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PAPER

Documentation of bioaerosol concentrations in an indoor composting facility in France†

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Bioaerosol concentrations were investigated in a totally indoor composting facility processing fermentable household and green wastes to assess their variability. Stationary samples were collected by filtration close to specific composting operations and then were analysed for cultivable mesophilic bacteria, thermophilic bacteria, mesophilic fungi, thermophilic fungi, endotoxins and total airborne bacteria (DAPI-staining). Indoor concentrations exceeded the background levels, between 500 and 5400 EU m^{-3} for endotoxins, 10^4 and 10^6 CFU m^{-3} for cultivable bacteria and generally below 10^5 CFU m^{-3} for airborne cultivable fungi. No significant ($p > 0.05$) differences were observed between the indoor composting operations. Successive 30 minute bioaerosol samples were collected to investigate the variation of cultivable mesophilic microorganisms over the work shift. Concentrations of mesophilic bacteria and fungi varied up to 1 log unit depending on the time at which they were collected in the day. Total airborne particles, counted using an optical particle counter, were present at up to 10^8 particles m^{-3} and several concentration peaks were noted. Values for total airborne bacteria were roughly 70-fold higher than cultivable bacteria. These results raise the question of the sampling strategy (duration of sampling; number of samples to be collected) used in similar studies. They provide new bioaerosol concentration data in a composting facility and suggest that the filtration sampling method might be a useful tool for exposure measurements in that occupational environment.

Introduction

Composting is an important part of solid waste management policy in France. It usually processes a wide variety of different wastes (green waste, household waste, sewage sludge, …) resulting in the reduction of their volumes and producing a valuable product which can be used as a soil amendment in agriculture and gardening. Composting is a natural self-heating process involving the biological degradation of organic matter under aerobic conditions. Installations for composting vary greatly in size (from domestic to large-scale facilities), degree of enclosure (open, partially enclosed, enclosed facilities), design (static windrow systems, aerated static piles, bioreactors, *etc.*) and the type of wastes composted.[1] The process is basically divided into several steps involving proliferation of microorganisms to high concentration, complex succession of bacterial and fungal microflora and increase in temperature up to 60–70 °C. Thus, thermophilic actinomycetes in compost have been reported up to 10^7 Colony Forming Units per gram of matter

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Environmental impact

This study investigated the ambient concentrations of bioaerosol in a totally indoor composting facility. We reported elevated airborne concentrations of endotoxins and of cultivable bacteria and fungi on the site, suggesting the need for further investigations of the occupational exposure to bioaerosols in enclosed composting plants. We also provided the first set of data describing the variation of both airborne microorganisms and total particles over the work shift in the composting environment. Our results constitute new helpful data for setting adequate sampling strategies for bioaerosol studies in composting facilities and also argue in favor of the filtration sampling method as a useful method for exposure measurements in that occupational environment.

(CFU g^{-1}) and thermophilic fungi, such as *Aspergillus fumigatus*, up to 10^5 CFU g^{-1}.[1,2]

Waste and compost handling during the process (compost turning, shredding, screening, …) has been shown to release airborne microorganisms and their associated compounds, called bioaerosols, in the ambient air of composting facilities. Thus, dust, mesophilic and thermophilic microorganisms as well as endotoxins and glucans have been found at high levels in numerous composting facilities.[3–8] Dispersion of these bioaerosols to residential populations surrounding composting plants has also been studied.[9] In addition, high personal exposure levels, up to 10^8 CFU m^{-3} for airborne bacteria, up to 10^7 CFU m^{-3} for fungi, and up to 37 000 endotoxin units (EU) m^{-3} of air for endotoxins, were measured among composting workers.[8,10,11]

There are neither dose/response relationships nor Occupational Exposure Limits (OELs) for bioaerosols admitted at the international level. Nevertheless, the exposure to bioaerosols has been associated with adverse effects on human health in several occupational environments.[12,13] Indeed, concentrations of bioaerosols in composting facilities have been associated with increased respiratory and dermal pathologies among compost workers.[10,14,15]

The enclosure of composting operations is a developing tendency to prevent their potential nuisance toward the neighboring inhabitants.[16] It is also considered that indoor plants provide a better control of the composting process. However, it also may create a confined environment that calls for specific prevention measures. In spite of the numerous studies published, bioaerosols in such composting facilities are still insufficiently characterized. Furthermore, methods and strategies used to perform such a characterization are not well established. While individual sampling is necessary for the assessment of workers' exposure, collection of ambient bioaerosol samples close to typical working activities is also useful for occupational safety purposes.[17] Indeed, ambient sampling may provide information on the behavior of airborne microbes in indoor environments and the collected data may be useful for the conception of collective preventive measures.

The aim of the present study was to assess the ambient bioaerosol concentrations in a totally indoor composting facility in France using the filtration sampling methods. An additional goal was to investigate the variation of airborne microorganisms during the work shift.

Material and methods

Description of the composting facility

The composting facility we have investigated was a totally indoor plant processing both fermentable household waste (approx. 6000 tons per year) and green waste (approx. 1000 tons per year) collected in the surrounding municipalities. The facility consisted of a large hangar-like building in which the different composting operations were performed (Fig. 1). Upon arrival at the facility, waste was unloaded in a dedicated area and manually sorted. Fermentable matter was introduced into a shredder using a mechanical shovel. Shredded waste was piled into the first box in the fermentation area by a mechanical shovel as they were shredded. The fermentation area contained four separated boxes (35 × 3.5 × 2.5 m) equipped with a floor-level forced aeration system. Compost was transferred once a week to the following box before being carried to the screener by a conveyor belt. Compost was transferred from one fermentation box to the next one by means of an automatic bucket wheel (approx. 2 h) and by the same system from the last box to the conveyor belt (approx. 8 h). The progression of the bucket wheel in the boxes also favored the turning, mixing and aeration of compost. A watering system placed above the piles in the fermentation area was used to manually adjust the humidity of compost. Two operators worked in the building during the study and the mechanical shovel used in the facility was equipped with a pressurised, air-conditioned cabin. No specific prevention measures were applied on the site apart from watering the compost during screening.

Methods for bioaerosol sampling

Airborne microbiological particles were sampled by the filtration method using 37 mm polystyrene 3-piece closed-face cassettes (CFCs) provided by Millipore®. Cultivable microorganisms were sampled with CFC mounted with a sterile polycarbonate filter (Nuclepore®, 0.8 μm pore size) and a backing cellulose pad (Millipore®, thick cellulose absorbent pad). Airborne endotoxins were sampled with CFC containing a pyrogen free glass fibre filter (Whatman®, GF/B glass microfiber filter) and the same backing cellulose pad. Before use, glass fibre filters were heated to 240 °C for 120 minutes to remove pyrogens. The cassettes were connected to portable constant flow pumps (Gilian®, GilAir-3) and sampling was performed at a flow rate of 2 L min^{-1}. The flow rate was calibrated before and after sampling using a bubble flow meter (Gilirator, Gilan®, USA).

Sampling strategy

The study comprised two different sampling campaigns (named campaign-1 and campaign-2) for which two different sampling plans were designed. For both campaigns, stationary samples were collected in the vicinity (1 m) of the main composting activities. Two cassettes (and eventually other instruments) were placed side by side, at a distance of 20 cm from each other, on a horizontal bar held at 1.7 m above ground level. The inlets were turned toward the activity being investigated. Temperature and relative humidity were monitored using a portable device (HygroPalm2, probe HS2, Rotronic) at the beginning and at the end of each sampling.

Description of sampling points. Six sampling points were placed inside the building and another one was placed outdoors (Fig. 1). The sampling point SP1 was located behind the shredder. SP2 and SP3 were placed close to the first fermentation box 12 m distant from each other. SP4 was located in a footpath between the conveyor belt and the 4th fermentation box. SP5 was located between the screener and the head of the fermentation area (between the boxes 2 and 3). The sampling point SP6 was placed close to a compost pile in the maturation area. The outdoor sampling point SP7 was placed 40 m upwind far from the building to determine the background level.

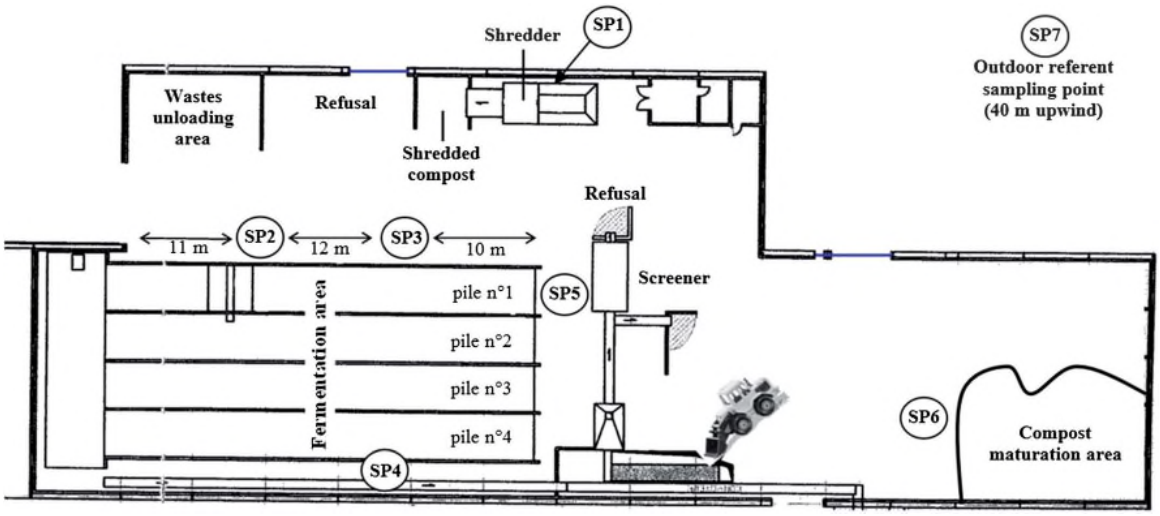

Fig. 1 Schematic organisation of the composting facility and localisation of the sampling points (SP1 to SP7).

Sampling plan for campaign-1. The sampling plan was designed to assess airborne endotoxins and cultivable microorganisms simultaneously and to collect 3 samples per sampling point. A CFC mounted with a polycarbonate filter and another one mounted with a pyrogen free glass fibre filter were placed on the horizontal bar. Bioaerosol samples were collected at SP1, SP2, SP4, SP5 and SP6 and at the referent point SP7 located outside during 3 different periods. The first set of samples (SET1) was collected on the 21st of May 2008 between 8 h 30 and 10 h 30 and the second one (SET2) on the same day between 11 h 45 and 14 h 00. The third set of sample (SET3) was collected on the 22nd of May 2008 between 9 h 00 and 11 h 00. Sampling time ranged between 86 and 163 minutes with a median of 131 minutes. Samples were brought to the laboratory for the analysis of endotoxins, mesophilic bacteria, mesophilic fungi, thermophilic bacteria and thermophilic fungi.

Sampling plan for campaign-2. The sampling plan was designed to assess the variation of airborne cultivable microorganisms over the work shift. Samples were collected on the 25th of June 2008 from 8 h 00 to 14 h 00. Two CFCs mounted with a polycarbonate filter were placed on the horizontal bar. One of the CFCs was replaced by a new one every 30 minutes while the other one was left for 6 hours. The trial was carried out for the same period of time at SP2, SP3 and SP5. No samples were collected at the referent point. An Optical Particle Counter (OPC, Grimm® G1108) was also placed at SP5 for continuous real-time monitoring of the total airborne particles. The 6 hour duration of sampling covered the main part of the work shift which started at 7 h 30 in the morning and ended at 15 h 30 in the afternoon. All the samples were brought to the laboratory for the analysis of mesophilic bacteria and mesophilic fungi. For 6 hour samples only, the analyses were completed with the enumeration of total bacteria by fluorescence microscopy.

Real time number concentration of airborne particles

An Optical Particle Counter (OPC, Grimm® G1108) was used for real-time monitoring of the particle number concentration of the ambient aerosol. This OPC works at a sampling flow rate of 1.2 L min^{-1} and classes particles according to size ("optical equivalent" diameter, dopt) between 0.30 and 32 μm, using 15 channels. During measurements, particle counting was integrated over 6 s and results were expressed in number of particles per cubic metre of air (# m^{-3}).

Transport and preservation of samples

Bioaerosol samples were transported to our laboratory in the day using a cold box and were then stored at 4 °C until analyses were conducted. Filters were kept in their respective cassettes. Special care was taken with the handling of endotoxin samples to limit particles deposition on the inner walls of the sampling cassettes. Samples were all analysed within 24 hours after being collected.

Analysis of bioaerosol samples

All equipment and dilution water used in our experiments were sterile and pyrogen free when required. The analyses were performed in a laminar flow cabinet.

Enumeration of cultivable microorganisms. A volume of 10 ml of a sterile solution (Tween 80 0.01%, peptone 0.1%, ultra-pure water) was introduced into the cassettes by the inlet. The cassette inlets and outlets were closed with a cap and placed on a rocker (Heildolph®, Multi-Reax shaker) at 2000 rpm for 20 min and at room temperature. The extracts were serially diluted in a tryptone salt sterile solution (NaCl 8.5 g L^{-1}, tryptone 1 g L^{-1}). Cultivable microorganisms were enumerated by the classical spread plating technique. Briefly, a 100 μl aliquot of the diluted extract was spread over Petri dishes (2 plates per dilution, 3 dilutions) containing the appropriate culture medium and the inoculated media were incubated. Thermophilic bacteria were cultivated on the Triptycase Soy Agar (TSA) medium and incubated at 56 °C for 3 days. Thermophilic fungi were cultivated on the Malt Extract Agar (MEA) medium and incubated at 47 °C for 3 days. Mesophilic bacteria and mesophilic fungi were cultivated on TSA plus cycloheximide and MEA, respectively, and incubated at 25 °C for 5 days. Cycloheximide (0.08 g L^{-1})

was added to prevent fungal growth on the TSA medium. Colonies grown on the media were enumerated every day and concentration of airborne cultivable microorganisms was calculated and expressed as Colony Forming Units (CFU) per cubic metre (CFU m^{-3}).

Endotoxin analysis. Glass fibre filters were carefully removed from cassettes, placed in 50 ml sterile and pyrogen-free polypropylene tubes (Cellstar tubes®, Greiner Bio-One) and extracted in 10 ml pyrogen-free water (Aqua B. Braun, B. Braun). Extraction was performed at room temperature on a rocker (Heildolph®, Multi-Reax shaker) at 2000 rpm for 60 min. It was completed with a final centrifugation step at 2000*g* for 10 minutes at 4 °C (3–18 K, Sigma). Endotoxin concentration in the extract was assayed (in duplicate) immediately after extraction using the kinetic chromogenic Limulus amoebocyte lysate (LAL). The LAL kit used across the study (Kinetic-QCL®, Lonza) came from a unique lot (kit lot no. GL0368; lysate lot number: G0032Q; endotoxin lot number GL0009; LAL reagent water lot GL0116). The RSE/CSE ratio was 10 EU ng^{-1}.

The samples were tested with no dilution and with the 1/10 dilution. A 100 µl aliquot was added to a microtiter plate (96 Well Clear Polystyrene Microplates, Costar®). The wells were filled out with 100 µl of the LAL reagent provided with the kit and the plate was immediately assayed. The assays were performed with an automated microtiter plate reader (ELx800 Absorbance Microplate Reader, BioTek®) interfaced with the WinKQCL® 1.20 software. Incubation was done at 37 °C and absorbance was read at 405 nm every 150 s. The standard curve obtained from the *Escherichia coli* (strain O55:B5) reference endotoxin was used to determine the concentrations in terms of endotoxin units (EU). Pyrogen-free water was analysed as the negative control and a 0.5 EU ml^{-1} CSE endotoxin solution as the positive control. Criteria for the validation of an assay were the lack of interferences, a coefficient of correlation $\geq$0.98 for the standard curve and a coefficient of variation $\leq$10% for samples. Samples were spiked with a known concentration of endotoxin to assess the interferences. Analytical interference was assumed to be not detected when endotoxins recovery was between 50 and 200%. The assay limit of detection (LOD) was 0.005 EU ml^{-1}. The concentration of airborne endotoxins was calculated and expressed as endotoxin units (EU) per cubic metre (EU m^{-3}).

Enumeration of total bacteria by fluorescence microscopy. Total bacterial counts in bioaerosol samples were done by epifluorescence microscopy using the fluorochrome 4′,6′-diamidino-2-phenylindole (DAPI) as a staining agent. The analyses were done on the extracts prepared for the enumeration of cultivable bacteria according to a method described previously.[18] Briefly, an aliquot of the extract was stained for 10 minutes in the dark with DAPI at the final concentration of 0.5 µL ml^{-1}. The stained solution was then filtered through a 0.2 µm pore size polycarbonate black membrane (Isopore™, Millipore®). The membrane was rinsed and mounted between slide and cover slip with paraffin oil. Total bacteria on the filter were counted using a fluorescence microscope (LEICA DM 2500 microscope), at 1000× magnification over 20–30 evenly spaced fields. Bacteria were recognised by shape and size (<1 µm) and aggregates were counted when possible.

Calculations and statistical analysis

The analysis of variance (ANOVA) was performed using the Statgraphics Centurion XV 15.2.00 software (StatPoint, Inc, USA) with log transformed data. Spearman correlations between the different microbial parameters were calculated using the same software.

Results

Sampling conditions

Campaign-1. Samples were collected on the 21st and 22nd of May 2008. During sampling SET1, the outdoor temperature rose 16 °C, and the relative humidity was around 55%. The temperatures monitored inside the building were similar to outdoor ones but the relative humidity values were higher (~60 to 65%). Wastes shredding, compost transfer from the last fermentation box to the screening area and compost screening were the main activities that were in process during the sampling SET1. Sampling SET2 was characterised by a higher outdoor temperature (25 °C) and a lower relative humidity (~30%) but a cooler atmosphere (21 °C) inside the building. The composting activities were intensified during sampling SET2 as compared to sampling SET1 with an additional transfer of compost from the screening area to the maturation one, achieved by the mechanic shovel. The atmospheric conditions monitored during the sampling SET3 were similar to those presented for sampling SET1. However the composting activities were reduced to the transfer of compost from one fermentation box to another.

Campaign-2. Samples were collected on the 25th of June 2008 from 8 h 00 to 14 h 00. Delivery of wastes in the dedicated area and their shredding were intense between 8 h 00 and 9 h 00. The main activities included successive periods of wastes shredding and their transfer to the first box of the fermentation area at 8 h 30, 9 h 45, 11 h 10 and 12 h 10. The automatic shovelled wheel started at 12 h 00 for 2 hours to transfer the compost from the first box of the fermentation area to the second one. No activity was recorded in the screening and in the maturation area. All the doors of the building were closed after 9 h 00. The evolution of the atmospheric conditions was similar from one sampling point to another. The temperature inside the building was close to 20 °C at 8 h 00 to reach 26 °C in the afternoon. In the same period of time, the relative humidity fell from 80 to 55%.

Results from campaign-1

Outdoor background levels. The concentrations of airborne endotoxins at the referent sampling point varied between 105 and 250 EU m^{-3} (Fig. 2). For cultivable microorganisms, the background levels were highly variable as a function of microorganisms (Fig. 3). The concentration of mesophilic microorganisms varied from 7.8×10^2 CFU m^{-3} to 7.8×10^3 CFU m^{-3} for bacteria and from 2.1×10^3 CFU m^{-3} to 4.6×10^4 CFU m^{-3} for fungi. The measurements of outdoor thermophilic bacteria were below the detection limit except for one sample which led to 2.1×10^3 CFU m^{-3}. The concentration of thermophilic fungi varied in a wide range from below the detection limit to 1.3×10^5 CFU m^{-3}.

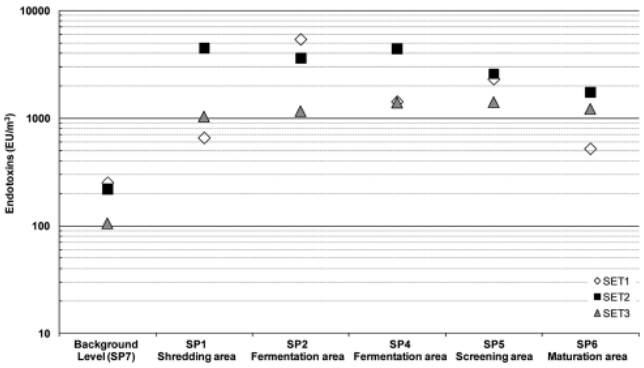

Fig. 2 Concentration of airborne endotoxins measured in the different working areas of the composting facility. ◇: values recorded during the sampling SET1; ■: values recorded during the sampling SET2; ▲: values recorded during the sampling SET3.

Indoor concentrations of endotoxins. The concentrations of airborne endotoxins measured inside the building were all over the background levels and exceeded 500 EU m^{-3} (Fig. 2). The highest concentration of endotoxins (5400 EU m^{-3}) was measured in the fermentation area, at the sampling point SP2 and during sampling SET1. Except for SP2, the concentrations of airborne endotoxins measured inside the building during the sampling SET2 exceeded those measured during the other sets. The coefficient of variation (CV) calculated with the three concentrations measured for each sampling point varied between 30 and 145%. The ANOVA showed that mean concentrations calculated for each composting activity we have investigated were not significantly different ($p > 0.05$).

Indoor concentrations of airborne cultivable microorganisms. Results for airborne microorganisms are presented in Fig. 3. Concentrations of mesophilic bacteria measured in the different areas all exceeded the background levels and varied in the range of 3.0×10^4 to 7.5×10^5 CFU m^{-3}. The CV calculated for each sampling point varied between 30 and 130%. The concentrations measured in the shredding area were generally below those measured in the other areas. No significant difference was observed between the mean concentrations of mesophilic bacteria measured in the different areas. Data from the analysis of thermophilic bacteria were only exploitable for one set of measurements due to overlapping of Petri dishes by invading bacterial colonies during the first day of incubation. The available data showed that concentrations of these bacteria in the air of the composting plant exceeded the background level and were between 5.5×10^4 and 6.2×10^5 CFU m^{-3}. Concentrations of airborne fungi were generally below those observed for bacteria. Concentrations of mesophilic fungi rarely exceeded 105 CFU m^{-3} and were in the range of those measured at the referent sampling point except for few samples. The CV for each sampling point for mesophilic fungi varied between 70 and 510%. Concentrations of thermophilic fungi were highly variable from one sampling set to another (230% < CV < 2300%). For SET2 and SET3, airborne thermophilic fungi were in the range of the background levels and did not exceed 10^4 CFU m^{-3}. For the first set of sampling the concentrations were higher.

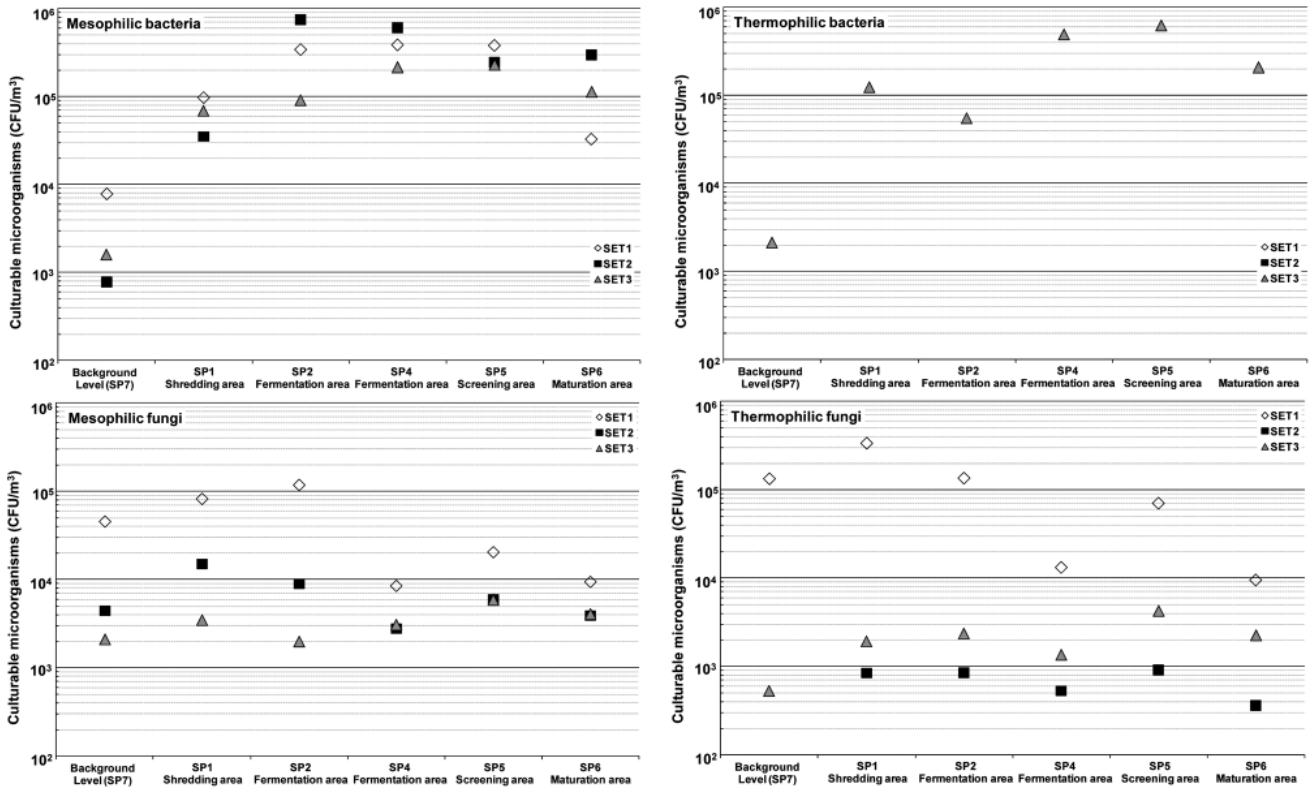

Fig. 3 Concentration of airborne cultivable microorganisms measured in the different working areas of the composting facility. ◇: values recorded during the sampling SET1; ■: values recorded during the sampling SET2; ▲: values recorded during the sampling SET3.

Results from campaign-2

Real time number concentration of airborne particles. The evolution of total airborne particles monitored with the OPC over the work shift is presented in Fig. 4. The number concentration of airborne particles whose "optical equivalent" diameter was superior to 0.3 μm reached 2.7×10^8 # m^{-3} at the beginning of the day and gradually declined to 3.0×10^7 # m^{-3} until 11 h 00. The highest peak of concentration recorded at 9 h 00 was assigned to wastes delivery and shredding. Several other peaks were monitored during the day but they were not assigned to a specific working task as several activities were in progress at the same time in the building (wastes shredding, compost transfer from the first fermentation box to the second one, and loading of the first fermentation box). The number concentration of particles with dopt >1.0 μm varied between 1×10^5 and 2×10^6 # m^{-3} over the work shift. Further analysis of size distribution of particles showed that the generated aerosol was predominantly dominated by particles with dopt <1.0 μm (data not shown).

Concentrations of airborne cultivable microorganisms over the work shift. Twelve successive 30 minute bioaerosol samples were collected to investigate the variation of cultivable mesophilic microorganisms over the work shift. The examination of measured data for each sampling point individually revealed increases and decreases of microbial concentration depending on the sampling period. Either bacterial or fungal concentrations varied in the sample range of 1 log unit over the work shift for the 3 sampling points (Fig. 5). Furthermore, the evolution of cultivable microorganisms globally showed a similar pattern from one sampling point to another. For SP5, cultivable microorganisms were also well correlated with the number concentration for particles with dopt >1 μm.

The highest concentration of airborne bacteria (6.6×10^5 CFU m^{-3}) was measured at SP5 just before 9 h 00 whereas the lowest one (2.0×10^4 CFU m^{-3}) was measured at SP2 just after 9 h 00. The geometric means calculated over the 12 consecutive measurements were 1.3×10^5, 1.3×10^5 and 1.2×10^5 CFU m^{-3} for sampling points SP5, SP3 and SP2, respectively. The coefficient of variation calculated over the same 12 samples varied from 68 to 125% from one sampling point to another. The analysis of linear correlation between sampling points revealed a significant and moderately strong correlation between SP5 and SP3 ($n = 12$; $r = 0.705$; $p = 0.0104$) and between SP2 and SP3 ($n = 12$; $r = 0.618$; $p = 0.0321$). No significant correlation ($p = 0.2628$) was observed for bacteria between SP5 and SP2.

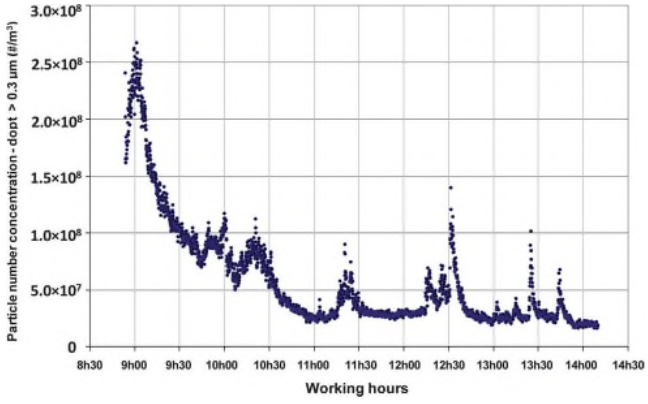

Fig. 4 Number concentration of total airborne particles (dopt >0.3 μm) monitored in the composting facility.

Airborne fungi concentrations were generally lower than those recorded for bacteria (Fig. 5). The highest fungal concentration (1.2×10^5 CFU m^{-3}) was measured at SP3 and the lowest one (9.1×10^3 CFU m^{-3}) was measured at SP5. The geometric means calculated from the 12 consecutive measurements were 3.3×10^4, 4.1×10^4 and 3.7×10^4 CFU m^{-3} for sampling points SP5, SP3 and SP2, respectively. The coefficient of variation calculated over the same 12 samples varied from 64 to 68% from one sampling point to another. Significant and moderately strong correlations were observed between all the sampling points, SP5 and SP3 ($n = 12$; $r = 0.836$; $p = 0.0007$), SP5 and SP2 ($n = 12$; $r = 0.596$; $p = 0.0405$), and SP2 and SP3 ($n = 12$; $r = 0.787$; $p = 0.0024$).

Further analysis of data indicated a significant and moderately strong linear correlation ($n = 36$; $r = 0.588$; $p = 0.0002$) between bacteria and fungi. No significant correlation ($p > 0.4$) was found between microbial and atmospheric parameters.

Measurements from continuous 6 hour duration of sampling. The concentrations of cultivable mesophilic fungi established with the continuous 6 hour sampling were 2.6×10^4, 3.5×10^4 and 3.0×10^4 CFU m^{-3} for SP5, SP3 and SP2, respectively. For cultivable mesophilic bacteria concentrations were 1.1×10^5, 1.6×10^5 and 1.5×10^5 CFU m^{-3} for the sampling points SP5, SP3 and SP2, respectively. Total bacteria counted by epifluorescence microscopy on the same samples were 7.8×10^6, 1.1×10^7, 1.1×10^7 cells m^{-3} for SP5, SP3 and SP2, respectively.

The concentrations of cultivable microorganisms from the 6 hour samples were compared to the mean concentrations calculated from the consecutive sampling presented in Fig. 5. To achieve that, the arithmetic mean concentration (named "calculated mean" in the following text) was calculated from the 12 consecutive 30 minute sampling and for each sampling points SP2, SP3 and SP5 individually. The sampling points were considered as replicates and the calculated means were compared to the corresponding concentrations measured for the continuous 6 hour duration of sampling. The ANOVA revealed a weak but significant ($p = 0.0347$) difference between the mean fungal concentration, continuously measured over the work shift (4.48 [log (CFU m^{-3})]), and calculated from the consecutive 30 minute sampling (4.63 [log (CFU m^{-3})]). No significant difference ($p = 0.3181$) was observed for airborne bacteria when comparing the mean of continuous measurements over the work shift (5.20 [log (CFU m^{-3})]) and the one calculated from the consecutive sampling (5.13 [log (CFU m^{-3})]).

Discussion

Indoor microbial and endotoxin concentrations

High concentrations of bioaerosols were monitored in the indoor composting facility we have investigated. The endotoxin concentrations measured in our study were all between 500 and 5400 EU m^{-3}, values between 3- and 22-fold higher than background levels. The presence of airborne endotoxins indoors was expected since Gram-negative bacteria have already been found

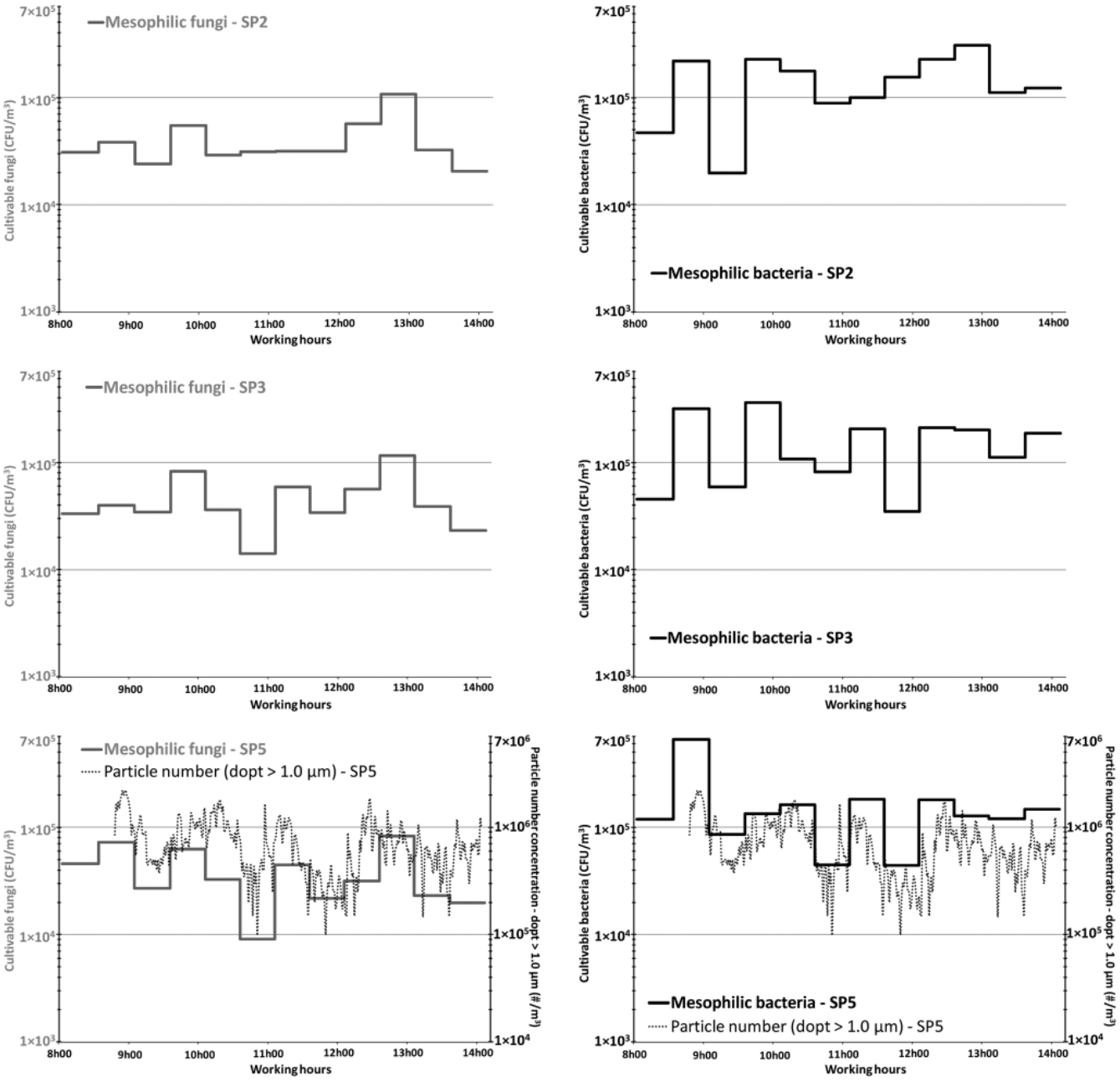

Fig. 5 Variation of concentration of airborne cultivable microorganisms and particle number concentration (SP5—dopt >1.0 µm) over the work shift.

in the bulk compost and in the air of composting facilities.[8,19,20] The concentrations measured in our study corroborate previous findings reporting ambient endotoxins between 0.2 and 18 000 EU m^{-3} in the air of a drum composting plant treating source-separated biowastes[3] and in the range of 289–59306 EU m^{-3} in an indoor sewage sludge composting facility.[4] Lower ambient endotoxin concentrations were reported by Sykes *et al.*[5] Airborne cultivable bacteria were also found at high concentrations (between 10^4 and 10^6 CFU m^{-3}) in our study (Fig. 3). Indoor levels of mesophilic bacteria exceeded background levels by between 4- and 960-fold. For thermophilic bacteria, this factor was between 25 and 290. These ambient concentrations were consistent with the range of cultivable bacteria (10^2–10^9 CFU m^{-3}) reported in other compost studies as spore-forming actinomycetes.[6–8] Airborne fungal concentrations were generally lower than bacterial concentrations. Concentrations measured for mesophilic airborne fungi (2.0×10^3 to 8.0×10^4 CFU m^{-3}) concurred with those generally encountered in the atmosphere of composting facilities.[3,20] Thermophilic fungi were present in low concentrations during the sampling SET2 and SET3 and in higher concentrations during the sampling SET1 (Fig. 3). Cultivable fungi generally exceeded the background level except for SET1 (see further discussion of the outdoor background levels). Thus, the composting operations in progress inside the building emitted large amounts of microbial particles, especially endotoxins and bacteria, in the atmosphere. The bioaerosol concentrations measured indoors were generally higher than the outdoor background levels. Our study provides a new set of data

confirming that bacteria, fungi, and microbial components are easily dispersed into the air during the operations involved in composting, leading to high bioaerosol concentrations.

Outdoor background levels

In the absence of any OEL for bioaerosols, the determination of the background level is of crucial importance for the interpretation of data. The ambient outdoor samples collected at the upwind boundary of the operational area of the site should reflect the background bioaerosol concentration at the time of sampling or the effects of neighbouring operations.[21] Several studies have suggested that background endotoxin concentrations are below 10 EU m^{-3} with a seasonal variation.[22–25] Outdoors mesophilic microorganisms have generally been reported at less than 1.6×10^3 CFU m^{-3} for bacteria, and less than 1.0×10^4 CFU m^{-3} for fungi.[26,27] Levels of thermophilic microorganisms are generally around or below 10^2 CFU m^{-3} for both bacteria and fungi.[26,27] Thus, the outdoor endotoxin concentrations measured in our study are between 10 and 25 times higher than the usual background level. Concentrations of airborne bacteria and fungi measured during the sampling SET2 and SET3 were generally in agreement with previously reported data. In contrast, mesophilic bacteria, mesophilic fungi and thermophilic fungi measured during the sampling SET1 were higher than the reported background levels. These results indicate that the referent sampling point was influenced by bioaerosol sources that affected the concentrations of endotoxins throughout campaign-1 and those of cultivable microorganism during SET1. Bioaerosols emitted by composting facilities are known to spread out downwind[9] and recommendations have been made to determine background levels at least 25 m upwind.[21] Although point SP7 complied with these recommendations, sudden changes in wind direction or meteorological conditions could have affected sampling. Furthermore, the composting facility was surrounded by agricultural activities (cultivated fields, grazing lands and dairy farms) which could also have contributed to the elevated bioaerosol concentrations at the upwind site. As a matter of fact, the non-identified sources did not cast doubt on our interpretation of data from campaign-1.

Overlapping of TSA medium

Counts of cultivable thermophilic bacteria on TSA medium were impaired by the overgrowth of colonies on the Petri dishes. This phenomenon was not observed for mesophilic bacteria and might be due to fast-growing thermotolerant bacteria belonging to the actinomycetes group or *Bacillus* genera. As a consequence data for this microbial parameter were unavailable for 2/3 of samples collected during campaign-1. This lack of data is problematic since thermophilic actinomycetes account for most prevalent microorganisms in the air of composting facilities. Plate masking is a well-known issue in traditional air microbiology and several authors have proposed alternative selective media and counting methods.[28,29] A variety of media and culture conditions have been used to assess airborne cultivable microorganisms in composting facilities, but no international consensus has yet been reached.

Concentrations of total airborne bacteria

Total airborne bacteria measured during campaign-2 were roughly 70-fold higher than cultivable mesophilic bacteria. This is in agreement with findings reported by Albrecht *et al.*,[30] in the same occupational environment. These results were expected as a similar trend (with different ratios) was observed in other environments, including indoor and outdoor residential areas,[31] swine buildings,[32] poultry houses and sewage treatment plants[33] when comparing cultivation with total counts following DAPI-staining. DAPI is a fluorochrome binding cellular DNA and direct counts using epifluorescence microscopy and DAPI-staining include both viable microorganisms (including viable but non-cultivable ones) and dead cells. In contrast, the cultivation method only allows viable and cultivable microorganisms to be counted, underestimating the naturally existing microflora.[34] Culture-based methods are useful but have limitations to assess the concentration of airborne bacteria. Thus, total count would give better estimates of exposure to bioaerosols. However, epifluorescence microscopy has its own limitations and biases. For example, DAPI total counts are not very useful in specifying the risks associated with exposure to specific microbial agents and cell debris. In addition, DAPI binds to DNA regardless of its biological origin. Thus, it is not always easy to distinguish bacteria from the other stained biological particles and to manage particle aggregation, particularly in heavily contaminated environments and with complex matrices including aerosols from compost.[35] Furthermore, as DAPI preferentially binds to DNA in AT-rich regions, total counts following DAPI-staining might underestimate G + C-rich microorganisms such as actinomycetes from compost. Thus, there is a need to develop analytical methods allowing to better estimate bioaerosol concentrations in composting environments. Recent progress has been made with the development of promising molecular methods.[36] Combining these methods with culture-based methods and techniques analysing microbial compounds such as endotoxins and glucans might be useful to assess exposure.

Real time total airborne particles over the work shift

Very few data are available on the real time monitoring of airborne particles in composting facilities.[37] Thus, the use of an OPC in our study was particularly interesting. Results from this device showed that high amounts of total particles (up to 10^8 # m^{-3}) were in the airborne state in the composting facility during campaign-2. Furthermore, the aerosol was dominated by submicron particles. These results are in total agreement with those published by Byeon *et al.*[37] As a comparison, total particle concentrations in an urban aerosol in Austria[38] were measured by the same OPC below 1000 # m^{-3}. OPC measurements also demonstrated fluctuating concentration of airborne particles over time, underlining several peaks throughout the work shift (Fig. 4). These peaks resulted from intermittent increases in the activity inside the building due to several simultaneous composting operations. The OPC measures physical particles whether of biological origin or not. Thus, it could be useful to further characterise aerosols in occupational settings in composting facilities. Our preliminary measurements should be repeated to support this conclusion.

Cultivable microorganisms over the work shift

Results from campaign-2 revealed that airborne cultivable microorganisms, particularly bacteria, remained at high concentrations throughout the work shift (Fig. 5). They also highlighted several peaks of concentrations that could not really be assigned to any specific events but occurred at the same time for the three investigated working areas. For the sampling point SP5, these peaks globally matched those observed with the OPC for particles >0.3 μm (Fig. 4). The correspondence is even more satisfactory when particles with dopt >1.0 μm are taken into account (Fig. 5). The results also showed that 30 minute sampling would lead to different concentrations depending on the period they are collected. These differences would reach 1 log unit in our example. They might explain a part of the variability, particularly high for fungi (described in the literature[8]), between intersampling sets in campaign-1. Results from campaign-2 provide an example of the variability of bioaerosol concentrations during the working day. To our knowledge, it is the first set of data published on the subject in the composting environment.

Sampling method and strategy

Sampling method and strategy are key factors in the assessment of the occupational exposure to airborne microorganisms and their components. Results from variations in airborne microorganisms over the work shift described in campaign-2 are particularly interesting for that purpose. They mean to suggest that short duration sampling should be repeated to better evaluate the exposure and to take variability into account. The duration of sampling could also be increased in order to average the variability of shorter sampling duration over the work shift. Several sampling devices are available for the measurement of bioaerosols but very few are suitable for both individual sampling and long time duration of sampling.[17] Indeed, one-stage impactors are easily overloaded in highly contaminated environments and dedicated to sampling duration under 5 minutes. Samplers collecting microbial particles in a liquid, such as impingers and CIP 10 M, are known to prevent stress due to sampling. However, duration of sampling is also limited due to the evaporation of the collecting fluid over time.[39] Besides, the filtration is known to be a stressful sampling method usually associated with a decrease in metabolic activity, viability and cultivability of sensitive microorganisms. Thus, long-duration filtration sampling might be expected to decrease cultivable counts in our study. As microbial concentrations measured during campaign-2 (6 hour sampling) were in the same range, we assumed that the three sampling points SP5, SP3 and SP2 were exposed to the same aerosol. Thus, the collected samples were considered as three replicates. Based on this assumption, we did not find any significant differences between bacterial cultivable counts from the 6 hour duration of sampling and those calculated with the consecutive 30 minute sampling. For fungi, we have found a significant but weak difference. These results are consistent with previous findings showing that duration of sampling by filtration did not affect the cultivable counts of airborne spore-forming bacteria such as bacillus and actinomycetes.[40,41] They are slightly different from published results suggesting that increased sampling time has no effect on the viability of airborne fungi.[40,42,43] Assuming that 30 minute sampling were less stressful conditions than 6 hour long sampling, we conclude that long duration of sampling did not affect dramatically microbial counts in our study. Thus, results argue in favour of the suitability of the filtration sampling method for the assessment of occupational exposure to bioaerosols in composting facilities.

Enclosure of composting operations

The results from campaign-1 did not demonstrate any obvious differences between the composting areas investigated. Furthermore, none of the composting activities appeared to release much more microbial particles into the air than another. In previous studies specific activities such as shredding, pile turning and compost screening were identified as bioaerosol sources.[9,44] In our case, several activities were in progress simultaneously and in a reduced and enclosed area. Thus, it can be assumed that all these activities contributed to global microbial pollution of the atmosphere inside the building. This assumption is supported by the results from the 6 hour samples collected during campaign-2. Indeed, similar microbial concentrations were measured in the three distinct areas in spite of their vicinity with different composting operations. High concentrations detected during the work shift are also indicative of the lack of efficient technical preventive measures to reduce airborne microbial pollutants. Our results suggest that the enclosure of composting operations favoured persistent high concentrations of airborne microbial particles. High levels might also be recurrent over time, as bioaerosol concentrations were very similar between the two sampling campaigns. Enclosure of composting facilities is often proposed to reduce bioaerosol dispersion to the surrounding residential populations.[16,45] Several studies have reported higher bioaerosol exposure when wastes and compost are handled indoors rather than outdoors.[8,11] Other studies focused on totally closed facilities and concluded that bioaerosol release was related to the type of waste (*i.e.* initial contamination of waste), the design of the plant and the composting activities used in the composting facility.[46–48] Our study provides new data in favor of a "containment effect" of composting operations on bioaerosol concentrations and highlights the recurrent occurrence of high microbial levels. It suggests further research to study the effect of enclosure of composting operations on the occupational exposure to bioaerosols and the required preventive measures.

Risks associated with measured concentrations

The exposure levels for the two employees working in the building cannot be extrapolated from our ambient data (no personal samples were collected in our study), but occupational exposure is expected as it was documented in previous works. Indeed, personal exposure to endotoxins has been reported from 8 to 340 EU m^{-3} for workers in composting household wastes.[10] Wouters *et al.*[11] have reported endotoxins exposure up to 37 000 EU m^{-3} in a plant processing the same type of wastes. Exposure to endotoxins has also been shown in plants processing other type of wastes.[5,8,49] Exposure to airborne bacteria and fungi was also demonstrated.[8,10] In spite of the recognition of health effects due to exposure to biological agents, there is no admitted Occupational Exposure Limit for bioaerosols at the international

level.[12,13] An occupational exposure limit of 50 EU m^{-3} was proposed for endotoxins in 1998 in the Netherlands.[50] Then in 2001, the Dutch social affairs ministry increased this value to 200 EU m^{-3}, which was used as a reference for a few years before being abandoned. In July 2010, an expert committee under the Dutch health ministry published a report proposing a new occupational exposure limit at 90 EU m^{-3}.[51] There are fewer data for cultivable microorganisms. Therefore, data from bioaerosol exposure are still interpreted with caution. However, the ambient concentrations measured in our study are in the range of occupational exposure measurements associated with adverse health effects in epidemiological studies and case report among workers.[10,14,15] The tasks of one of the employees were to clear spilt soil and perform maintenance, both of which have already been described as exposing ones.[8] The other employee was in the cabin of the mechanical shovel, which is supposed to be a clean environment due to the cabin protection system. However, this workstation should be further investigated as high bioaerosol exposure of vehicle drivers has been reported in several studies.[8,10,19,52] Consequently, further preventive measures might be required in the composting plant we investigated to improve employee protection against exposure to bioaerosols.

Conclusions

This study investigated ambient bioaerosol concentrations in a totally enclosed composting facility. High airborne concentrations of endotoxins and cultivable bacteria and fungi were measured on the site but no apparent relationships were found with the activities known to generate bioaerosols. The results suggest that the use of totally indoor processes, in the absence of any specific individual or collective preventive measures, may lead to high and persistent bioaerosol concentrations. The study highlights the need to further investigate occupational exposure to bioaerosols in enclosed composting plants. The variability of concentrations measured indoor recalls that both the number of samples and the duration of sampling are two important parameters to take into account when assessing exposure to bioaerosols. Furthermore, we also provide the first set of data describing the variation in both airborne microorganisms and total particles over the work shift in the composting environment. These results constitute new data which would be helpful in defining sampling strategies for bioaerosol studies in composting facilities. They also present the filtration sampling method as a useful tool to measure exposure in this particular occupational environment.

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