with loading height with the highest ratios for low loading height ($p = 0.013$). The ratios for fungi were associated with intervention with the highest ratios for reference workers ($p = 0.046$). The ratios for endotoxin were or tended to be associated with loading height ($p = 0.017$) and intervention ($p = 0.063$) with the highest ratios for low loading height and reference workers.

3.5. Microorganisms on palms

The concentrations of fungi on the workers' palms were between bd and 270 cfu/sample (GM = 59 cfu/hand sample). The concentrations of yeasts were between bd and 1800 cfu/sample (GM = 23 cfu/hand sample), and the concentrations of bacteria between 400 and 4.1×10^4 cfu (GM = 3507 cfu/hand sample). At baseline, the concentrations of yeasts and fungi were not significantly different between the intervention and the reference group ($ps > 0.05$), but for bacteria the concentration was highest for the future intervention group ($p = 0.028$).

On Day C, 9 of the 10 intervention workers told us that they had used hand sanitizers 1–7 times during the day of measurement. None of the reference workers used hand sanitizer. The number of fungi/hand sample was significantly affected by the intervention ($p = 0.017$), day of sampling ($p = 0.0093$), and type of waste handled ($p = 0.0044$) (Fig. 4a). The number of bacteria/sample tended to be affected by the type of waste handled ($p = 0.097$) with highest concentrations for household waste. The number of yeasts/hand sample was not affected significantly by the intervention ($p > 0.05$). There was no significant correlation between the number of bacteria/hand sample and the number of bacteria in the personal air samples ($p > 0.05$). However, the concentration of fungi on hands and in the personal samples correlated significantly ($r = 0.62$, $p < 0.0001$). Concentrations of fungi and bacteria on the workers' hands did not correlate significantly ($r = 0.20$, $p = 0.20$), but concentrations of yeasts correlated with concentrations of fungi ($r = 0.39$, $p = 0.0098$) and bacteria ($r = 0.55$, $p = 0.0002$) on the workers' palms.

3.6. Steering wheel surface

As with the hand samples, yeasts constituted a considerably fraction of the fungi sampled from the steering wheel surface, and yeast and fungus data are treated separately. The concentrations of fungi and bacteria correlated significantly ($r = 0.70$, $p = 0.0057$), but neither correlated with concentrations of yeasts ($ps > 0.05$). At baseline, no difference was found between what would become the reference and the intervention groups for the steering wheel samples for fungi ($p = 0.66$), bacteria ($p = 0.11$), and yeasts ($p = 0.92$). The interventions tended to be or were associated with lower concentrations of fungi ($p = 0.056$) (Fig. 4b), bacteria ($p = 0.012$) (Fig. 4c), and yeasts ($p = 0.0068$) (Fig. 4d), and in addition the yeast concentrations tended to be associated with the day ($p = 0.066$) while bacteria concentrations were associated significantly with day of measurement ($p = 0.041$).

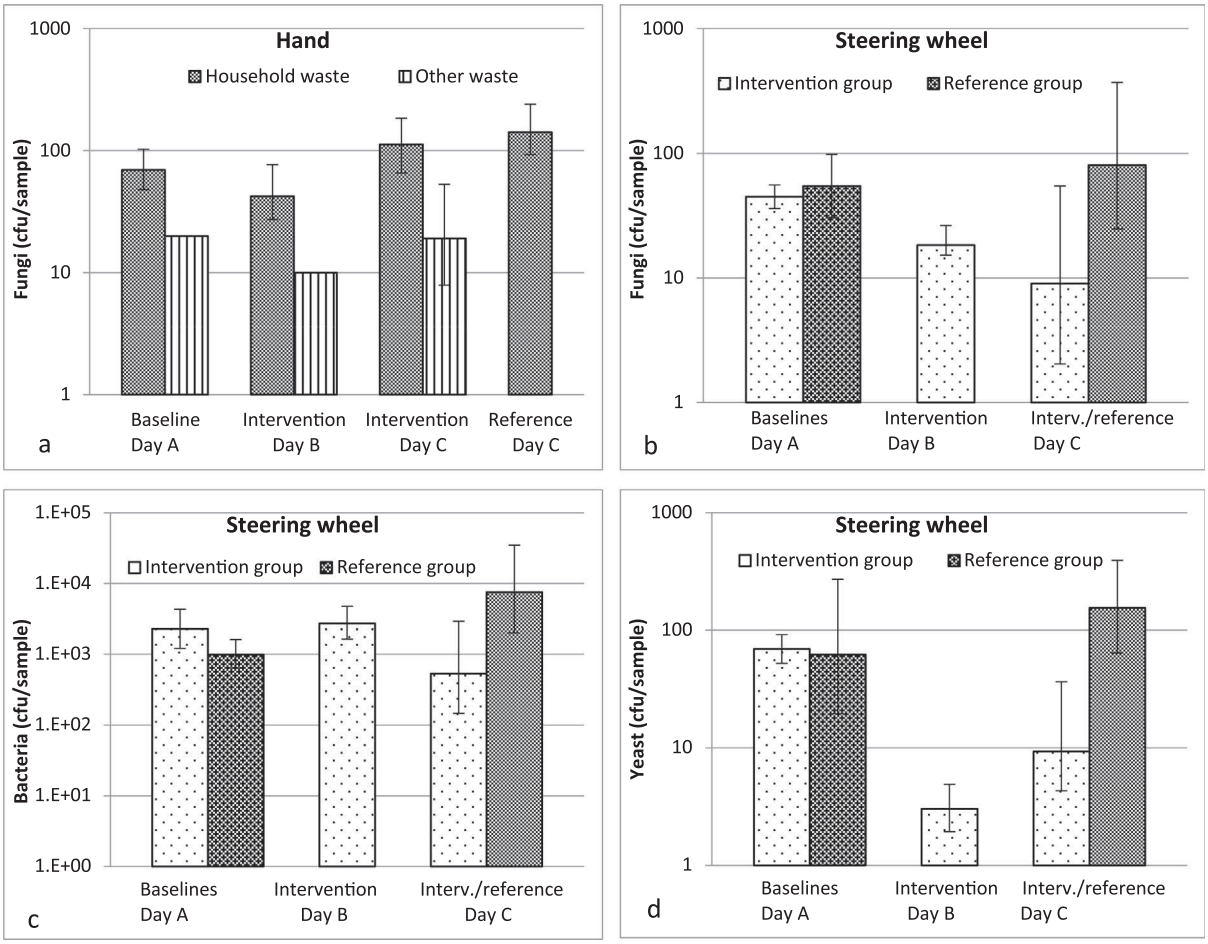

Fig. 4. Concentrations (GM with confidence limits) of fungi on hands (a); on the steering wheels of intervention and reference trucks, fungi, bacteria, and yeasts (b, c, d). Other waste = bulk, cardboard, and paper waste. For number of samples, see Table 1. At baseline, the interventions were not yet implemented.

4. Discussion

Waste workers are expected to be exposed to microorganisms primarily via the air and hand-to-mouth. For exposure via air, this study shows that the TIP of the personal exposure was reduced post implementation of interventions. For the hand route, concentrations of fungi on the workers' palms were reduced for the intervention waste collection workers, and in addition, the truck cab hygiene was improved.

The GM exposure to endotoxin during collection of 'other waste' was 22 EU/m^3 ($n = 5$) and the GM exposure for household waste collection was 109 EU/m^3 ($n = 37$). In a previous study, workers collecting different types of waste in the summer and fall were exposed to 40 EU/m^3 (GM), and the group working as loaders collecting residual waste were exposed to 49 EU/m^3 (GM) (Wouters et al., 2006). The workers in the present study worked as both drivers and loaders throughout the day. The suggested occupational exposure limits for endotoxin are 50 EU/m^3 (Douwes and Heederik, 1997) and 150 EU/m^3 (Smid et al., 1992), which were exceeded for respectively 35 and 11 out of 42 personal exposure measurements.

In the winter, the waste collection workers were exposed to up to 3.3×10^5 cfu bacteria/m^3 air (GM = 8.9×10^3 cfu/m^3) while the average outdoor reference was 124 cfu bacteria/m^3. In a previous study, we found that 13 waste workers were exposed to bacterial concentrations between 112 and 4.8×10^4 (GM = 1.1×10^3) cfu/m^3 air in the winter (Madsen et al., 2016). In a study from Denmark published in 1995, 20 waste workers collecting mixed household waste were exposed to $\leq 2 \times 10^5$ cfu bacteria/m^3 air (mean = 6.4×10^3 cfu/m^3 air; the season was not mentioned (Nielsen et al., 1995). In the winter, personal exposure to fungi was $\leq 1.1 \times 10^5$ cfu/m^3 air (GM = 1.1×10^4 cfu/m^3 air) while the average outdoor reference was 217 cfu/m^3 air. In the previously mentioned study (Madsen et al., 2016), the 13 waste workers were exposed to $\leq 4.6 \times 10^4$ cfu/m^3 air (GM = 5.7×10^3 cfu/m^3 air) in the winter, while the 20 workers (Nielsen and Breum, 1995) were exposed to $\leq 5 \times 10^5$ cfu fungi/m^3 air (mean = 7.7×10^4 cfu/m^3 air). Thus, the exposure levels seem not to have changed considerably. In a study from South Africa, the exposure of 20 waste collection workers ranged from 5.8×10^3 to 1.36×10^5 cfu/m^3 fungi (Ncube et al., 2017). There are no occupational exposure limits for fungal and bacterial exposure, which is probably because the health effects caused by the microorganisms differ greatly at species level.

At baseline, the future intervention group's exposure to airborne microorganisms was at the same level as that of the reference group, and therefore differences are expected to be related to the interventions. The interventions had significant effects on the waste collection workers' exposure to bacteria measured on SSI-agar and to the TIP of the exposure, but not to fungi and bacteria in general or to endotoxin. The workers themselves may also be the sources of a, probably small, portion of their own bacterial exposures and a fraction of the airborne bacteria in the truck cab, but this alone cannot explain why the interventions did not affect the personal exposure to bacteria. The TIP and exposures correlated, and the TIP measurement combines the effects of all exposures, and as the fungal exposure on Day C tended to be higher for the reference group this may have contributed to the measured effect of the intervention on TIP.

The fungal exposure inside the truck cabs was reduced for intervention workers, and the bacterial and endotoxin concentrations inside the truck cabs were lower on Day B than before the implementation of the interventions. A previous study shows that waste collection workers spend 37% of their working day inside the truck cab (Madsen et al., 2016), and therefore it is also relevant to reduce exposure inside the truck cabs. On Day C, which was in the summer, windows were open in the truck cabs, and a high air change rate is expected to cause a reduced bacterial concentration as seen in indoor air (Madsen et al., 2018). This may have contributed to or caused the lack of difference between intervention and reference cabins for bacteria and endotoxin on Day C. The GM concentrations of bacteria and fungi in the truck cabs were 1.5×10^3 cfu bacteria/m^3 air and 1.2×10^3 cfu fungi/m^3 air, respectively, and thus these exposures are at the same levels as previously found inside truck cabs (Nielsen et al., 1995).

In this study, samples from the workers' hands had $\leq$270 cfu fungi/hand sample, $\leq$1800 cfu yeasts/hand sample, and $\leq$3507 cfu bacteria/hand sample, which is lower than what was found on the palms of three waste workers in South Korea (means = 6.4×10^6 cfu fungi/cm^2 palm and 1.4×10^7 cfu bacteria/cm^2 palm) (Madsen et al., 2016; Park et al., 2011). The workers with access to hand sanitizer had significantly fewer fungi on their hands, but there was no significant effect on bacteria and yeasts. Previous studies of health care workers have shown a significant effect of hand sanitizer on the total number of bacteria (Larocque et al., 2016), and *in vitro* studies have found that a 15 s exposure of e.g. *Aspergillus flavus* and *Penicillium citrinum* spores to hand sanitizer kills the spores (Fendler and Groziak, 2002). The lack of effect on bacteria and yeasts seen in this study could be because the bacteria and yeasts might be aggregated in clusters and thus are better protected, as well as presence of yeasts and bacteria inside the gloves, but it could also reflect the workers' own skin bacteria and yeasts. Some of the intervention workers told us that they had introduced a routine where they cleaned the inside of the truck cab while refilling the gas tank. Accordingly, the concentrations of fungi, yeasts, and bacteria on the steering wheels were also lowest in the intervention truck cabs. In a study concerning bacteria on smartphones, cleaning was also found to have a significant effect on the number of bacteria (Egert et al., 2015).

In this study, using a truck with a high waste loading height for collection of household waste in containers caused lower exposure levels to bacteria and a lower TIP of the exposure compared to using a truck with low waste loading height. The effect of loading height was large enough that even inside the cabs of trucks with high loading height, exposure to endotoxin was lower. Top loading trucks have previously been observed to cause lower exposure to fungi than trucks which are loaded at the same level with the workers' breathing zone (Nielsen et al., 1997). Waste type also had an effect on the workers' exposure to fungi, bacteria, endotoxin, and on the associated TIP with higher exposure levels during collection of mixed household waste than during collection of 'other waste' (cardboard, paper, and bulk categorized together). Waste collection workers also had more fungi on their hands if they collected household waste rather than 'other waste'.

The exposures to fungi and bacteria on SSI-agar as well as the TIP of the exposures were significantly higher in the summer than in the winter. This is expected to be related to a faster growth rate of fungi during higher temperatures. Concentrations of bacteria, fungi, and endotoxin were low inside the truck cabs on Day B when the outdoor temperatures were low and the interventions had been implemented. Seasonal or temperature variations in exposure are in accordance with previous studies (Madsen et al., 2019; Nielsen et al., 1997) and of relevance as temperatures are in general rising in many regions of the world. In relation to this, it should be noted that gastrointestinal symptoms among waste workers collecting mixed household waste are most abundant in the summer (Ivens et al., 1999).

In conclusion, waste workers are exposed to high concentrations of airborne fungi, bacteria, and endotoxin. By simple interventions, it was possible to reduce the TIP of the workers'

exposures, the concentration of fungi on hands, concentrations of airborne fungi inside the truck cabs, and concentrations of fungi, bacteria, and yeasts on the steering wheels. However, the type of waste handled, the type of truck used (high or low loading height), and season also had significant effects on more of the exposures. In general, household waste, low waste loading height, and summer were associated with the highest exposures.

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Declaration of Competing Interest

All authors declare no conflicts of interest in this paper.

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