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Protection of the vehicle cab environment against bacteria, fungi and endotoxins in composting facilities

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ABSTRACT

Microbial quality of air inside vehicle cabs is a major occupational health risk management issue in composting facilities. Large differences and discrepancies in protection factors between vehicles and between biological agents have been reported. This study aimed at estimating the mean protection efficiency of the vehicle cab environment against bioaerosols with higher precision. In-cab measurement results were also analysed to ascertain whether or not these protection systems reduce workers' exposure to tolerable levels.

Five front-end loaders, one mobile mixer and two agricultural tractors pulling windrow turners were investigated. Four vehicles were fitted with a pressurisation and high efficiency particulate air (HEPA) filtration system. The four others were only equipped with pleated paper filter without pressurisation. Bacteria, fungi and endotoxins were measured in 72 pairs of air samples, simultaneously collected inside the cab and on the outside of the cab with a CIP 10-M sampler.

A front-end loader, purchased a few weeks previously, fitted with a pressurisation and high efficiency particulate air (HEPA) filtration system, and with a clean cab, exhibited a mean protection efficiency of between 99.47% CI 95% [98.58–99.97%] and 99.91% [99.78–99.98%] depending on the biological agent. It is likely that the lower protection efficiency demonstrated in other vehicles was caused by penetration through the only moderately efficient filters, by the absence of pressurisation, by leakage in the filter-sealing system, and by re-suspension of particles which accumulated in dirty cabs. Mean protection efficiency in regards to bacteria and endotoxins ranged between 92.64% [81.87–97.89%] and 98.61% [97.41–99.38%], and between 92.68% [88.11–96.08%] and 98.43% [97.44–99.22%], respectively. The mean protection efficiency was the lowest when confronted with fungal spores, from 59.76% [4.19–90.75%] to 94.71% [91.07–97.37%]. The probability that in-cab exposure to fungi exceeded the benchmark value for short-term respiratory effects suggests that front-end loaders and mobile mixers in composting facilities should be fitted with a pressurisation and HEPA filtration system, regardless of whether or not the facility is indoors or outdoors. Regarding the tractors, exposure inside the cabs was not significantly reduced. However, in this study, there was a less than 0.01% risk of exceeding the bench mark value associated with fungi related short-term respiratory effects during an 1-h per day windrow turning operation.

Pressurisation and a HEPA filtration system can provide safe working conditions inside loaders and mobile mixer with regard to airborne bacteria, fungi and endotoxins in composting facilities. However, regular thorough cleaning of the vehicle cab, as well as overalls and shoes cleaning, and mitigation of leakage in the filter-sealing system are necessary to achieve high levels of protection efficiency.

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1. Introduction

Moving large quantities of materials in composting facilities generates emission and dispersion of dust. All practices involving waste handling and extensive mechanical disturbance have reported elevated concentrations of bioaerosol emission: feedstock unloading, manual sorting, shredding, mixing, breakdown of piles, windrow turning, screening, truck loading with compost, cleaning activities (Epstein et al., 2001; Millner et al., 1994; Sanchez-Monedero et al., 2005; Schlosser et al., 2009; Spencer and Alix, 2006; Swan et al., 2003; Wouters et al., 2006). In addition, vehicle movement across dust-covered surfaces generates dispersion, which is expected to increase when the dipper of the front-end loader is lowered to scrape and clean the dusty floor.

Composting operations are associated with the growth of micro-organisms that enables the decomposition of organic materials. When large quantities of materials are agitated, large amounts of live and dead micro-organisms and related compounds, i.e., microbial fragments, toxins and metabolites, are

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released into the air. These airborne biological agents, constituting bioaerosols, are attached to dust particles, or occur as aggregates of cells, or as single cells such as moulds and actinomycetes spores (Górny et al., 1999). Published data indicate that the sizes of particles supporting colony forming bacteria or fungi collected in the vicinity of composting plants were generally less than 7–8 μm (Byeon et al., 2008; Chiang et al., 2003; Stagg et al., 2010; Streib et al., 1996), with the mass median aerodynamic diameter being approximately 4 μm (Darragh et al., 1997). The aerodynamic diameter range of fungi genera spores frequently identified in the vicinity of composting facilities, such as *Aspergillus, Penicillium* and *Cladosporium,* is between 1.7 μm and 3 μm (Madelin and Johnson, 1992). Bioaerosols in composting facilities can thus be inhaled, and a substantial part can reach bronchioles and alveoli in the deep lung. In addition, bioaerosols can be ingested by workers and deposited onto their eyes and skin.

Exposure to bioaerosols has been associated with infectious, allergic and toxic effects (ACGIH, 1999; Douwes et al., 2003; Dutkiewicz, 1997). In composting materials, most bacteria and fungi are not pathogens, i.e., they do not cause infection in a host with a healthy immune system. *Aspergillus fumigatus* can cause invasive infection, but it is an opportunistic pathogen which mainly targets immuno-compromised subjects. In fact, the major concern in non-immunocompromised individuals is the occurrence of adverse effects on the respiratory tract through immuno-allergic and toxic mechanisms. Causative agents of respiratory allergies are found in moulds and thermophilic actinomycetes (Allmers et al., 2000; Bünger et al., 2007; Weber et al., 1993). Regarding the toxic effects of exposure to airborne biological agents, the causative agents are primarily microbial cell wall agents, and mainly endotoxin from the cell wall of Gram negative bacteria because of its high pro-inflammatory potential (Rylander, 2002). The release of inflammatory mediators, like cytokines, in mucous membrane leads to the irritation of airways and eyes (Douwes et al., 2000; Heldal et al., 2003).

Numerous scientific papers have been published on the level of occupational exposure to bioaerosols in composting facilities, such as reviewed by Deloraine et al. (2002), Schlosser et al. (2009), Stagg et al. (2010), and Swan et al. (2003). Widely varying concentrations in air of mesophilic bacteria, thermophilic actinomycetes, fungi and endotoxins have been reported, mostly from stationary samplings. When personal sampling was carried out, results indicated high levels of individual exposure to micro-organisms and endotoxins, consistent with inflammatory and allergic respiratory outcomes among workers (Bünger et al., 2007; Epstein et al., 2001; Schlosser et al., 2009; Stagg et al., 2010; Sykes et al., 2011; van der Werf, 1996; Wouters et al., 2006).

High levels of exposure to bioaerosols by workers in composting facilities emphasise the need for source and pathway control and personal protection. The main operating task in composting facilities consists of driving vehicles, such as loaders, mobile mixers, large mobile windrow turners, and agricultural tractors which pull small windrow turners. The quality of air inside the vehicle cab is thus a major objective in the management of health risks in compost facilities regarding bioaerosols.

In the field, large discrepancies between the protective equipment in vehicle cabs can be observed. Some vehicle cabs are well-sealed and fitted with a pressurisation and high efficiency particulate air (HEPA) filtration system, whereas others are insufficiently air tight and fitted with a poorly designed filtration system. During operations, if the vehicle is not fitted with a pressurisation system, particles can penetrate into the cab through the various leaks in the cab housing. Particles can also penetrate through air filters of moderate efficiency. Moreover, even if the cab is fitted with a pressurisation and HEPA filtration system, leakage in the filter-sealing system enables penetration of particles into the cab. However, vehicle cabs are also contaminated by particles entering when doors and windows are opened and from workers' dirty clothes and shoes. Finally, if the cab is not cleaned regularly and thoroughly, particles can accumulate inside the cab, and then be re-suspended, contributing to air contamination.

The performance of vehicle cab filtration systems has mainly been investigated in the agricultural field. The purpose of these studies was to investigate protection against pesticides aerosols, with field studies (Bémer et al., 2007; Hall et al., 2002; Heitbrink et al., 2003; Moyer et al., 2005) and laboratory testing (Bémer et al., 2007, 2009; Heitbrink et al., 2003), or protection against grain dust, bacteria and fungi during harvesting (Thorpe et al., 1997). Mining operations have also been studied, with regards to protection against silica particles (Cecala et al., 2005).

By contrast, protection of the vehicle cab environment against bioaerosols in composting facilities is poorly documented. Bünger et al. (2007) compared exposure levels in shovel loader cabs with filtered air and without filtered air. Concentration ranges of filamentous fungi and actinomycetes were one order of magnitude lower in the cab with filtered air. In three works that studied levels of exposure to bioaerosols in composting facilities, bioaerosol concentrations inside vehicle cabs were compared with concentrations of bioaerosol outside of the cab. Schlosser et al. (2009) and Stagg et al. (2010) compared personal sampling results inside the cab with the results of stationary sampling in the nearby operational area using similar samplers, for dust, bacteria, fungi and endotoxins (Schlosser et al., 2009) and for bacteria, total fungi and *A. fumigatus* (Stagg et al., 2010). Median values of the ratios between out of the cab and in-cab concentrations were in the same order of magnitude for these two studies, ranging between 1.5 and 5.6. However, each of these studies showed large differences in the ratios both between the vehicles and between airborne agents. Depending on the vehicle and the airborne agent, the ratios ranged from 0.03 to more than 500,000 (Schlosser et al., 2009), and from 0.01 to 871 (Stagg et al., 2010). Recently, in a study that characterized compost workers' exposure to dust, endotoxin and β-(1–3) D glucan, Sykes et al. (2011) compared personal sampling results inside the cab to measurements with samplers positioned at the air intake on the outside of vehicles. Similarly, considerable differences in protection factors between vehicles and between airborne agents were observed. In summary, all the afore-mentioned studies in composting facilities have reported a high variability in the reduction of exposure to bioaerosols inside vehicle cabs and interpretation of results is, thus, limited. However, none of these field studies had been specifically designed for the assessment of the protection of the cab environment. Information on the reliability of the estimated protection factor was limited, often from single measurements, and the observed discrepancies have not been explained.

The objective of this study was therefore to further estimate the protection of vehicle cab environment against airborne biological agents in composting facilities. A specific sampling strategy was designed to consider the variability of bioaerosol concentration in air and to gain precision in the estimate of the protection level descriptor. Protection levels were then compared between vehicles and between airborne biological agents. In-cab measurement results were also analysed to ascertain whether or not these protection systems reduce workers' exposure to tolerable levels.

2. Materials and methods

2.1. Site and vehicle selection

Airborne biological agents sampling was carried out in four large-scale composting facilities: two as enclosed facilities (A and

B), and two as open-air facilities (C and D). All facilities compost sewage sludge, using ground pallet and/or green waste as a bulking agent. In the enclosed facilities, all processes are indoors, except maturation and storage in site A and storage in site B. The first phase of degradation is operated in open boxes with negative aeration. Real capacities are 21,000 t and 40,000 t, respectively. In the open-air facilities, degradation is operated in windrows, turned once a week with a turner pulled by an agricultural tractor. Real capacities of C and D facilities are 13,000 t and 10,000 t, respectively.

A total of eight vehicles were investigated, including five front-end loaders, one mobile mixer, and two agricultural tractors (Table 1). All vehicles were equipped with a cool-air system.

Vehicles in enclosed facilities were all equipped with a pressurisation and HEPA filtration system, located on the roof of the vehicle or on one of its fenders (Fig. 1). Originally these vehicles were not pressurised, were not supplied with filtered air, and were only equipped with an outside recirculation ventilation system with a pleated paper filter inside the cab. The pressurisation and HEPA filtration system was fitted at a later date by a specialised manufacturer. With this system, a fan moves the fresh air through three separate filters (H13 class borosilicate filter protected by a pre-filter and a F7 class filter) and then through an activated carbon cartridge for protection against gases, especially ammonia. Activated carbon cartridges have negligible filtration efficiency against particles, except against the very largest ones (Thorpe et al., 1997). Concerning the front-end loaders, the filtered air is transported through ventilation ducts to the air cooling unit. At this point, the filtered air is drawn into the cab by the blower. In these vehicles, the recirculation ventilation system in not operative. Conversely, in the mobile mixer, the filtered air is directly blown into the cab through an inlet louver located behind the operator seat. The air cooling unit uses the recirculation system of air inside the cab. In all vehicles, air flows out of the cab through the various leaks in the cab housing, such as leaks due to mechanical and electrical connections and potential defects of the housing seals. According to the manufacturer, the airflow rate was 50–60 m^3/hour. Air blowing maintains positive pressure inside the cab. During the sampling runs, the differential pressure was measured using a portable electronic manometer (model M100, Kimo, France). Overpressure inside the cab was high at more than 50 Pa (Table 1).

In vehicles in open air facilities, the fresh air was only filtered through a pleated paper filter, and no cab pressurisation system had been fitted.

The frequency of filter changes differed between the facilities: five weeks in site B, four months in sites C and D and one year in site A. In site A, filters were all changed five days before the sampling was carried out. On each site, cabs were regularly cleaned with an air gun. The cleaning frequency ranged from an half a week to one month depending on the site (Table 1). Vehicle cab had been cleaned before the sampling runs in sites B, C and D, but not in site A. It is noteworthy that the front-end loader in site B was purchased as a new vehicle two months beforehand, and thus filters were still recent.

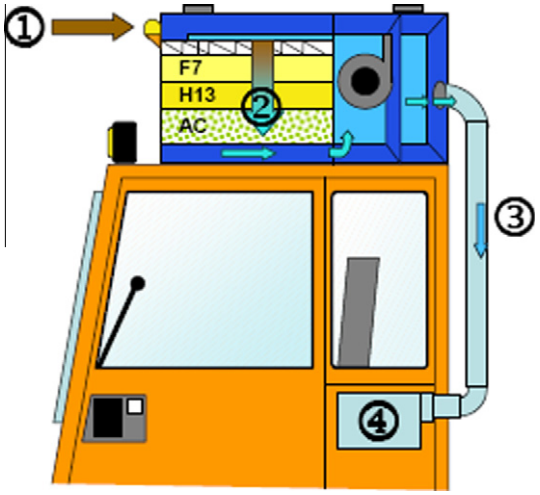

Fig. 1. Schematic of cab inlet airflow in front-end loaders equipped with a pressurisation and HEPA filtered system. A fan moves the fresh air (①) through three separate filters (②) (a pre-filter, a F7 class filter, and then a H13 class filter) and then through an activated carbon cartridge (AC). The filtered air is transported through ventilation ducts (③) to the air cooling unit (④).

No specific protocol for the assessment of cleanliness of vehicle cabs, clothes, shoes and filters was performed. Workers' clothes had not been changed just before the sampling runs. However, visual inspection indicated that they were apparently clean.

Examination of the vehicles showed no sign of impact and no major defect of the housing seals.

In the following text, the term "equipped vehicles" means vehicles equipped with a pressurisation and HEPA filtration system.

2.2. Sampling procedure

Bioaerosol sampling was carried out during the warm-season, between April and October 2010. Outdoor temperature ranged from 12.8 °C to 27.4 °C, and outdoor relative humidity ranged from 36.3% to 82.9%. Inside the enclosed facility B, mean temperature was 26.3 °C and 20.3 °C and relative humidity at the sampling points was 46.3–54.4%. For technical reasons, no climatic data were measured during sampling on site A.

The collection of culturable mesophilic bacteria, culturable mesophilic fungi and endotoxins was performed with CIP 10-M samplers (Tecora) equipped with the inhalation fraction selectors. This sampler has been described elsewhere (Görner et al., 2006; Nieguitsila et al., 2011). Briefly, this sampler is based on the rotative cup system with the rotation of the cup maintaining a flow rate of 10 l/min. For collection of micro-organisms and endotoxins, the CIP 10-M cup was filled with 2 ml of pyrogen-free sterile water (Versol, Aguettant Laboratory) containing 0.05% of Tween 20

Table 1
Description of the vehicles studied in composting facilities.

Site	Vehicle	Vehicle activity	Cab filtration system	Overpressure AM (SD), Pa	Cab cleaning frequency
A	Front-end loader	Mixing, piles breakdown, screening	Overpressure and HEPA filer	50.7 (2.3)	1/month
A	Front-end loader	Mixing, screening, compost loading	Overpressure and HEPA filter	149.3 (2.3)	1/month
B	Mobile mixer	Mixing	Overpressure and HEPA filter	210.0 (8,7)	1/week
B	Front-end loader	Screening	Overpressure and HEPA filter	279.9 (3.2)	1/week
C	Front-end loader	Screening, compost loading	Pleated paper filter	3.8 (3.5)	1/week
C	Agricultural tractor	Pulling of windrow turner	Pleated paper filter	<0.1	1/week
D	Front-end loader	Screening, compost loading	Pleated paper filter	1.0 (0.0)	1 or 2/week
D	Agricultural tractor	Pulling of windrow turner	Pleated paper filter	<0.1	1 or 2/week

(Polyoxyethylen sorbitan monolaurate, Merck). The cup was rinsed out three times to optimise particles recovery. The physical collection efficiency of the CIP 10-M is 50% for particles of 1.8 μm in aerodynamic diameter, and higher than 95% for particles larger than 2.8 μm. As emphasised by Görner et al. (2006), the collection efficiency of the CIP 10-M is similar to that of many single-stage microbiological impactors. The CIP 10-M was chosen for its capability one to be used as a personal sampler and two because it can easily be placed on the outside of the cab, as well as for its flow rate consistent with sampling lasting less than an hour in the vicinity of composting facilities. Moreover, the configuration of the CIP 10-M air flow also caused minimal stress to micro-organisms (Görner et al., 2006), and avoided desiccation of vegetative bacteria that is observed when collecting onto filters (Crook, 1995; Eduard and Heederik, 1998). However, in previous studies in composting facilities (Schlosser et al., 2009) and in sludge drying units (Schlosser et al., 2011), the results of endotoxin measurement observed with the CIP 10-M were higher than those reported in studies where the collection method was by filtration. Such differences regarding to the collection technique could be explained by: an increased amount of the free part of endotoxins when collected into a liquid (Rylander, 2002), the rotating movement of the sampler cup, and the absence of an extraction step during the analytical procedure. A significant log-linear correlation was observed in endotoxin measurements in composting plants between CIP 10-M and 25 mm IOM glass fibre filters (SKC Inc) (results not shown), but further experiment is needed for better characterization.

For each vehicle investigated, sampling was performed simultaneously inside the cab and on the outside of the cab. Inside the cab, the driver held a CIP 10-M with the sampler head placed near the breathing zone. On the outside of the cab, the CIP 10-M was placed next to the air inlet. All drivers, during the sampling period, were observed, and had been instructed not to open the door of the vehicle during the sampling run. However, during one of the sampling period on site A and on site B, drivers opened the vehicle door and came out of the cab. Therefore, these two samples have been excluded from analyses. Vehicle activity during sampling is described in Table 1. Regarding windrow turning in open-air facilities, on site C, during sampling, the tractor first moved facing the wind as in usual operation, and then with a tail wind as required by the study. The wind speed was between 0.0 m/s and 2.0 m/s. On site D, during sampling, the wind turned quickly, with a speed between 0.0 m/s and 3.1 m/s.

Past experiments indicate that the collecting efficiency of CIP 10-M equipped with the inhalable fraction selector is not affected by 5 m/s wind speed (Tecora, personal communication). On site vehicle speed was less than 20 km/h, and it is unlikely that the CIP 10-M collection efficiency was affected when placed on the outside of a moving vehicle.

CIP 10-M calibration was checked in the field by measuring the revolutions per minute of the rotating cup. Depending on the conditions under which the investigated vehicle was operated, six to eleven pairs of samples were collected (Table 2). The mean sampling duration ranged between 25 min 22 s (SD = 7 min 36 s) and 36 min 34 s (SD = 3 min 42 s). All samples were refrigerated and analysed within one to three days.

2.3. Sample analysis

Mesophilic aerobic heterotrophic bacteria (called mesophilic bacteria in the text) and mesophilic fungi were quantified by culture isolation. Mesophilic bacteria culture was carried out in Tryptone Soya Agar medium (TSA, Oxoid Ltd) incubated at 22 ± 2 °C for 72 h following the ISO 6222. Mesophilic fungi were recovered in Rose-Bengal 100 mg/L chloramphenicol (Oxoid Ltd); plates were incubated at 22 ± 2 °C for 7 days. Endotoxin was assayed with a quantitative kinetic chromogenic Limulus Amaebocyte Lysate (LAL) method (Biotek WinKQCL, Lonza), according to the European Pharmacopoeia (2010). Results from assay were expressed as Endotoxin Units/mL, which is a measure of the biologically available endotoxin in the sample. Three different lots of LAL assay kits were used during the study. However, for each vehicle, the same lot was used to analyse endotoxin from the air samples inside the cab and outside of the cab.

For data analysis, samples with values below the limit of detection (LOD) were assigned a value of 0.5 of the LOD of the sampling run in that period. The choice of the 0.5× LOD mode of substitution was made according to geometric standard deviation values of around 3 (Hornung and Reed, 1990).

2.4. Descriptors of the level of vehicle cab environment protection

For each pair of samples, the level of protection of the cab environment against mesophilic bacteria, mesophilic fungi and endotoxin was described by the measure of efficiency (*Eff%*), which is

Table 2
Concentrations of bacteria, fungi and endotoxins inside and on the outside of the vehicle cab.

Vehicle	Location of the samplers	N of samples	Bacteria (10^3 cfu/m^3) AM GM (GSD) Min–max	Fungi (10^3 cfu/m^3) AM GM (GSD) Min–max	Endotoxins (EU/m^3) AM GM (GSD) Min–max
A-loader1	In-cab	8	17 9.1 (3.3) 1.9–78	2.0 1.6 (2.1) 0.71–5.1	207 169 (2.1) 36–394
	Out-cab	8	2000 950 (4.3) 56–7100	58 37 (2.8) 8.2–170	3438 2780 (2.1) 800–8360
A-loader2	In-cab	11	6.2 3.2 (3.3) 0.75–26	0.99 0.77 (2.2) 0.22–2.0	172 147 (1.8) 55–478
	Out-cab	11	1800 610 (4.8) 63–8300	34 26 (2.1) 11–110	5000 3100 (2.7) 740–18,000
B-mixer	In-cab	9	30 18 (2.6) 4.9–140	18 11 (2.9) 3.0–61	310 212 (2.6) 85–900
	Out-cab	9	6900 3100 (4.9) 190–19,000	180 120 (2.9) 21–570	27,000 23,000 (1.9) 8900–52,000
B-loader	In-cab	10	12 3.5 (4.6) 0.76–74	0.54 0.33 (3.4)<0.033–2.1	631 78 (8.1) 12–3910
	Out-cab	10	15,000 14,000 (1.5) 7200–25,000	240 220 (1.6) 130–430	170,000 160,000 (1.5) 98,000–330,000
C-loader	In-cab	10	140 45 (5.2) 3.6–770	7.2 5.4 (2.3) 1.6–20	231 176 (2.2) 66–657
	Out-cab	10	2700 1900 (2.4) 470–8000	39 27 (2.5) 5.2–110	6200 5200 (1.8) 2200–19,000
C-tractor	In-cab	6	27 18 (3.0) 3.3–68	6.3 5.6 1.6) 3.4–14	113 71 (3.0) 25–281
	Out-cab	6	3300 130 (31.4) 5.6–16,000	39 13 (4.4) 3.4–180	4805 253 (32.0)<13–18,600
D-loader	In-cab	10	250 130 (3.0) 35–1300	3.5 2.0 (2.8) 0.48–12	730 490 (2.5) 177–2340
	Out-cab	10	31,000 18,000 (3.4) 2500–90,000	110 56 (3.7) 11–230	70,000 50,000 (2.4) 11,000–230,000
D-tractor	In-cab	8	34 13 (4.7) 1.8–150	1.7 1.3 (2.1) 0.53–3.8	21 20 (1.4)<16–35
	Out-cab	8	560 110 (12.6) 1.7–2200	9.2 3.4 (4.2) 0.75–47	2917 424 (12.0) 16–14,360

AM: arithmetic mean; GM: geometric mean; GSD: geometric standard deviation; Min: minimum; Max: maximum; cfu: colony forming unit; EU: endotoxin unit.

the reduction in exposure to the biological agent inside the cab compared with the outside of the cab:

$$Eff\%(\text{in}\%) = (Co - Ci) \times 100/Co,$$

where *Co* = concentration of airborne biological agents on the outside of the cab and *Ci* = concentration inside the cab. *Eff%* is related to the penetration *Pen%* and the protection factor *PF* as follows:

$$Eff\% = 100 - Pen\% = 100 \times (1 - 1/PF),$$

where *Pen%* (in %) = 100x*Ci*/*Co*, and *PF* = *Co*/*Ci*.

In this formula, *Ci* describes both the particles which have penetrated into the cab and the particles inside the cab which have accumulated and were re-suspended during the vehicle operation.

2.5. Health risk evaluation

For airborne micro-organisms and microbial agents, no regulatory occupational exposure limits (OEL) have been set in Europe and North America. However, measurement data can be compared with benchmark values and guidelines proposed by authors and institutes for bacteria (Malmros et al., 1992; Poulsen et al., 1995; Dutkiewicz, 1997), fungi (Dutkiewicz, 1997; Eduard, 2009) and endotoxins (Górny and Dutkiewicz, 2002; Health Council of the Netherlands, 2010), but such a comparison would require similar methods of collection and analysis (Eduard and Heederik, 1998). CIP 10-M and filtration methods performed similarly with culturable fungi measurements (Nieguitsila et al., 2011). Exposure levels to fungi were therefore compared with the proposed lowest observed effect levels (LOEL) of 10^4 fungi cfu/m^3 for short-term respiratory effect in non-sensitised populations and 10^6 fungi cfu/m^3 for hypersensitivity pneumonitis (Eduard, 2009).

In contrast, because the use of the CIP 10-M led to potential differences in bacteria and endotoxins measurements compared to impaction and filtration methods, related health risk evaluation was not undertaken.

Personal exposure was estimated as a time-weighted value depending on the duration of the related task over the working day. The operation of front-end loaders and the mobile mixer lasted up to 6 h per day. Windrow turning in open air sites lasted not more than one hour per day.

2.6. Statistical analysis

Concentration data were log-transformed before analysis. Concentration descriptive statistics were calculated as arithmetic mean (AM), geometric mean (GM) and geometric standard deviation (GSD). Non-parametric permutation exact tests were used to compare *Ci* and *Co* for each vehicle. As a Spearman rank correlation test did not indicate significant correlation between *Ci* and *Co* for any of the biological agents in any of the vehicles, data samples were considered as independent for comparison testing. A vehicle cab was considered as "non-protected" if the null hypothesis was not rejected for each of the biological agents.

As the theoretical distribution of *Eff%* was unknown, the bootstrap resampling method was used to approximate the sampling distribution of the arithmetic mean of *Eff%*. Arithmetic mean was chosen as the central tendency value of *Eff%* as it was the least biased when compared with GM and median. Bootstrap samples were drawn up with 5000 replacements, and the 95% confidence interval limits of AM were estimated from bootstrap "bias-corrected and accelerated" 2.5 and 97.5 percentiles.

Non-parametric rank tests (Kruskal & Wallis, Mann & Whitney) were used for *Eff%* comparisons between airborne biological agents, first in overall analysis, and then adjusting for the vehicle type. *Eff%* was also compared between vehicles according to the airborne biological agent, with overall analysis and subsequent pairwise comparisons.

In overall analysis, P-values were considered significant when less than 0.05. Multiple comparisons for hypotheses tests were corrected by the False Discovery Rate (FDR) control method (Benjamini and Hochberg, 1995). In the following text, when comparison was multiple, "*P*-value < X" means "*P*-value less than FDR controlled α".

Regarding the comparison of in-cab exposure to fungi with the bench marks, the probability that the benchmark values were exceeded (the exceedance fraction) was calculated assuming a log-normal distribution. The upper limit of the one-sided confidence interval of the exceedance fraction was calculated using Gibbs sampling method with Monte Carlo simulation (Wild et al., 1996). The 70% confidence level is a good balance between a biased conclusion when a higher level of confidence is chosen, and low credibility when a lower confidence level is chosen (INRS, 2008).

StatXact.8 (Cytel studio) software was used for descriptive and inferential analysis, and bootstrap resampling was performed with XLSTAT 2010 software (Addinsoft). The exceedance fraction was calculated with Altrex Chimie. 2.0.1 version software (Institut national de recherche et sécurité).

3. Results and discussion

3.1. Concentration measurement results

Counts of bacteria, fungi and endotoxin measurements below the LOD were few (0, 1 and 4 out of 144 samples, respectively), and descriptive statistics were calculated with the substitution of values below LOD by 0.5× LOD with negligible influence (Table 2).

High variability over time of exposure to bioaerosols in the work environment is well known. However, in the present study, dispersion of data from sample runs was generally low to moderate (GSD $\leqslant$ 3), both for in-cab and outside-of-the-cab sampling. These findings suggest a relative homogenous exposure during sample runs, and thus increased the power of the comparison tests and gained precision in exceedance fraction estimates. Samples collected on the outside of the cab of the two tractors C-tractor and D-tractor were exceptions, as they were probably exposed to changes of wind direction during sampling. Excluding the tractors, dispersion of out-of-the-cab concentration values between airborne biological agents within vehicles was similar (Levene's test, all *P*-values > 0.05).

The front-end loaders B-loader and D-loader both presented the highest concentrations of biological agents in the samples collected on the outside of the cab. These vehicles were involved in screening and/or compost loading operations, which are known to be associated with high particle emission. It is noteworthy that B-loader operated in an enclosed facility, whilst D-loader operated at an open air one, demonstrating that bioaerosols concentration on the outside of a front-end loader associated with screening operations can be as high in open air facilities as in enclosed ones.

The benchmark associated with short-term respiratory effects associated with exposure to fungi was exceeded in almost all samples taken outside the loader and the mobile mixer cabs, and in 3/6 and 2/8 samples for C-tractor and D-tractor, respectively. These results emphasise the need for vehicle cab protection against bioaerosols.

Inside the cab, there was a decrease of all biological agent concentrations for each of the front-end loaders (Table 2). This decrease was statistically significant regardless of whether or not the loader was equipped with a pressurisation and HEPA filtration system (all *P*-values $\leqslant$ 0.005). Similarly, concentrations of all biological agents were lower inside the mobile mixer cab than on

the outside of the cab (all *P*-values ⩽ 0.005). On the other hand, concentrations were not significantly decreased in the cab for the two tractors, except for endotoxins in D-tractor (P-value = 0.005).

3.2. Measure of the protection efficiency of the cab environment and comparison between airborne biological agents and between vehicles

Concerning the front-end loaders and the mobile mixer, mean *Eff%* of the vehicle cab protection system ranged from 59.76% CI 95% [4.19–90.75%] to 99.91% [99.78–99.98%] depending on the vehicle and the biological agent (Fig. 2).

With regards to the two tractors, protection of the cab environment against bioaerosols was low with high uncertainty in *Eff%* estimates because of wide dispersion of the values (Fig. 3). Therefore, because of a low power in comparison tests, no further analysis of *Eff%* data for tractors was undertaken.

The equipped vehicle B-loader exhibited the highest measures of protection efficiency against bioaerosols compared with the other front-end loaders and the mobile mixer. Mean *Eff%* for bacteria, fungi and endotoxins was 99.91% [99.78–99.98%], 99.78% [99.68–99.87%] and 99.47% [98.58–99.97%], respectively, and did not differ from one another (all *P*-values > 0.05). Consistency of *Eff%* measures between sampling runs was high for each biological agent, reducing uncertainty in mean estimates. The B-loader vehicle is noteworthy because it had been purchased a few weeks beforehand and had a clean cab. Consequently, in-cab concentrations of biological agents in B-loader mainly represented the performance of the pressurisation and HEPA filtration system and were unlikely to have been significantly affected by re-suspension of particles. Moreover, as the levels of B-loader *Eff%* were high, penetration of particles through leakage in the filter-sealing system was thought to be minimal for this vehicle cab, in agreement with the measurement of leakage in the order of 0.5% observed by Thorpe et al. (1997) from laboratory tests. B-loader data could thus be used as the reference values in order to explain differences in protection level of cab environment between vehicles.

Regarding the other loaders and the mobile mixer, dispersion of *Eff%* values within sampling runs was wider, and uncertainty in *Eff%*

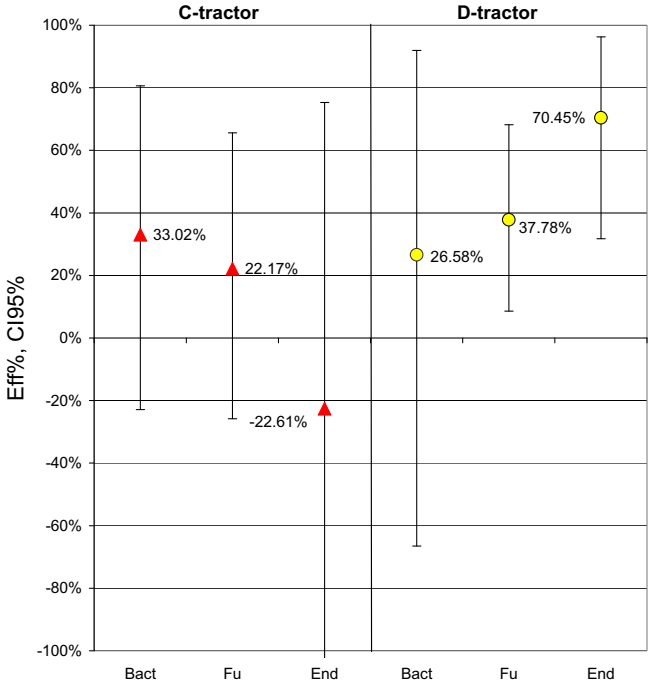

Fig. 3. Protection efficiency of agricultural tractor cabs environment (*Eff%*). Arithmetic mean and 95% confidence interval from bootstrap resampling method. Bact: bacteria, Fu: fungi, End: endotoxins.

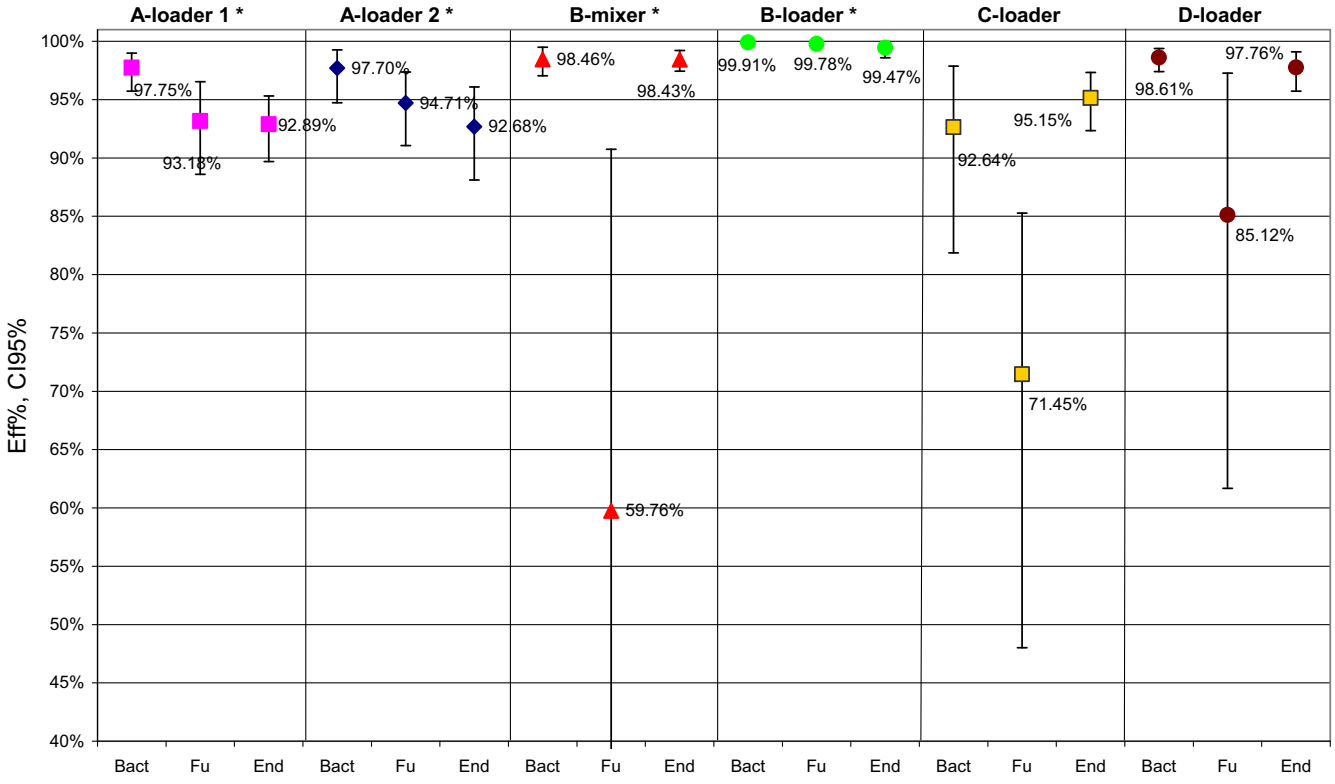

Fig. 2. Protection efficiency of front-end loader and mobile mixer cabs environment (*Eff%*). Arithmetic mean and 95% confidence interval from bootstrap resampling method. Bact: bacteria, Fu: fungi, End: endotoxins. *Vehicles equipped with a pressurisation and HEPA filtration system.

mean estimate was thus higher than that of the B-loader. However, in our study, *Eff%* did not exhibit such wide ranges as in other works (Schlosser et al., 2009; Stagg et al., 2010), and no extreme outliers was measured. Out of 288 pairs of analyses, a higher concentration inside the cab than outside the cab (2.1 times) was measured only once, and nearby similar in-cab and out-cab concentrations (ratio between 0.5 and 1.1) were observed no more than five times. These values were yet responsible for only moderate precision in mean *Eff%* estimates for fungi in B-mixer, C-loader and D-loader, and for bacteria in C-loader. Lower filter performance, absence of pressurisation, leakage in the filter-sealing system and re-suspension of particles that had accumulated inside the cab were factors which could explain the lower protection efficiency demonstrated in equipped loaders in site A, non-equipped loaders, and the mobile mixer than for B-loader.

Comparison between vehicles did not show a single profile of *Eff%* ranking depending on the biological agent. However, several tendencies may be highlighted, as follows.

Firstly, for all vehicles except tractors, the cab was better protected (or equally for B-loader) against bacteria than against fungi. This finding is consistent with results from Thorpe et al. (1997) in agricultural vehicles during harvesting. In accordance with these authors, two explanations may be put forward.

First, bacteria in air are often clustered or fixed onto particles and thus could be larger than fungal spores, which are frequently free in air (Górny et al., 1999). Chiang et al. (2003) showed that in air samples collected in a sludge composting facility, aerobic bacteria tend to exist in larger particle sizes than mesophilic and thermophilic fungi. Consequently, bacteria may be more easily captured by filters than fungal spores. However, vehicles fitted with HEPA filtration system did not support this hypothesis, since B-loader data showed similar performances for bacteria and fungi. Free fungal spores could also more easily penetrate through leakage in the filter-sealing system than bacteria. Studies on respiratory filters which have shown that particles smaller than 2 μm in diameter pass easily through leaks may support this hypothesis (Hinds and Bellin, 1987; Brown, 1992). However, fungal spores may be released into the air aggregated as chains or clumps, and could therefore be larger in size than single spores (Eduard, 2009). Following air collection with a six-stage impactor, Byeon at al. (2008) suggested that the majority of bioaerosols from a municipal composting facility were suspended as agglomerates.

The second explanation below is just as plausible. Vegetative bacteria are known to be more fragile to desiccation than fungal spores, and could therefore accumulate and be suspended at lower concentrations of viable micro-organisms inside the cab. Hence, this low re-suspension of viable bacteria raises the *Eff%* value. Fungal spores are less susceptible to desiccation and can accumulate in the vehicle cab as viable micro-organisms. It is thus likely that concentration of fungi in the cab of the two equipped loaders in site A, whose cabs had not been cleaned before sampling was carried out, was mainly influenced by the suspension of settled fungal spores that penetrated into the cab when the door was opened and from the driver's dirty clothes and shoes, and through potential leakage in the filter-sealing system.

Secondly, regarding endotoxins, data from the level of protection of the cab environment showed marked differences when compared with that of bacteria and fungi depending on the type of vehicle. In the group with equipped loaders, protection against endotoxins was similar to that of fungi, but lower than the protection against bacteria (all adjusted *P*-values $\leqslant 0.0004$). In the group with the two non-equipped loaders and the mobile mixer, protection against endotoxins was similar to that of bacteria, but higher than the protection against fungi (all adjusted *P*-values $\leqslant 0.0003$). As presented below, the hypothesis of differences in particle sizes between fungal spores and endotoxin-bearing particles can be put forward to explain such variations in the *Eff%*.

The performance of the pressurisation and HEPA filtration system against endotoxins was high, as evidenced by *Eff%* in B-loader (99.47% [98.58–99.97%]). In addition, since endotoxins are borne by bacteria wall fragments and are not fragile in environment in contrast with vegetative bacteria, endotoxins were expected to accumulate in dirty cabs just as fungi did.

As the two loaders in site A were equipped with a pressurisation and HEPA filtration system, *Eff%* data suggest that a lower protection level for fungi and endotoxins than for bacteria in these vehicles may have been associated with accumulation inside the cab and re-suspension during the operation. In contrast, the cabs of C-loader and D-loader were not equipped with a pressurisation and HEPA filtration system, but had been cleaned before the sampling run so that low protection against fungi was not expected to be strongly associated with re-suspension of accumulated fungi inside the cab. Higher level of protection for these two non-equipped loaders against endotoxins than against fungi may rather suggest that, on these sites, endotoxin-bearing particles were larger and captured more efficiently by the filter than fungal spores, and/or passed through leakage in the filter-sealing system with more difficulty. This hypothesis is consistent with the observation that endotoxin-containing aerosols represent a broad spectrum of particle sizes and distribution (Jacobs, 1997).

For B-mixer, the profile of *Eff%* according to the biological agent appears to be comparable to that of the two non-equipped loaders. However, the fitting of B-mixer with a HEPA filter does not support the hypothesis that low protection efficiency against fungi was due to low capture by the filter. Explanation for low protection against fungi could be the easier penetration by spores through leakage of the filter-sealing system than that of bacteria and endotoxin-bearing particles, and secondary accumulation of fungal spores inside the cab and re-suspension. Confirmation would require further studies with the check of the filter-sealing system tightness.

One of the main findings of this study is that protection of the vehicle cab environment was the lowest when confronted with fungal spores. This was the lowest either because fungi had accumulated in a dirty cab, or because free fungal spores were not as well captured by only moderately efficient filters, or again because fungal spores easily penetrated through leakage of the filter-sealing system. When the vehicle was equipped with a pressurisation and well-fitted HEPA filtration system and when the cab was clean, as was the case for B-loader loader, the protection against bacteria, endotoxins and fungi was high. Using a step-by-step, one-sided permutation exact test to compare *Ci* and $Cox(1 - Eff\%/100)$ variables, *Eff%* was calculated to be at least 99.98%. This field evaluation was consistent with the 99.95% required efficiency of the constituting H13 filter in this system according to the EN 1822 standard (2009). Due to the re-suspension of particles that had accumulated inside the cab and possible leakage of the filter-sealing system, real *Eff%* in vehicles fitted with pressurisation and HEPA filtration system was below this value most of time. Our observations are in agreement with the statement by Thorpe et al. (1997) that the performance of agricultural vehicle cab protection systems could reasonably achieve a protection factor of 100, i.e., an efficiency of 99%. They also agree with results from laboratory methods applied by Bémer et al. (2009), which showed efficiency values of more than 99.5% for cabs correctly designed for ventilation, cleaning and leak tightness.

3.3. Health risk assessment

The probability that LOEL for short-term respiratory effects with regard to fungi was exceeded was high in the mobile mixer

Table 3
Probability that the exposure level in the vehicle cab exceeded fungi lowest observed effect level for short-term respiratory effects (upper limit of one-sided 70% confidence interval).

Vehicle	Short-term respiratory effects
A-loader1	1.0%
A-loader2	0.08%
B-mixer	49%
B-loader	0.62%
C-loader	20%
C-tractor	<0.01%
D-loader	7.0%
D-tractor	<0.01%

and in the two non equipped loaders (Table 3). In the mobile mixer's cab, the benchmark value was expected to be exceeded during half of the working time. Inside each of the three equipped loaders, the level of exposure to fungi was not expected to exceed LOEL for short-term respiratory effect for more than 1% of time. As indicated in Table 3, the operation of tractors for windrow turning in the open air sites for one hour per day was associated with a very low risk of exceeding short-term respiratory effects LOEL. One-hour tractor operation had negligible influence on the risk that total exposure over the full-shift period exceeded the LOEL.

Regarding hypersensitivity pneumonitis, the probability that level of exposure to fungi inside the cab exceeded the related LOEL was quite low (<0.01%) for all vehicles.

3.4. Strengths and limitations

This study was designed in order to consider environmental variability in the concentration of bacteria, fungi and endotoxins in air so as to provide information on the precision in the estimate of the reduction in exposure to bioaerosols inside the vehicle cab, which describes the level of protection of the cab environment. The descriptor of the level of protection was thus estimated with its uncertainty and allowed for comparison between vehicles and between airborne biological agents. In contrast, the relevance of comparison with data from literature is limited because of the lack of information in most papers on the uncertainty of estimates and on the protection equipment fitted to the vehicles. At most, it may be pointed out that *Eff%* mean values for B-loader in our study were consistent with the highest protection factor values (and the equivalent *Eff%*) reported by authors from studies in composting facilities. Observed maximum *Eff%* ranged between 99.89% and 99.95%, depending on the vehicle and the biological agent (Schlosser et al., 2009; Stagg et al., 2010; Sykes et al., 2011). Two *Eff%* values exceeded 99.99% and one exceeded 99.999% and were probably extreme outliers due to inappropriate comparison of mean personal sampling results and a single stationary sampling measurement in the nearby operational area made at a different time (Schlosser et al., 2009).

In a field study during harvesting that involved 8 vehicles and 12 filtration systems, 4/12 efficiency measures for bacteria and 3/12 for fungi exceeded 99% (Thorpe et al., 1997). Depending on the vehicle and the installed filter, maximum efficiency for bacteria and fungi was 99.96% and 99.69%, respectively, and thus was also consistent with mean *Eff%* for B-loader in our study.

In contrast, when minimum protection factor values were reported, the data were as low as 0.01, and thus appeared to be extreme outliers due to inappropriate comparisons as mentioned above (Schlosser et al., 2009) or to huge differences between values of single measurements inside and on the outside of the cab (Stagg et al., 2010).

In the two tractor cabs, no significant reduction of exposure to bioaerosols was shown. However, interpretation of results is limited, as variability in the concentrations outside of the tractor cabs was large and mean *Eff%* estimates exhibited quite high uncertainty. Further sampling would be of interest to better characterize the level of protection of tractor cab environment in composting facilities.

This field-based study had not been designed to carry out data analysis adjusted for the different factors that could contribute to contamination of the cab environment. Further work with a more suitable approach could contribute to a better assessment of factors such as the cleanliness of the cab, overalls and boots.

In this study, temperature of bacteria and fungi culture was at 22 ± 2 °C. This temperature enabled the measurement of mesophilic fungal and actinomycetes genera that include species with low-size spores which are frequently identified in air in composting facilities, such as *Penicillium*, *Aspergillus* mesophilic species, *Cladosporium* and *Streptomyces* (Fischer et al., 1998; Hryhorczuk et al., 2001; Tolvanen et al., 1998). Therefore, this growth temperature was relevant for the assessment of the protection factor of the filtration system against low size sporulated micro-organisms. However, this culture temperature was not optimal for the growth of thermophilic species, such as thermophilic actinomycetes and *A. fumigatus*. Given that these micro-organisms may be a concern for the health of compost workers, further sampling could be worthwhile.

A limitation in this study was that no health risk evaluation could be carried out with regard to endotoxin exposure because proposed guidelines are related to filtration as the collection method. Nevertheless, *Eff%* measures in this study can be used to simulate in-cab exposure levels from results of endotoxin personal measurements that have been reported in other works using the filtration method. In an overview of personal occupational exposure levels in domestic waste composting enclosed facilities, Wouters et al. (2006) reported endotoxin measurement results in operators involved in operation inspection. Assuming these exposure levels were comparable to concentrations on the outside of the vehicle cab, potential in-cab concentrations could be estimated by applying related *Eff%*. The lowest levels of operators' exposure to endotoxins were described by GM = 527 EU/m^3 and GSD = 1.7, and GM = 285 EU/m^3 and GSD = 3.0. In these situations, the probability that in-cab exposure for 6 h per day exceeded the proposed occupational exposure limit of 90 EU/m^3 (Health Council of the Netherlands, 2010) was less than 5%, or close to 5% for the two equipped loaders whose cabs had not been cleaned (with the second data set, 5.3% and 5.6% for A-loader1 and A-loader2, respectively). On the other hand, for the highest level of exposure described by GM = 1038 EU/m^3 and GSD = 4.8, probability was below 5% (2.5%) for the B-loader *Eff%* measure only. For the other loaders and the mobile mixer, probability would have been between 10% and 39%. These simulations emphasise that in such a situation of high level exposure to endotoxins, the fitting of a high pressurisation and HEPA filtration system on vehicles and regular cleaning of the cab would be necessary. Further work with filtration collection methods for in-cab endotoxin measurements would help in the assessment of associated health risks and compliance with the proposed OEL.

In this study, investigated vehicles were representative of most vehicles operating in composting facilities. However, large machines are used to straddle and turn windrows and are equipped with a cab for operation. Such machines were not included in our study, but the corresponding cab can be fitted with a similar protection system making the possibility of reaching a 99% performance level likely. In addition, the practices observed under these study conditions were very representative of the ones usually performed by the vehicle operators on the facility, except when the tractor on site C was moving with a tail wind.

4. Conclusion

In the field, repeated measurements inside and on the outside of composting facilities' vehicle cabs for bacteria, fungi and endotoxins highlighted that a pressurisation and HEPA filtration system can provide a protection efficiency of more than 99.9%. An appropriate design and good fitting procedures are needed to limit leakage of the filter-sealing system, and efforts should be made to check the filter-sealing system leak-tightness. Nevertheless, it is important to acknowledge that the pressurisation and filtration system is only one of a series of control measures required. Conditions of cleanliness of the cab, the operator's overalls and boots are factors that could significantly contribute to contamination of the vehicle cab. It is probable that if a multifaceted control strategy was not adopted, such high levels of protection of the cab environment against bioaerosols would not be achieved in composting plants.

This study also emphasised that performance of vehicle cab protection systems was at it lowest when confronted with fungal spores. This observation is probably linked to the low size of free spores and their ability to survive inside the cab when they settle. Thermophilic actinomycetes were not included in this study. However, since thermophilic actinomycetes are sporulated microorganisms and because spore size is around 1 μm (Reponen et al., 1998) it is likely that the performance of the vehicle cab protection system would demonstrate the same limits as with fungal spores.

Moderate reduction of fungi concentration inside the cab of vehicles that were not fitted with a pressurisation and HEPA filtration system was associated with high probability that exposure levels exceeded the short-term respiratory effects benchmark value. Similarly, the results of calculations of exposure to endotoxins with data from other works indicated that the proposed occupational exposure limit could be too often exceeded over the working time. These findings suggest that front-end loaders and mobile mixers in composting facilities should be fitted with a pressurisation and HEPA filtration system, regardless of whether or not the facility is indoors or outdoors. Assuming that the vehicle cab is carefully and regularly cleaned with a HEPA vacuum cleaner, this type of protective equipment was shown to be efficient in controlling the risk of short-term respiratory effects from exposure to fungal spores and in complying with the proposed occupational exposure limit for endotoxins.

In order to better estimate the protection of tractor cab environment during windrow turning on open air sites, further sampling is necessary. However, in this study, levels of exposure to fungal spores inside the tractor cabs were associated with a very low risk of exceeding the short-term respiratory effects benchmark value over a 1-h per day windrow turning operation.

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