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TECHNICAL PAPER

Assessment of waste workers occupational risk to microbial agents and cytotoxic effects of mixed contaminants present in the air of waste truck cabin and ventilation filters

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ABSTRACT

Workers in the waste-processing industry are potentially exposed to high concentrations of biological contaminants, leading to respiratory and digestive problems and skin irritations. However, few data on the exposure of waste collection truck (WCT) drivers are available. The goal was to document the microbial risk of the waste collection truck (WCT) workers while in the vehicle cab. Long-period sampling using the truck air filters (CAF) and short time ambient air sampling in the cab were used. The potential release of microbial particles from CAFs was also investigated since it could contribute to the microbial load of the cabin air. A combination of analytical methods also helped assess the complex mixture of the biological agents. *Aspergillus* sections *Fumigati and Flavi, E. coli, Enterobacter* spp. *and Legionella* spp. were detected in the CAF of trucks collecting three types of waste. The highest levels of bacteria and fungi were found in the CAF from organic WCT. The highest endotoxin concentrations in CAF were 300 EU/cm^2. Most of the CAF showed cytotoxic effects on both lung cells and hepatocytes. Only one mycotoxin was detected in a CAF. The maximal concentrations in the ambient WCT air varied according to the type of waste collected. The highest proportion (84%) of the air samples without cytotoxic effects on the lungs cells was for the recyclable material WCTs. The results revealed the potential microbial risk to workers from a complex mixture of bio-contaminants in the cabs of vehicles collecting all types of waste. The sustained cytotoxic effect indicates the potential adverse health-related impact of mixed contaminants (biological and non-biological) for the workers. Overall, this study highlights the benefits of using complementary sampling strategy and combined analytical methods for a the assessment of the microbial risk in work environments and the need to implement protective measures for the workers.

Implications: Exposure to microbial agents is a well-known occupational hazard in the waste management sector. No previous study had evaluated the cytotoxicity of ambient air and ventilation filters to document worker exposure to a combination of contaminants during waste collection. This research confirms the usefulness of ventilation filters for long-term characterization of exposure to infectious agents, azole-resistant fungi, coliform bacteria and mycotoxin. Overall, this study highlights the importance of using several sampling and analysis methods for a comprehensive assessment of microbial risk in work environments, as well as the need to implement appropriate protective measures for collection workers.

Highlights

- Complementary sampling strategy and combined analytical methods are helpful in risk assessment.
- Air filter analysis (long-term sampling) assesses the presence of airborne biological contaminants over a long period.
- The type of waste collected influences the microbiological hazard of the workers.
- Waste collection workers are potentially exposed to infectious and mycotoxin-producing fungi.
- Cytotoxic assays revealed that waste collection workers are potentially.

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Introduction

Concerning environmental protection, waste burial must be minimal, for which municipalities carry out selective collections intending to revalorize residues by composting, biomethanization, or recycling, leaving only the final residues of the domestic waste for burial or incineration. In Europe, the total waste treatment increased from 45.9% to 59.1% between 2004 and 2020 (Eurostat Waste Statistics 2023). The Circular Economy Action Plan adopted in 2020 by the European Commission defines specific measures to promote circular economy processes, encourage sustainable consumption, and ensure that wastage is prevented and the resources used are maintained in the European Union economy for as long as possible (European Commission 2023). In Quebec, since 2018, recycling has increased by 3%, the amount of household waste sent to landfills decreased by 5%, and the municipal sectors recycle 48% of their organic waste generated (green waste, third lane [brown bins] as well as municipal sludge) (Recyc-Québec, La collecte sélective 2023; Recyc-Québec, Les matières organiques 2023). Although there is a great interest in improving waste management to protect humans and the environment, the impacts of the exposure of waste industry workers to biological agents remain neglected (Viegas et al. 2022).

Waste collection workers can be exposed to biological agents not only during waste handling but also within the waste collection truck (WCT) cabin. This microbial risk can occur through the resuspension of particles deposited on the surfaces or through the emission of microbial agents from the ventilation system (Aquino et al. 2018; Gołofit-Szymczak et al. 2023; Gołofit-Szymczak, Stobnicka-Kupiec, and Górny 2019; Simmons et al. 1997). Studies have identified a significant fungal diversity in the filters of vehicles. Furthermore, the levels of fungal particles emitted into the cars could be twice as high when the filters of the air-conditioning system were aged (Aquino et al. 2018; Vonberg et al. 2010). The air passing through the filtration material can reaerosolize the bioburden of the filters, thereby potentially increasing the exposure of the occupants of the vehicle cabin (Gołofit-Szymczak, Stobnicka-Kupiec, and Górny 2019; Li et al. 2013; Viegas et al. 2017).

Numerous studies have indicated that the workers in the waste industry are exposed to relatively high concentrations of biological contaminants, further confirmed by a recent study (Madsen et al. 2021). Respiratory health problems (coughing, nose irritation), digestive disorders, and skin irritations have been associated with exposure to biological agents and organic dust in these environments (Heldal and Eduard 2004; Heldal et al. 2003; Ivens et al. 1999; Madsen et al. 2021; Poole and Basu 2017; Wouters et al. 2002). Many studies have evaluated the biological agents in domestic, recyclable, and organic waste treatment facilities (Bonifait et al. 2017; Dubuis et al. 2017; Krajewski et al. 2002; Madsen et al. 2019; Malmros, Sigsgaardt, and Bach 1992; Marchand, Lavoie, and Lazure 1995; Park et al. 2011; Poulsen et al. 1995; Viegas et al. 2022; Wikuats et al. 2022). However, only a few studies have focused on the microbial risk of the workers (drivers, collectors) engaged in the collection. Numerous studies have particularly analyzed the endotoxins and cultivable bacteria and fungi from air samples (Dias and Viegas 2021; Krajewski et al. 2002; Lavoie et al. 2006; Madsen et al. 2016; Neumann et al. 2002; Nielsen, Nielsen, and Breum 1995; Park et al. 2011; Salambanga et al. 2022; Szulc et al. 2022).

Recent studies have highlighted the benefits of combining sampling and analytical protocols as this provides a better understanding of the biological risk (Dias and Viegas 2021; Madsen et al. 2020; Viegas 2018; Viegas et al. 2022). Short and long-term sampling sampling have advantages and disadvantages and are recommended for biological risk assessment (Gomes et al. 2023; Viegas 2018; Viegas et al. 2016, 2018). Long-term sampling reveals a potential exposition over days or months, is influenced less by temporal fluctuations, and recovers higher biodiversity (Hoisington et al. 2014; Li et al. 2013; Viegas 2018). Active methods documented concentrations during work shifts, allowing workers tasks to be characterize and quantify probable inhalation (Hoisington et al. 2014; Madsen et al. 2021; Viegas et al. 2018).

While cultivable analysis is crucial to determine the risk of infection, it provides an incomplete biological risk assessment since it represents a small percentage of the total microbial contaminants and should be complemented by molecular tools. Thus, molecular biology assays that allow efficient, accurate, rapid, and sensitive detection and speciation of dead, non-cultivable species without the limitations of cultivable methods have been widely used (Dias and Viegas 2021; Dubuis et al. 2017; MacNeil, Kauri, and Robertson 1995). In addition, inhalation of microbial contaminants (endotoxins, mycotoxins, and antigens) is associated with health concerns (Dias and Viegas 2021; Eduard et al. 2012; Viegas et al. 2015); hence, the endotoxins and mycotoxins have been quantified in many occupational settings (Duquenne, Marchand, and Duchaine 2013; Franco et al. 2020; Viegas et al. 2020). Due to the complex nature of any occupational environment, the "one contaminant at a time" approach for biological agents may

result in a limited understanding of the health risks associated with the simultaneous presence of several contaminants (Madsen et al. 2021; Viegas et al. 2022; Vinggaard et al. 2021).

The study aimed to document the microbial agents present in the cabin air filters (CAFs) and the air of the WCT cabin to assess the microbial risks to the workers. This was performed by 1) using the WCT CAF, 2) sampling of the ambient air within the WCT cabin, and 3) a combination of several analytical methods to assess the complex mixture of biological agents and to determine their cytotoxicity. The hypothesis was that the type of collected waste and location can significantly impact the levels and the diversity of microbial concentrations in the cabs and the CAFs.

Materials and methods

Sampling conditions

The sampling was performed in 2019 and 2021 in the province of Quebec. Quebec has a continental climate with freezing winters and warm and humid summers. As humidity and warm temperatures promote microbial growth compared to cold and dry environmental conditions, sampling was performed in the summer to deal with the worst-case scenario. No sampling could be done during the summer of 2020 because of the COVID-19 pandemic. In total, 28 WCTs from urban and rural areas were studied (Table 1). The types of waste collected were mixed domestic, organic, or recyclable. Domestic waste consisted of a mixture of wastes from households or small businesses. In some communities, it could include putrescible organic waste if the separate collection of organic waste was unavailable. The organic fraction was a mixture of putrescible, organic, and green waste from households that would be directed to composting or biomethanization plants. Recyclable waste could be a combination of paper, cardboard, plastic, glass, and metals from households and small businesses. All waste types were collected weekly during the summer. At least once a day, the truck was driven to the disposal site to empty the accumulated waste. A workday lasted between three and eight hours, depending on the density of the population and the amount of trash collected.

Table 1. Number of trucks studied and samples collected per waste type.

Waste types	Waste collecting trucks	Cabin air filters	Ambient air
	N (Urban-Rural)	N	N
Organic	6 (5–1)	6	24
Recyclable	10 (6–4)	10	40
Domestic	12 (6–6)	11	46

For each WCT, the waste collection team comprised a driver and, in some municipalities, one or two runner collectors. When no runners were on the team, the trucks were equipped with automatic clamps that allowed the garbage to be automatically emptied into the bin. Some drivers disembarked to help the collectors or to solve problems with the automated arm, the frequency of which varied daily. Few personal protective equipment was used. Workers wore safety shoes, some wore gloves, but none wore respiratory protection.

Sample collection

Two types of samples using filtration but providing different and complementary information on drivers' potential exposure to microbial agents were collected as part of this project. Firstly, the SASS3100 sampler, which takes air samples from the truck cab enclosure over a short period and provides information on the concentrations of microbial agents in the air surrounding the driver, i.e., the air workers are likely to inhale. And secondly, the CAF that can provides an overview of the microbial agents present in the working environment and enables samples to be taken over a long period, highlighting sporadically present microbial agents that might not be detected by short-term sampling. CAF also provides information on microbial agents likely to be released during the use of ventilation systems and possibly inhaled by workers. The information produced by the two types of samples combine to give a picture of the potential exposure.

Cabin air filters (CAFs)

After the workdays, the research team requested to collect the CAF samples during the day. One of the domestic WCTs was not equipped with any air filter. The next day, 4 cm^2 samples were cut off with a sterile scalpel and were placed in 50 mL Falcon® tubes for analysis.

Cabin air sampling

The sampling of cabin air was performed with the SASS 3100 air sampler (Research International Instrument Inc., Washington, DC). The collection substrates used were 44 mm-diameter polypropylene electret filters. These filters had a collection efficiency of 50% for aerosols of diameter 0.3–0.5 µm and 75% for those of 1 µm when the SASS 3100 was operated at a flow rate of 300 L/min (CBR Technology Evaluation Branch Test Report 2008; US Department of Defense 2012). The samplers were installed on the passenger seat adjacent to the driver throughout the working day. Depending on the duration of the working day, three or four sequential air

samples were taken, which lasted for one or two hours each. Before extraction, all samples were placed in 50 mL Falcon® tubes (Thermo Fisher Scientific, Burlington, ON, CA). Half of the samples were used for culturing and endotoxin analysis along with detection of microbial risk indicators employing ddPCR conducted on a Q×200Droplet Digital PCR System (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada), while the other half was used to analyze the mycotoxins, cytotoxicity, and fungal resistance to azole.

Sample extraction

The CAF and ambient air samples were extracted in 10 mL PBS with 0.05% Tween 20, for culture on tryptic soy agar (TSA) and malt extract agar (MEA), endotoxin analysis, and detection of microbial risk indicators by ddPCR. For ascertaining azole resistance, culture on violet-red bile agar [VRBA], 18% dichloran-glycerol agar [DG18], and sabouraud dextrose agar [SAB]); and cytotoxic assays, the samples were extracted in 10 mL of 0.9% NaCl with 0.05% Tween 80 (Frilabo, Maia, Portugal), and 20% glycerol was added for the cultivation assays. All extractions used a multivortex (Fischer Scientific, Ottawa, ON, Canada) for 10 min at 2,500 rpm. For detecting mycotoxins, the samples were stored at – 20°C until extraction and analysis. The extraction was performed with 4 mL of an extraction solvent (ACN:H_2O:AcOH at 79:20:1, v/v/v) for 60 min using a rotary shaker.

Samples analyses

The samples were extracted and plated on the TSA and MEA media the same day. CAF and ambient air samples were stored with glycerol at – 80°C until plating on VRBA for enteric bacteria and on DG18 and SAB to evaluate the fungal diversity. Fungal azole resistance was screened by culturing on SAB (Frilabo, Maia, Portugal) supplemented with 4 µg/mL itraconazole (ITRA), 2 µg/mL voriconazole (VORI), 0.5 µg/mL posaconazole (POSA), and incubated at 27°C for 2–3 days, as previously described (Viegas et al. 2022). Detailed protocols for cultivation analyses published in previous reports were employed (Salambanga et al. 2022).

The endotoxins were either analyzed the same day as sample extraction or stored at 4°C for 24 h. A chromogenic assay was performed using the *Limulus* amoebocyte lysate following the IRSST method 332 (Marchand 2009). Mycotoxins were analyzed by the Nexera HPLC-MS [Shimadzu, Tokyo, Japan] with an API 4000 mass spectrometry detector [Sciex, Foster City, CA, USA], following the procedures described in previous papers (Vinggaard et al. 2021). The mycotoxin concentrations were calculated using external calibration. The limits of detection (LOD) and quantification (LOQ) for each mycotoxin were obtained with the analytical method used before (Salambanga et al. 2022).

Before DNA extraction, the samples were concentrated by centrifugation to detect the microbial risk indicators. The extracted DNA samples were kept at – 20°C until analysis. Total fungi (Fungi) and bacteria (Bacteria), *Aspergillus* sections *Fumigati* and *Flavi, Aspergillus/Penicillium* (Asp/Pen), *Escherichia coli*, *Enterococcus*, and *Legionella* spp., were chosen as microbial risk indicators. They were detected by ddPCR with the Q×200Droplet Digital PCR System (Bio-Rad, Hercules, California, USA) and an internal inhibition control (Bio-Rad, Hercules, CA, USA) was used to control for false negatives. Wells with < 12,000 accepted droplets were excluded. The Detection Limit was set at three copies/20 µL. For each detection system, non-template and positive controls were also included for each run. The complete protocol and the sequences of the primers and probes used (Integrated DNA Technologies, Coraville, IA, USA) were reported previously (Salambanga et al. 2022).

The cytotoxicity of the air and CAF samples was evaluated using the 3-(4,5-dime thylthiazol-2-yl)-2.5-diphenyltetrazolium bromide (MTT) colorimetric assay. This assay determines the metabolic activity of cells employing the enzymatic reduction of the tetrazolium dye, MTT, observed at 510 nm and is related to the number of viable cells (Hanelt, Gareis, and Kollarczik 1994). Cytotoxicity was determined using human lung epithelial (A549) and human hepatocarcinoma (HepG2) cells. The A549 alveolar epithelial cancer-derived cells have been commonly used to study the toxicity of airborne mixtures both by passive and active samples (Viegas et al. 2022). HepG2 are non-tumorigenic cells with high proliferation rates and are widely used for studying drug metabolism and hepatotoxicity (Viegas et al. 2022). A549 or HepG2 cell suspensions, 100 µL each, were transferred into 96-well plates at 2.5×10^5 cells/mL. The suspension was exposed for 48 h to five or ten serial binary (1:2) dilutions of the samples in a humid atmosphere under 5% CO_2 and 37°C. The cytotoxicity level was determined through spectrophotometric analysis using an ELISA LEDETECT 96 microplate reader (Biomed Dr. Wieser GmbH, Salzburg, Austria), and the data was analyzed using the MikroWin® 2013SC software (https://www.bioconcept.ch). The lowest concentration causing a reduction of < 50% in the absorption (IC_{50}) was considered the threshold toxicity level. The results were reported in the units of surface filter mm^2/mL. The

complete protocol has been described (Salambanga et al. 2022).

Microbial release analyses under laboratory settings

CAFs were also used to evaluate the potential release of microbial particles when exposed to a sudden airflow surge simulating the start of the cabin ventilation system. For this, round samples of each CAF, 37 mm, were punched and inserted into a stainless filter holder. The filter holder was connected directly to the inlet of a six-stage Andersen impactor to assess the release of cultivable microbes. The system was connected to two 1.2 m Versapor HEPA capsules (Pall Corp., New York, NY, USA). The first one was placed upstream of the filter holder to eliminate the microbes from the laboratory air, and the other one was placed downstream of the Andersen impactor to avoid the contamination of the laboratory air by the particles released from the CAF and not contaminating the culture media in the Andersen impactor (Figure 1). The sudden surge in airflow through the CAF sample was triggered by starting the pump of the Andersen impactor. The airflow rate was 28.3 L/min, which simulated the highest filtration velocity measured in real-life trucks, i.e., around 50 cm/s. For each assay, the Andersen impactor was run for two min. The cultivable bacteria released from the CAF were collected on TSA, and the cultivable fungi on MEA

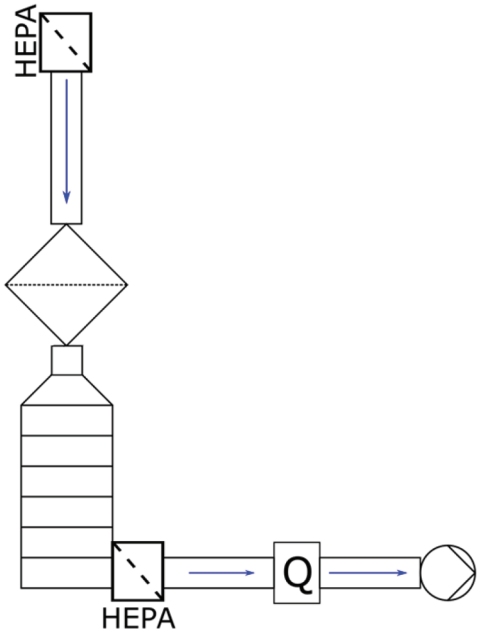

Figure 1. Schematic of the release of cultivable microbial particles from the CAF.

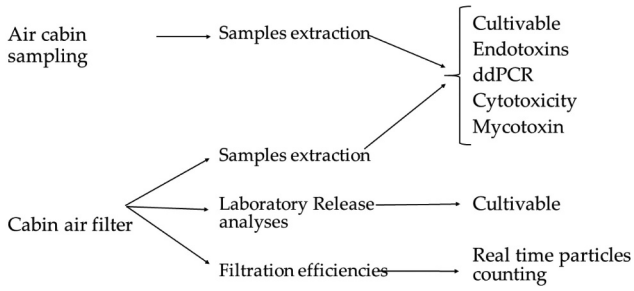

Figure 2. Project experimental design.

were incubated at 25°C for 48 h in the case of the bacteria and 5–7 days for the fungi.

CAF filtration efficiency was determined by measuring the concentrations of the polydispersed particles before and after the filtering media using direct reading particle counters (SMPS and APS). The experimental design is presented in Figure 2

Data analyses

Statistical analyses were performed with NCSS™ 12 (NCSS NCSS Statistical software 2018). The Martinez-Iglewicz test was used to evaluate the normality of data distribution, and the log-transformed data showed a normal distribution. All statistical tests were therefore performed on log transformed data. The concentrations reported below the LOD varied depending on the microbial agent and the collection medium. To facilitate the data treatments, one substitution method was chosen regardless of their occurrence. In this regard, the LOD divided by two was used for all the statistical analyses (Hornung and Reed 1990; Lavoué et al. 2021). The two-way general linear model analysis of variance (GLM-ANOVA) and the Tukey-Kramer multiple-comparison tests were used to assess the effects of the type of waste collected, the location (rural or urban), and their interaction. The impact of the filter model was investigated for the three most frequently used models, but this factor was not included in the two-way GLM-ANOVA. A significant level of 0.05 was used for all statistical tests.

Results and discussion

Microbial agents in CAF

The detection of culturable microorganisms in CAF is subject to influencing factors such as: desiccation, high air speed and aging. The assumption that the degradation of growth capacities is uniform between microorganisms can lead to certain biases. Therefore, the cultivable results are considered representative of the

filters and conditions during the tests. Microbial agents in CAF were analyzed by a two-way ANOVA to examine the effects of the type of waste, the location (rural, urban) and their interactions. There was no significant interaction between the type of waste and the location for all the microbial agents 'concentrations studied in the CAF.

Waste type (domestic, organic, recyclable)

The concentration of microbial agents in CAF of trucks collecting the three types of wastes are reported in Table 2. The *Aspergillus* sections *Fumigati* and *Flavi*, *E. coli*, *Enterobacter*, and *Legionella* spp. were measured in the CAFs from trucks collecting the three types of wastes. Regarding the ddPCR method results, the CAF of the organic waste truck proved to have significantly higher concentrations. Although not significantly different, *Aspergillus* section *Fumigati* demonstrated the highest concentrations with average 18S-rDNA copies between 6,000 (recyclable) and 82,600 (domestic) per cm^2 of filters. The average *PEPO*-gene copies for *Aspergillus* section *Flavi* was lower, ranging from 80 (domestic) to 400 (organic) DNA copies/cm^2 of CAF. The concentration difference was explained by the multicopy 18S rDNA compared to the single-copy *PEPO* per cell. *A. fumigatus* isolates could carry 38–91 copies of the 18S rDNA gene per genome (Herrera et al. 2009). Since ddPCR reports the absolute quantification of gene copies, this method could indicate concentrations for *Aspergillus* section *Fumigati* 38–91-fold higher than those of *Aspergillus* section *Flavi*. *Enterobacter* spp. were detected at 3,000–4,000 16S copies/cm^2, followed by *Legionella* spp. at 60–300 16S copies/cm^2 by 16S rDNA targeting systems; the levels of *E. coli* were only 100 uspA copies/cm^2. As mentioned previously, the copy-number variations between the single-copy *uspA* and the multicopy *16S rDNA* also explained a few differences. Nevertheless, it is worth noticing that the highest concentrations of all the target microbes except *Aspergillus* section *Fumigati* were detected in the CAFs from organic WCTs. The presence of the enteric bacteria *Legionella* spp., *Aspergillus* section *Fumigati*, and *Aspergillus* section *Flavi* highlights the potential risks faced by waste collection workers upon exposure to infectious microbial agents and mycotoxin-producing fungi. Their presence in filters from automobile air-conditioning systems was also reported as a severe health risk by Golofit-Szymczak et al (Gołofit-Szymczak et al. 2023). The detection of *Legionella* spp. contrasts with a previous report that could not find *Legionella* spp. in the CAFs of personal cars (Li et al. 2013). For a complete microbial risk assessment, this finding enhanced the importance of using a combination of culture and molecular biology methods that do not depend on the growth capacity of the microorganisms. The isolation of cultivable *Legionella* spp. in widely diversified and high bioburden samples is complicated (Marchand et al. 2018). The analysis of *Legionella* spp. faces several limitations and potential false negatives. An underestimation of the occupational risk may be frequent if only the culture method is used. *Legionella* spp. serve as an example, but the limitations

Table 2. Concentration of microbial agents in CAF of trucks collecting the three types of wastes (domestic, organic, recyclable).

	All types			Domestic			Organic			Recyclable		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
		CFU/cm2			**CFU/cm2**			**CFU/cm2**			**CFU/cm2**	
Bacteria TSA	28	2.9E4	5.4E 4	11	2.3E4	4.4E 4	7	6.3E4	8.8E4	10	1.3E4	1.2E4
Bacteria VRBA	27	2.1E3	6.0E3	10	2.4E3	7.0E 3	7	7.0E2	1.0E3	9	2.9E3	8.0E3
Fungi MEA	28	7.9E3	1.8E 4	11	4.2E3[b]	5.0E 3	7	2.2E4[a]	3.4E4	10	2.0E3[b]	1.0E3
Fungi DG18	26	3.2E3	3.0E3	10	2.5E3	3.0E3	7	3.7E3	2.0E3	9	3.5E3	4.0E3
Fungi SAB	26	8.0E2	7.0E2	10	6.0E2	5.0E2	7	1.4E3	9.0E2	9	7.0E2	40
Fungi ITRA	26	2.0E2	3.0E	10	2.0E2	3.0E2	7	2.5E2	3.0E2	9	1.0E2	1.0E2
Fungi VORI	26	7.0E2	1.0E3	10	1.0E3	1.0E3	7	7.0E2	6.0E2	9	4.0E2	7.0E2
Fungi POSA	26	30	50	10	10	10	7	40	60	9	4.0E2	60
		EU/cm2			**EU/cm2**			**EU/cm2**			**EU/cm2**	
Endotoxins	27	2,0E2	2,0E2	11	1,0E2	2,0E2	7	3,0E2	4.0E 2	9	90	50
		DNA/cm2			**DNA/cm2**			**DNA/cm2**			**DNA/cm2**	
Bacteria	27	8.8E5	2.0E6	11	6.1E5	1.2E6	7	2.1E6	3.4E6	9	2.5E5	1.8E5
Fungi	27	6.5E5	1.2E6	11	4.6E5	9.8E5	7	1.4E6	1.9E6	9	2.5E5	2.5E5
Asp./Pen.	27	2.6E5	5.1E5	11	2.7E5	6.4E5	7	4.8E5	5.6E5	9	6.9E4	4.6E4
A.s. Fumigati	27	5.0E5	1.4E5	11	8.3E5	2.1E5	7	5.3E4	6.8E4	9	6.0E3	4.0E3
A.s. Flavi	27	2.0E2	3.0E2	11	80[b]	2.0E2	7	4.0E2[a]	5.0E2	9	100[b]	200
Enterobacter	27	4.0E2	7.0E3	11	3.0E3	8.5E3	7	4.0E3	7.0E3	9	4.0E3	5.0E3
Legionella	27	2.0E2	2.0E2	11	2.0E2	2.0E2	7	3.0E2	3.0E2	9	60	30
E. coli	27	1.0E2	2.0E2	11	1.0E2	4.0E2	7	1.0E2	1.0E2	9	40	30

a, b, c: different letters mean significantly different from one another at $p < 0.05$ SD: Standard deviation; A.s. *Fumigati*: *Aspergillus* section *Fumigati; Aspergillus* section *Flavi: A.s. Flavi*; Asp./Pen.: *Aspergillus Penicillium* group; Asp: *Aspergillus; E.coli: Escherichia coli;* TSA: tryptic soy agar; VRBA: violet-red bile agar; MEA: Malt extract agar; DG18: Dichloran-Glycerol agar; SAB: Sabouraud dextrose agar; ITRA: itraconazole supplemented; POSA posaconazole supplemented; VORI: voriconazole supplemented; CFU: Colony forming units; EU, Endotoxins units; DNA: DNA copies by ddPCR.

to the analysis of cultivable microbiota fully justify the prerequisite of combining sampling and analysis methods to achieve a complete microbial risk assessment of workplaces (Viegas et al. 2022), as performed in the present study.

Similar to the results of molecular detection, the highest average concentrations for the cultivable microorganisms were observed in the CAFs of organic WCT (Table 2). 100% of the WCT filters were contaminated with cultivable fungi and bacteria, as was previously reported with CAFs from personal cars (Aquino et al. 2018; Li et al. 2013). These frequencies are, however, two to five-fold higher than those reported by other studies in CAFs from taxis (21.1%) and personal cars (53.6%) (Viegas et al. 2018). Climate change, methodological variations, and differences in vehicle function could explain some of these discrepancies. Organic and microbial contaminants originating from the waste materials could influence microbial load. In other studies, the concentration of fungi in CAFs from taxis (184 CFU/cm^2) was lower than in the WCT filters (7,900 CFU/cm^2) (Viegas 2018). However, the one measured in personal cars (3.4×10^3 CFU/cm^2) (Viegas 2018), 5.4×10^4 CFU/m^2 for summer and 2.4×10^4 CFU/m^2 for winter (Gołofit-Szymczak et al. 2023) mainly were comparable. Another interesting difference concerns the cultivation of enteric bacteria in WCT filters, which are absent from the CAFs of taxis and personal cars. The absence of enteric Gram-negative bacteria (GNB) grown on VRBA media was explained by the fragility of their cell membranes and the fact that they could not survive the dehydration caused by the air-conditioning system (Viegas et al. 2018). In the present study, enteric bacteria were grown from > 50% (15/26) of the CAFs, demonstrating their capacity to resist some of the stress caused by the system. The higher inherent microbial contamination and organic dust near the WCTs may offer nutrients, thus providing superior growth conditions and supporting bacterial metabolism, improving the recovery of cultivable bacteria. Microbes are common contaminants of waste materials (Madsen et al. 2021; Neumann et al. 2002). Since the ventilation system draws outside air around the truck, those contaminants could be aspired in the CAF by the truck's ventilation system. This could, at least, partially explain the results specific to the WCTs compared to taxis and personal cars.

Endotoxin concentrations measured were between 90 and 300 EU/cm^2 of a filter but were not significantly different between the three types of waste collected. To our knowledge, no other study has reported endotoxin concentrations in CAFs from vehicles. However, a study concerning HEPA-air-purifier filters found negligible concentrations (0.19 to 1.57 EU per cm^2) compared to the CAFs of WCTs (Niu et al. 2020). The presence of GNB cultivable on VRBA may explain some of the endotoxin concentrations observed. However, endotoxins were also detected in CAFs without any cultivable GNB, suggesting that GNB may not be considered a risk sentinel of endotoxin concentrations.

As for mycotoxins, only fumonisin was detected in a CAF sample. Fumonisin B1, mainly produced by *Fusarium* species, was present at a concentration above the LOD (>2.5 µg/kg) while below the LOQ (<8.3 µg/kg). Fumonisin is classified as possible human carcinogenic (B2) by the International Agency for Research on Cancer (International Agency for Research on Cancer List of Classifications – IARC Monographs on the Identification of Carcinogenic Hazards to Humans Volume 56 1993; Viegas et al. 2016). It highlights a potential health risk for these workers. The toxin was detected in a CAF sample from a truck collecting recyclable wastes in a rural community. Since *Fusarium* is often present in maize, wheat, and other cereals, its detection in rural environments may be explained by large agricultural fields.

The screening of azole resistance in fungal isolates of *Alternaria, Aspergillus, Chrysonillia, Chrysosporium, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus*, and *Trichoderma* from CAFs (*A*. section *Fumigati* was not detected), identified *Fusarium*, *Mucor*, and *Rhizopus* which are considered high priority pathogens based on the WHO fungal priority pathogens list (WHO 2022). The WHO report aims to garner more attention and resources toward developing policies and driving research efforts focused on strengthening the global response to these fungal pathogens, particularly regarding the prevention of antifungal resistance, as fungal infections keep increasing (Auberger et al. 2012; Bitar et al. 2009; Caetano et al. 2017; Fisher et al. 2022; Gow et al. 2022; Kontoyiannis et al. 2005). Of note, our results might be underestimated considering the presence of the fast-growing fungi: *Mucor, Rhizopus*, and *Trichoderma* (Walther et al. 2020) in the culture media, which can hinder the growth of other fungal species through nutrient competition. Even though insignificantly different, Table 2 shows that concentrations of voriconazole-resistant species exceed more than three-fold those of itraconazole-resistant species and more than twenty-fold those of posaconazole-resistant species.

Most of the samples from CAFs of the WCTs demonstrated particular cytotoxic effects both on A549 (75%)

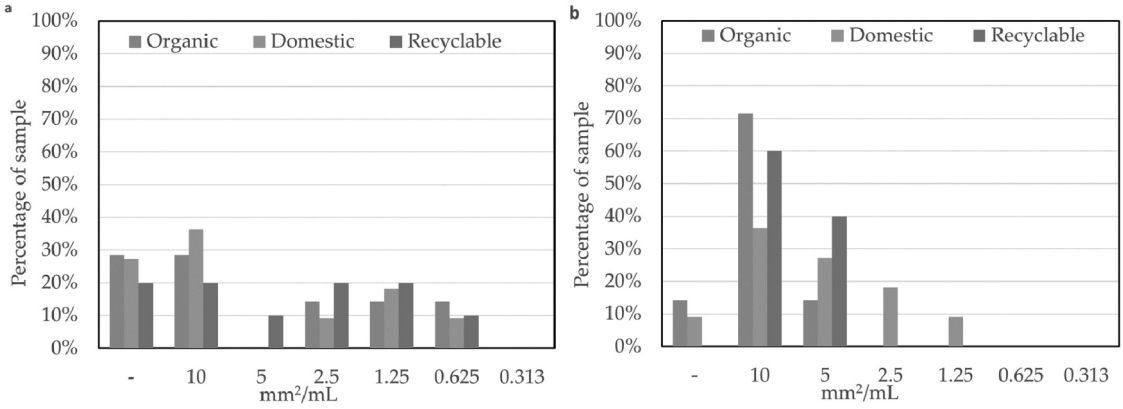

Figure 3. Cytotoxicity levels (LC50-mm^2/mL) of CAF samples from waste collecting trucks. a: A549 lung epithelial cells; b: HepG2 human hepatocyte carcinoma cells "–": no cytotoxicity effect.

and HepG2 (93%) cells (Figure 3). In the CAFs from the organic WCT, 71% of the samples showed toxic effects on the lung cells, many at lower surface-sample concentrations, attesting to a more potent toxicity. Most effects in hepatocytes were observed with the concentrated extracts, corresponding to sample surface of 10 and 5 mm^2/mL. Nearly 75% of the CAF samples from the domestic WCTs demonstrated toxic effects on lung cells, and > 90% had some impact on the hepatocytes. Once again, the effects on the lung cells were observable at lower samples concentrations. The effects in hepatocytes were primarily visible with the undiluted samples, demonstrating a lower toxic effect. Regarding the recyclable materials, >80% of the samples affected lung cells and 100% the hepatocytes. The lung cells were again affected by the samples with the least concentration. Although HepG2 cells experienced a toxic effect by a higher proportion of samples, they generally required more concentrated samples to exhibit any interference in metabolic activity. The cytotoxicity assay clusters the effects of multiple contaminants and provides an integrated risk assessment for the workers. Even if the impacts of individual contaminants were not examined, cytotoxicity results demonstrated that waste workers were exposed to cytotoxic pollutants that can affect the metabolism of both lungs and liver cells.

Location (urban or rural)

In addition to the types of waste collected, the location (rural or urban) was identified as a potential influencing factor on the microbes and the cytotoxic effects of the CAF samples (Figure 4). Location significantly influenced voriconazole resistance in cultivable fungi ($F = 6.21$, $p = 0.022$), the fungi ($F = 5.47$, $p = 0.003$), and *A.* section *Flavi* ($F = 4.8$, $p = 0.044$). The levels of voriconazole-resistant fungi were significantly higher for CAF samples from rural compared to urban environments. The excessive use of azole fungicides in agriculture has been proposed as a potential inducer of azole resistance in the environment (Caetano et al. 2017; Chowdhary et al. 2013; Pena et al. 2021; Snelders et al. 2012). This might partially explain the higher prevalence of voriconazole resistance in the countryside samples. However, more information on the fungicides used in these regions would be needed to confirm this association. The presence of azole-resistant strains in the CAF indicates a potential risk of infection for immunocompromised workers. Even though the screening method used for azole resistance is not the reference method by microdilution (EUCAST), it nevertheless underlines the relevance of determining the presence of azole-resistant strains of microbes during risk evaluations (Arendrup et al. 2014).

In contrast, all other variables showing significant differences had the highest concentrations in CAF samples from urban WCTs. The higher population density and waste load in urban areas compared to rural areas could partially explain this. The influence of the tonnage loading per day on the fungal concentrations was reported during the collection of recyclable waste (Neumann et al. 2002).

Cytotoxic effects according to the type of agglomeration (urban/rural) demonstrated that 87.5% of CAF samples from rural trucks affected liver cells compared to only 50% on lung cells. On the urban side, 95% of the CAF samples had a cytotoxic effect on liver cells and 85% on lung cells. For A549, the average surface area of CAF samples producing a toxic effect was 6.6 mm^2/mL for urban areas compared to 13.9 mm^2/mL for rural areas, demonstrating a significant difference between the two areas. For HepG2 cells, the average surface area causing a toxic effect was 9.4 mm^2/mL for the CAF samples from WCTs in the rural areas and 8.1 mm^2/mL for the

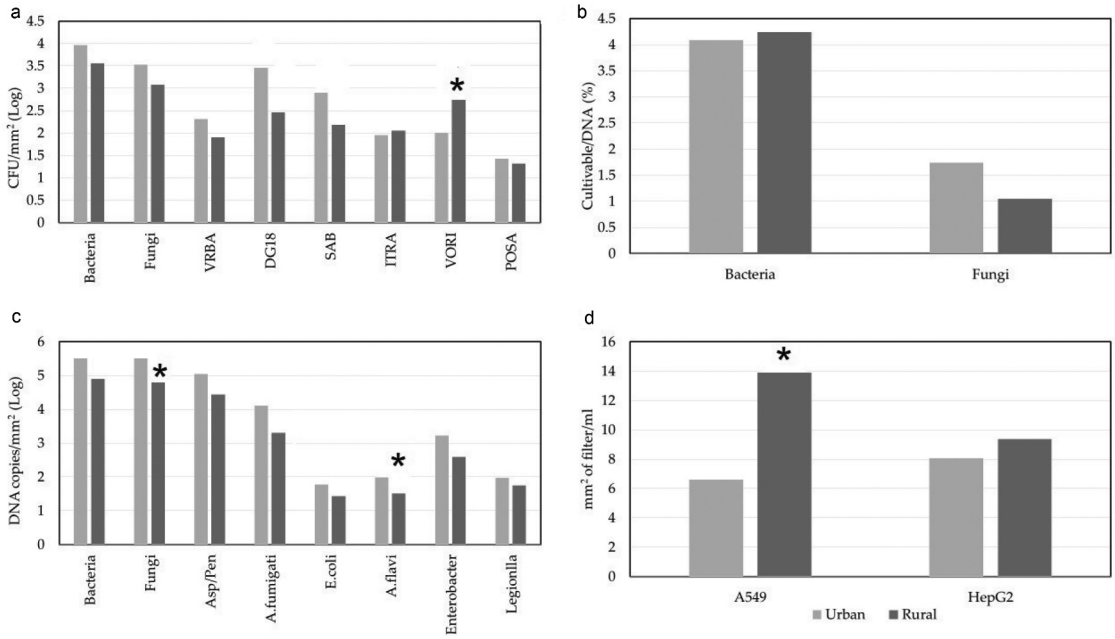

Figure 4. Concentrations of microbial agents and cytotoxic effects on the lung (A549) and hepatic cells (HepG2) produce by the CAF (ventilation system filters) of waste collecting trucks from urban and rural agglomerations. * significantly different at $p < 0.05$. See Table 2 for abbreviations.

trucks from the urban areas. As in many other occupations, waste workers are exposed to various pollutants. Cytotoxicity assessment plays an essential role in occupational health and safety because it helps identify and better understand the influence of co-exposure on the health of workers (Viegas et al. 2022). Indeed, cytotoxicity evaluations may reveal the toxic effects of an interaction between several contaminants. The complete model on the two-way GLM-ANOVA could not demonstrate any significant differences in the mean concentrations of the microbial agents nor the cytotoxic effects for ascertaining the interaction between the type of waste and the location.

Influence of the CAF models

The CAF model might potentially impact microbial contamination in the filtration media. Unfortunately, since many of the filter models recovered from the trucks were present in only one copy, it was impossible to validate this hypothesis. Only three models were collected on more than one occasion (4466, 9082, and ABP). For these models, an ANOVA analysis was performed to confirm the relevance of investigating the model impact since significant differences were found for some microbial agents (ddPCR: *A.* section *Fumigati* and culture: bacteria, fungi MEA, DG18 and SAB) (Figure 5). The Tukey-Kramer multiple-comparison test demonstrated that the CAF model 4466 had the lowest cultivable bioburden, while the model 9082 had the highest.

Filtration efficiency influenced the collection of the particles, their location, and migration in the filter. To determine if it impacted the microbial contamination, their fractional filtration efficiency was evaluated at two flow rates (5 and 30 L/min), simulating the minimum and the maximum filtration velocities based on measurements performed in real-world WCTs. The filtration efficiency of the filter model 4466 was

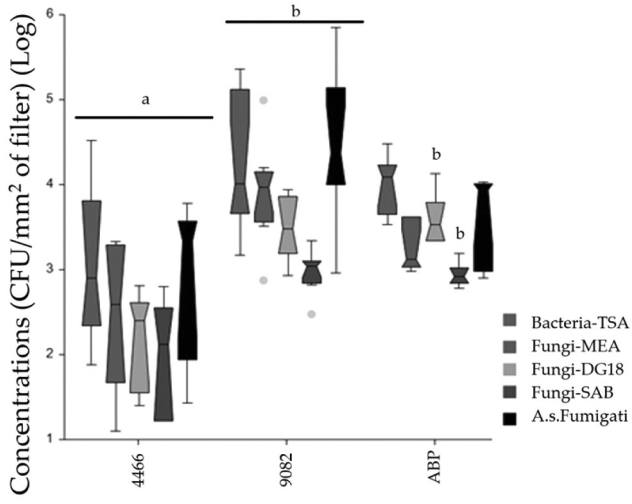

Figure 5. Microbial agents significantly differ in their cultivable bioburden load between the three models of CAF studied. The middle line of the box: Median; gray dots: outliers. a, b: different letter means significantly different from one another at $p < 0.05$. See Table 2 for abbreviations.

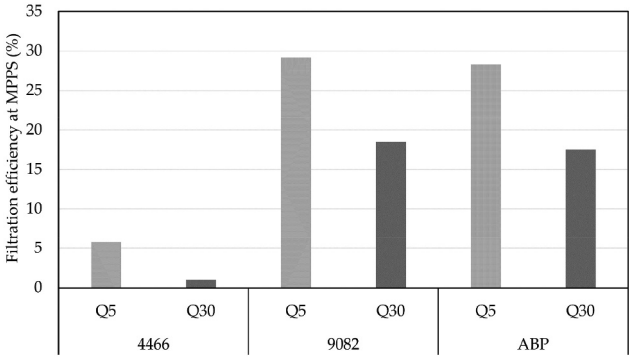

Figure 6. Minimum fractional filtration efficiency at two different flow rates for three CAF models removed from waste collecting trucks. Q5: 5 L/min; Q30: 30 L/min.

negligible for the most penetrating particle size at both flow rates compared to a minimum efficiency of ~ 30% of the filter models 9082 and APB at 5 L/min flow rate and close to 20% at 30 L/min (Figure 6). This low efficiency of filter 4466 could explain the fewer microorganisms recovered. The filter 4466 probably could not stop particles, which may end up in large quantities in the truck cabin. An in-depth paper on filtration performance is currently being written.

Microbial released from CAF

It is important to emphasize that the recovery of culturable microorganisms is subject to influencing factors, and it must be recognized that the impact of desiccation and the high air velocity in the CAFs could affect the recovery of the culturable microorganisms. The assumption of uniform degradation between microorganisms can also cause a bias. For these reasons, the results are considered estimates and representative of the filters and conditions during the tests. Nevertheless, the ANOVA analysis demonstrated that the CAF model influenced the release of both bacteria and fungi (Figure 7). The filter model 9082 presented the highest reemission of particles, and the model 4466 was the lowest. This behavior can be thought of logically with microbial contamination (Figure 5) and minimum filtration efficiency (Figure 6). Indeed, a lack of efficiency implies difficulties in retaining particles and, consequently, a few particles available for release. The measured airflow resistance CAF also agreed with the particle release measurements. Indeed, at a flow rate of 30 L/min, model 4466 exhibited a pressure drop almost three-fold lower than 9082 and ABP. A lower airflow resistance could imply a more downward drag force experienced by the filter sample, a lower air velocity inside the porous media, and potentially less energy to be transferred to the particles for their release.

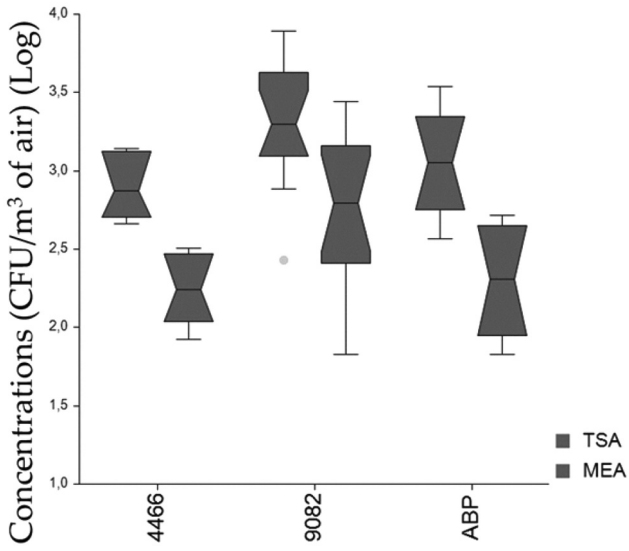

Figure 7. Concentration of cultivable microbial agents releases from ventilation filters and collected with a six-stage Andersen impactor adjusted at a flow rate of 28 L/min. TSA: tryptic soy agar for bacteria, MEA: malt extract agar for fungi. Box middle line: median; gray dots: outliers.

Microbial agents in the ambient air of truck cabins

Microbial agents in the ambient air of trucks cabins were analyzed by two-way ANOVA to examine the effects of the type of waste collected, the location (rural, urban) and their interactions (Table 3). Unlike the statistical analyses conducted on the filters, these results enlightened that many of the variables had significant interactions between the two principal factors. Of those, GNB and fungi showed no significant differences when individual factors were evaluated but when interactions were included with p-values of 0.004, 0.002, 0.002 and 0.005, respectively. For the cultivable fungi, the concentrations measured in the recyclable truck cab were higher in the rural conditions, while for the same type of waste, the order variable concentrations were usually higher in the urban agglomerations. For most of the other variables, except cultivable bacteria and endotoxins, the concentrations in the air of the trucks collecting the organic waste were higher in the rural environments. In contrast, that environment presented the lowest concentrations for the recyclables and the domestic waste. The collection of organic wastes in rural agglomerations is still not a common practice in the province of Quebec. Therefore, the effectiveness of that combination of rural-organic is a critical limitation for the interaction interpretation.

Waste type (domestic, organic, recyclable)

As for the CAFs, air samples in the cabin allowed the detection of targeted microbial risk indicators (*A.* section *Fumigati*, *A.* section *Flavi*, *E. coli*, *Enterobacter* spp.,

Table 3. Two-way ANOVA F ratio for the microbial agents and the cytotoxicity assay analyzed on the air of waste collecting trucks cabins.

	Location		Type of Waste		Location*Type of Waste	
	F ratio	Probability	F ratio	Probability	F ratio	Probability
Bacteria TSA	5.15	0.025*	0.57	0.568	1.64	0.199
Bacteria VRBA	3.85	0.062	1.75	0.795	7.19	0.004*
Fungi MEA	0.26	0.615	3.04	0.052	6.94	0.002*
Fungi DG18	8.78	0.005*	3.9	0.030*	7.71	0.002*
Fungi SAB	0.71	0.403	2.47	0.098	6.06	0.005*
Fungi ITRA	2.06	0.281	1.2	0.237	0.97	0.197
Fungi VORI	0.91	0.348	0.13	0.883	0.28	0.754
Fungi POSA	2.65	0.121	0.56	0.56	0.08	0.08
Bacteria	12.3	0.001*	4.76	0.012*	10.97	0.000*
Fungi	0.15	0.698	13.5	0.000*	1.94	0.153
Asp./Pen.	2.45	0.123	18.29	0.000*	11.04	0.000*
A.s. Fumigati	0.02	0.878	12.18	0.000*	9.82	0.000*
A.s. Flavi	0.56	0.459	10.36	0.000*	1.8	0.176
Enterobacter	7.89	0.007*	0.91	0.407	4.43	0.016*
Legionella	6.01	0.018*	1.21	0.307	6.65	0.003*
E. coli	2.66	0.109	0.44	0.646	2.96	0.06
Endotoxins	40.06	0.000*	1.46	0.241	4.84	0.012*
A549	1.95	0.17	0.32	0.726	1.13	0.333
HepG2	4.06	0.05	2.55	0.09	4.73	0.014*

*significant at $p \leq 0.05$.
See footnote of Table 2 for abbreviations.

and *Legionella* spp.) in at least one sample for the three types of wastes. Table 4 presents the mean concentrations, the standard deviation, and the significant difference reported for the types of waste as principal factors for the microbial agents in the air of WCTs. The average concentrations measured in the air of the cabin of organic WCTs were significantly higher than the two other types of waste for cultivable fungi on DG18 and bacteria, fungi, *Asp/Pen*, *A section Fumigati*, and *A section Flavi*. However, not significant cultivable bacteria were also higher in the organic WCT cabins, while the *Legionella* spp and the enteric bacteria (VRBA) were higher during the collection of recyclables.

The highest average bacterial (16S) concentrations measured during the domestic waste collection (1.0×10^5 DNA/m^3 of air) were at the lower range of the concentrations reported in wastewater treatment and composting plants in Quebec (Bonifait et al. 2017; Duchaine et al. 2019). The levels of *A.* section *Fumigati* DNA were the highest among the targeted microbial risk indicators, and the highest average concentrations in the cabin of WCTs collecting the organic waste reached 8,000 DNA copies/m^3. The domestic waste concentrations were also significantly higher than in the air of WCTs managing the recyclable wastes. These findings demonstrate the influence of the waste

Table 4. Concentration of microbial agents in the air of waste collecting trucks cabins.

	All types			Domestic			Organic			Recyclable		
	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD
		CFU/m3			**CFU/m3**			**CFU/m3**			**CFU/m3**	
Bacteria TSA	108	5.2E3	6.0E3	46	5.2E3	6.3E3	23	6.1E3	7.4E3	39	4.6E3	4.6E3
Bacteria VRBA	45	9.0E2	2.1E3	19	1.0E2	3.0E2	11	1.2E2	2.6E3	17	1.6E3	2.6E
Fungi MEA	107	1.3E3	2.2E3	45	1.3E2	1.9E3	22	2.3E3	3.4E3	39	9.0E2	1.5E3
Fungi DG18	47	8.0E2	1.0E3	19	8.0E2^b	9.0E2	11	1.3E3^a	1.4E3	17	4.0E2^b	6.0E2
Fungi SAB	47	4.0E2	4.0E2	19	4.0E2	4.0E2	11	7.0E2	4.0E2	17	3.0E2	3.0E2
Fungi ITRA	47	80	1.0E2	19	70	1.0E2	11	1.0E2	1.0E2	17	80	2.0E2
Fungi VORI	47	1.7E2	3.0E2	19	2.0E2	3.0E2	11	3.0E2	5.0E2	17	80	1.0E2
Fungi POSA	24	1.0E2	2.0E2	19	90	3.0E2	11	1.0E2	2.0E2	17	90	2.0E2
		EU/m3			**EU/m3**			**EU/m3**			**EU/m3**	
Endotoxins	58	10	10	25	12	10	24	6	5	20	7	10
		DNA/m3			**DNA/m3**			**DNA/m3**			**DNA/m3**	
Bacteria	61	6.8E4	1.2E5	25	1.0E5^a	1.6E4	13	3.7E4^a	2.7E4	23	4.9E4^b	9.0E4
Fungi	61	1.7E5	3.1E5	25	1.2E5^b	9.54	13	5.0E5^a	5.6 E 5	23	4.7E4^c	5.0E4
Asp./Pen.	61	3.3E5	1.3E6	25	3.4E5^b	1.4E6	13	9.0E5^a	2.1E6	23	1.6E4^c	5.1E4
A.s. Fumigati	58	3.5E3	1.1E4	25	3.5E3^b	9.2E3	13	8.0E3^a	1.8E4	20	5.6E2^c	1.1E3
A.s. Flavi	61	10	40	25	20^b	60	13	90^a	10	23	10^b	10
Enterobacter	61	30	80	25	10	10	13	90	150	23	10	10
Legionella	61	4.3E2	1.0E3	25	3.8E2	1.0E3	13	1.3E2	1.0E2	23	6.6E2	1.2E3
E. coli	61	20	60	25	30	90	13	10	5	23	10	10

See footnote of Table 2 for significant of statistics and abbreviations.

type on the concentration of *A*. section *Fumigati* in the truck's air. To our knowledge, only one study reported *A*. section *Fumigati* in the ambient air of a work environment using ddPCR quantification (Salambanga et al. 2022). Still, a study carried out in three composting centers reported DNA concentrations of *A*. section *Fumigati* using qPCR, which were in the same order of magnitude as those measured here, apart from the carcass composting center where the concentrations were about an order of magnitude higher (51,800 DNA/m^3). These results support the relevant role of *A*. section *Fumigati* as an indicator in the professional environment. Even more so, it induced hypersensitivity pneumonitis in waste workers, significantly influenced their peak expiratory flow, and caused alveolitis or bronchial asthma (Albrecht et al. 2007; Dutkiewicz et al. 1994; Hagemeyer et al. 2013; Lacey and Crook 1988; Madsen et al. 2021; Poulsen et al. 1995; Wéry 2014).

Although their detection remained weak, it is crucial to underline the presence of *Legionella* spp., a notorious pathogen, in the air of domestic WCTs. Studies on *Legionella* have long been limited to cooling towers. Still, others demonstrated their presence in other environments (Bonifait et al. 2017; Casati et al. 2010; Conza et al. 2013; Hughes and Steele 1994; Picard-Masson et al. 2016), highlighting the importance of including *Legionella* spp. as targeted risk indicators when assessing specific working environments.

The average concentration of cultivable fungi measured in the air of the organic and domestic WCTs was higher than the recommended limits for high-load working environments (10^3 CFU/m^3 of air) (Marchand 2021; Poulsen et al. 1995; SUVA 2021). 60% (17/28) of WCTs had ambient loads in the cabin above the recommended action levels for cultivable fungi at least once a day. Some studies performed in Copenhagen, in the air of domestic WCTs, reported airborne cultivable fungal concentrations varying from 219 to 15,000 CFU/m^3 when grown on DG18 media (Madsen et al. 2016, 2020). In the present study, the measured concentrations on DG18 were lower, varying from < 1 to 5,100 CFU/m^3; nonetheless, the fungi cultivated on MEA reached equivalent concentrations (12,600 CFU/m^3 of air). These findings underline the critical importance of multimedia plating (culturomics) to obtain a widespread representation of cultivable fungal contamination in environmental samples. Even if higher airborne concentrations of fungi were reported on DG18 than on MEA (Jo et al. 2008; Salambanga et al. 2022), others reported the opposite (Ren et al. 2001). These variations of culture media performances may be attributable to the environments' specific microbiota and their growth requirements or even to competition among all the microorganisms present. These findings supported using an extended strategy using combined sampling and analysis methods to improve the documentation of workers' potential exposition to biological agents and appreciate the risks incurred by them.

Regarding the cultivable bacteria, even if the average concentration in the air of the WCTs was lower than the recommended limits, almost 20% of the samples exceeded the proposed threshold concentrations (1,0E 4 CFU/m^3) (Marchand 2021; Marchand, Lavoie, and Lazure 1995; Suva 2021). Air sampling in the domestic WCTs most often exceeded the proposed limit, with a maximum concentration of 33,560 CFU/m^3. The geometric means and the maximum concentrations observed in Copenhagen WCTs are much lower (261 and 2,600 CFU/m^3) than those measured in Montréal (1,790 and 33,560 CFU/m^3). As explained for the fungi, the dissimilarity between the culture media can explain some of the differences (Madsen et al. 2020). In addition to the culture media, the impacts of other factors were anticipated, namely the different air samplers (Gesamtstaubprobenahme vs. SASS 3100), membrane (polycarbonate vs. electrets), flow rate (3.5 L/min vs. 300 L/min), geographic locations (Denmark vs. Canada), climates (oceanic vs. continental), and waste types collected between the two studies.

It is also worth mentioning that the concentrations of endotoxins in the air of all types of WCTs never exceeded the recommended limits of 90 EU/m^3 of air (Nordic Expert Group for Criteria Documentation of Health Risk from Chemicals (DECOS) 2020). The highest concentration [50 EU/m^3 of air] was measured in the air of a domestic WCT. No mycotoxins were measured above the detection limits on any air samples.

Although insignificant, the mean concentration of fungi resistant to voriconazole was the highest among the three tested azoles, considering all types of waste. The trucks collecting organic and domestic wastes had the highest concentrations of resistant fungal species. The genera identified in CAFs, except for *Fusarium*, were also found in the cabin air samples. However, the species diversity for resistant *Aspergillus* strains was higher in air samples than in CAFs. In the ambient air, *Aspergillus* sections *Nigri*, *Candidi*, and *Nidulantes* were identified, compared to only *A*. section *Nidulantes* in the CAF samples. Although no resistant strains of *A*. section *Fumigati* were identified in the air, the presence of *A*. section *Nigri* is of concern since this species is also responsible for causing infections (Public Health Agency of Canada).

The toxic effects of cabin ambient air samples were more frequent in HepG2 cells [85% air samples] than in A549 cells [<25% air samples] [Figure 8]. The highest

proportion of samples without toxic effects on lung cells was observed for trucks that collected recyclable materials [84%]. Most samples produced toxic effects on hepatic cells regardless of the type of waste collected, with a maximum observable effect for organic waste collection [100%]. Notably, most of the toxic effects are observed with low-diluted and concentrated samples, indicating lower cabin air toxicity. This observation differs from what was observed for the CAF samples since several samples showed toxic effects even at high dilutions. This confirms the value of using CAFs, as they can provide information about a potential exposition over a long period and allow sensitive analysis. Sampling over a short period does not allow risk assessment of prolonged exposition nor document the infrequently appearing microbial agents.

Location (urban, rural)

The two-way GLM-ANOVA analysis on the ambient air in the cabin determined that location significantly impacted some of the microbial agents measured. For all the significant variables (bacteria TSA and DNA, fungi on DG18, *Enterobacter* spp., *Legionella* spp, and endotoxins), regardless of the type of waste, higher concentrations were observed in the air samples from the urban trucks. However, when the interactions and the kinds of waste were considered, the concentration measured in the organic collecting truck were lower in the urban agglomeration for most of those independent variables (excluding endotoxin).

For the rural trucks, 68% of the air showed some cytotoxic effect on HepG2 cells, compared to only 10% on A549 cells. In the urban context, 100% of air samples had a cytotoxic effect on HepG2 cells and 34% on A549 cells. For A549 cells, the average surface area of samples producing a toxic effect was 15.54 mm^2/mL for the air from the urban areas compared to 19.13 mm^2/mL for air from the rural areas. For HepG2 cells, the average surface area of samples causing a toxic effect was 8.75 mm^2/mL for air samples in the urban areas and 12.83 mm^2/mL for the trucks from the rural areas.

Conclusion

The results revealed the presence of a complex mixture of biological contaminants during the collection of all types of waste. More explicitly, relatively high concentrations of *Aspergillus* section *Fumigati*, the presence of azole-resistant fungal strains specifically in the rural locations, the higher concentration of fungi and bacteria during the collection of organic waste, and the presence of *Legionella* spp. and enteric bacteria during the collection of all types of wastes, highlighted the importance of targeting multiple microbial contaminants in the waste sector to assess the microbial health risk.

In addition, this study emphasizes the value of employing complementary sampling strategy and combined analytical methods for a assessment of microbial risk in working environments. Culture, molecular biology, mycotoxins, endotoxins, and cytotoxicity assay are all valuable tools. *Aspergillus* section *Fumigati* has proven a prudent choice as a risk sentinel for this work environment. The sustained presence of cytotoxic effects through the samples confirms the potential adverse health impact of a cocktail of contaminants for the waste collection workers.

This project also confirms the usefulness of sampling using CAFs since they provide complementary information about the presence of microbial risk for an extended period. CAFs represent the presence of contaminants over a long period, while ambient air samples characterize the concentration variations of a working day. Combining both sampling methods contributed to a sensible and deeper characterization of microbial risk in this industry.

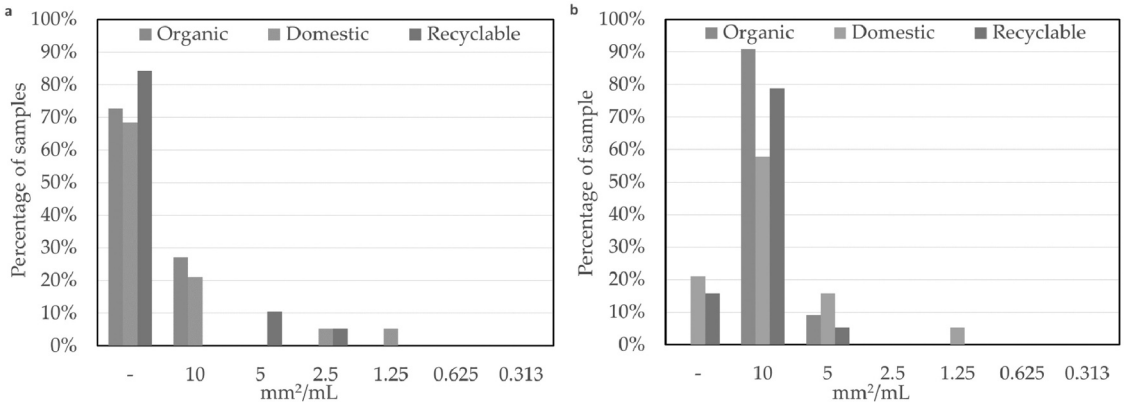

Figure 8. Cytotoxicity levels [IC50-mm^2/mL]) in the ambient air from the cabin of waste collecting trucks. a: A549 lung epithelial cells; b: HepG2 human hepatocyte carcinoma cells. "–": no cytotoxicity effect measured.

The results show the need to implement better risk management measures to reduce workers' microbial risk. These control measures could include more effective filters, better cabin maintenance, or respiratory protection equipment, but this project did not allow us to assess their impact.

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Author contributions

Conceptualization: G.M., M.D.a, L.W., C.V., S.V., L.C.; Funding acquisition: G.M., M.D.a, L.W., C.V., S.V., L.C.; Project administration: G.M., L.W., M.D.a, C.V., S.V., L.C.; Investigation: G.M.; L.W., M.D.a, N.L., D.L., I.V.,F.G; Formal analysis: L.W., N.L., D.L., E.S., M.T., R.K., M.D.b; Re-sources: G.M., M.D.a, C.V., S.V., L.C, M.T.; Data Curation: G.M., L.W.; Writing – Original Draft: G.M.; Writing Review & Editing: L. W., M.D.a, C.V., L.C., S.V., M.T.; N.L., D.L., I.V., F.G.

All authors have approved the final version.

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No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the findings of this study are available from the corresponding author (GM), upon request.

Informed consent statement

Informed consent was obtained from all workers involved in the study.

Institutional review board statement

The study was conducted in accordance and approved by the Ethics Committee of Université de Montréal (protocol CERC-19-039-D on may, 28 2019).

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