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Concentration of Bioaerosols in Composting Plants Using Different Quantification Methods

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ABSTRACT

Background: Bioaerosols (organic dusts) containing viable and non-viable microorganisms and their metabolic products can lead to adverse health effects in exposed workers. Standard quantification methods of airborne microorganisms are mainly based on cultivation, which often underestimates the microbial burden. The aim of the study was to determine the microbial load in German composting plants with different, mainly cultivation-independent, methods. Second purpose was to evaluate which working areas are associated with higher or lower bioaerosol concentrations.

Methods: A total of 124 inhalable dust samples were collected at different workplaces in 31 composting plants. Besides the determination of inhalable dust, particles, and total cell numbers, antigen quantification for moulds (*Aspergillus fumigatus, Aspergillus versicolor, Penicillium chrysogenum,* and *Cladosporium* spp.) and mites was performed. Concentrations of β-glucans as well as endotoxin and pyrogenic activities were also measured. The number of colony forming units (cfu) was determined by cultivation of moulds and actinomycetes in 36 additional dust samples.

Results: With the exception of particle numbers, concentrations of all determined parameters showed significant correlations ($P < 0.0001$; $r_{Spearman}$: 0.40–0.80), indicating a close association between these exposure markers. Colony numbers of mesophilic moulds and actinomycetes correlated also significantly with data of cultivation-independent methods. Exposure levels showed generally large variations. However, all parameters were measured highest in dusty working areas like next to the shredder and during processing with the exception of *Cladosporium* antigens that were found in the highest concentrations in the delivery area. The lowest concentrations of dust, particles, antigens, and pyrogenic activity were determined in wheel loader cabins (WLCs), which were equipped with an air filtration system.

Conclusion: It was possible to assess the microbial load of air in composting plants with different quantification methods. Since allergic and toxic reactions may be also caused by nonliving microorganisms,

cultivation-independent methods may provide additional information about bioaerosol composition. In general, air filtration reduced the bioaerosol exposure shown in WLCs. Due to the fact that the mechanical processing of compost material, e.g. by shredding or sieving is associated with the generation of high bioaerosol concentrations, there is still a need of improved risk assessment and state-of-the-art protective measures in composting plants.

KEYWORDS: bioaerosol; composting; cultivation-independent methods; microorganisms; organic dust; working areas

INTRODUCTION

Composting is the biological decomposition of organic material, which is mainly driven by a complex microbial community. The qualitative analysis of the microbial composition in composting plants' bioaerosols revealed many different species of moulds, bacteria, including actinomycetes, a type of high GC gram-positive bacteria (Swan *et al.* 2003) and methanogenic archaea (Thummes *et al.* 2007). Bioaerosols generated at composting plants contain up to 8×10^6 colony forming units (cfu) of bacteria and 2×10^7 cfu of moulds per m^3, which can be inhaled by the employees of composting plants (Albrecht *et al.* 2007).

During the handling of fresh green waste, the bioaerosol is dominated by *Cladosporium* and *Alternaria* species (Swan *et al.* 2003). However, during compost maturation, the composition of microbial community changes rapidly in the compost heaps and is affected by parameters such as kind of the input materials and operational treatment processes but as well by self-heating and final cooling process of heaps (Ishii and Takii 2003; Thummes *et al.* 2007). It can be assumed that the microbial community in bioaerosols will vary in a similar manner. In the air of German composting plants, hundreds of different species of moulds and saprophytic bacteria, including thermophilic actinomycetes, have been identified. Frequently found moulds were *Aspergillus fumigatus* and *Penicillium* spp. (Göttlich *et al.* 1994).

It is known that the occupational exposure to bioaerosols may cause adverse health effects, particularly respiratory symptoms (Domingo and Nadal 2009). There is increasing evidence that symptoms are mainly of a non-allergic inflammatory nature caused by a complex mixture of several microbial cell wall agents (e.g. endotoxin and β-(1–3)-glucans) (Schlosser *et al.* 2009). The primary health effect is an inflammatory response of the upper airways and eyes, the so-called mucous membrane irritation syndrome (MMIS) (van Kampen *et al.* 2012). In addition, cases of hypersensitivity pneumonitis, organic dust toxic syndrome, and allergic bronchopulmonary aspergillosis have been reported (Weber at al. 1993; Allmers *et al.* 2000; Bünger *et al.* 2007). Especially allergens of moulds can trigger symptoms of a type I allergy (Bünger *et al.* 2007) but also skin and gastrointestinal problems were observed (Ivens *et al.* 1999; Bünger *et al.* 2000). Thus, the minimization of exposure to bioaerosols is an important protective measure in waste management. A prerequisite for this task is an adequate monitoring of exposures.

In Germany, the technical standards and working conditions at composting plants are regulated by the Technical Rule for Biological Agents 214 (TRBA 214). According to this rule, a Technical Control Value (TKW) for mesophilic moulds of 50 000 cfu m^{-3} is applicable for sorting cabins, control rooms, driver's, and other cabins in composting plants. The application of measurement methods and technical control values for airborne biological agents in the workplace is regulated by the TRBA 405. Measurements of moulds and bacteria have to be performed by cultivation according to a standard protocol of the 'Institute for Occupational Safety and Health of the German Social Accident Insurance' (IFA-Arbeitsmappe no. 9420 and 9430).

This cultivation on solid agar media (as cfu) is the most commonly used method for quantification of airborne microorganisms. Due to the fact that only viable microbes are detected, the classic cultivation-based detection method can significantly underestimate the numbers of microorganisms (Martin and Jäckel 2011). Additionally, the cultivation is time-consuming, requires a high expertise, and could be biased by the used cultivation method. Furthermore, it has been shown that also non-viable cells or microbial fragments are able to cause health effects like allergies or toxic reactions in exposed workers (Douwes 2005; Wouters *et al.* 2006; Rylander 2010).

Therefore, further methods have been used like the total cell counting by fluorescence microscopy after DNA staining using acridine orange (Durand *et al.* 2002) or diamidinophenylindole (DAPI) (Martin and Jäckel 2011), the determination of spore concentration using a Kramer–Collins spore sampler (Hryhorczuk *et al.* 2001), or more recently by analysis of PCR-amplified 16S rRNA (Dees and Ghiorse 2001; Schäfer *et al.* 2013). Also enzyme-linked immunosorbent assays (ELISAs) based on antibodies specific for antigenic components of microorganisms are usable tools for a sophisticated detection of components in bioaerosols. These tests can be based on monoclonal or polyclonal antibodies against the whole fungal extract, isolated fungal extracellular polysaccharides (Douwes *et al.* 1999), or single allergens like Asp f 1 (Arruda *et al.* 1992) or Asp v 13 (Shi *et al.* 2011).

A number of studies suggest that the potency of organic dust to cause adverse health effects is most likely related to their endotoxin and β-(1–3)-glucan contents (Wouters *et al.* 2006; Rylander 2010; Sykes *et al.* 2011). Endotoxins, also known as lipopolysaccharides, are outer surface membrane components present in almost all Gram-negative bacteria. Up to now, the endotoxin levels are mainly measured using the Limulus amoebocyte lysate (LAL) assay that is well established. Additional information, e.g. concerning the pyrogenic activity of bioaerosols, may be delivered by the whole blood assay (Swan *et al.* 2003; Liebers *et al.* 2009). The β-(1–3)-glucans are cell wall compounds of fungi, some bacteria, and plants, consisting of glucose polymers. Glucans are potent inflammatory agents that induce non-allergic inflammatory reactions and may also act as a respiratory immunomodulator (Douwes 2005). In addition to a modified LAL assay, glucans can be detected by ELISA (Noss *et al.* 2010).

To assess the microbial load in various working areas in German composting plants, measurements of inhalable dust, particles, total cell numbers, different antigens, as well as endotoxin and pyrogenic activity were performed. In a part of the samples, also moulds (total fungi and *A. fumigatus*) and actinomycetes which play an important role in the composting process were quantified by cultivation. So far, data on pyrogenic activity and on airborne antigens using newly developed ELISAs in composting plants are not available in literature. Results obtained with the different methods were compared especially to evaluate whether the cultivation-independent methods correlate with the traditional microbiological method. A further aim of the study was to clarify which process and workplace were associated with the highest or lowest concentrations in ambient air and to assess the compliance with the German regulation TRBA 214, especially with regard to the exposure levels in vehicle cabins.

METHODS

Inhalable dust sampling

Sampling 1 (124 samples)

In 31 composting plants located in Northwestern Germany, stationary pumps with Gesamtstaubprobenahme (GSP) sampling heads were installed (May–October 2009) to collect samples for inhalable dust and particle measurement, total cell counting, the quantification of different antigens (moulds, domestic mites [the combined group of storage mites living in the home environment and of house-dust mites], and β-(1–3)-glucans) and for the determination of endotoxin and pyrogenic activity. In each plant, samples were taken on 1 day at four different workplaces; generally in sorting cabins, in wheel loader cabins (WLCs), at the delivery place/hall, and at piles. However, also other workplaces were chosen for sampling because in some plants, for example, no sorting cabin existed. For data analysis, places of sampling were grouped in six working areas: sorting cabin ($n = 6$), WLC ($n = 33$), delivery place ($n = 26$), piles ($n = 26$), shredding ($n = 14$), and processing ($n = 19$). The last group subsumes different sampling places and activities like vehicles and sorting places without a cabin, halls and places where compost was moved, and used for filling compost into sacks for delivery. This first sampling period (sampling 1) resulted in 124 samples.

Because more than one filter was needed to permit the full range of tests, at each place of sampling, three sampling units were installed in parallel at breathing level. All pumps were started simultaneously. The flows were regularly checked, and actual running times of samplers were documented. Details of sampling are shown in Table 1.

Sampling 2 (36 samples)

To investigate whether results differ depending on sampling date, in 9 (29%) of the 31 composting plants at the same workplaces, additional dust samples were

Table 1. Characteristics of different sampling procedures at breathing level in composting plants using stationary pumps and GSP-sampling heads

Samples for	Duration of sampling	Sampling pumps	Flow rate	Filter material (pore size)	Number of samples
Total cell count	At least 4 h	SG-10 air	10 l min^{-1}	Polycarbonate (0.8 µm)	124
Inhalable dust and antigen[a] quantification	2–3 h	SG-10 air	10 l min^{-1}	Polytetrafluoroethylene (1.0 µm)	124 36[b] 7 (reference)
Endotoxin and pyrogenic activity	2–3 h	HFS-513 Gilian	3.5 l min^{-1}	Glass fibre (0.5 µm; sterile)	124 36[b] 7 (reference)
Moulds and bacteria (cfu)	At least 1 h	224-PCXR8	3.5 l min^{-1}	Polycarbonate (0.8 µm)	36[b] 9 (reference)

[a]Antigens: *Aspergillus fumigatus, Penicillium chrysogenum, Cladosporium* spp., *Aspergillus versicolor,* domestic mites, β-(1–3)-glucans.
[b]From identical sampling places as 36 of the 124 samples.

taken with a time shift of 5–47 days (median 16 days). Thus, 36 samples for quantification of inhalable dust, particles, antigens (moulds, domestic mites, and β-(1–3)-glucans), endotoxins, and pyrogenic activity had been collected twice. Simultaneously, dust samples for the cultivation of moulds and bacteria were taken at these 36 sampling points enabling the comparison of cultivation-based and cultivation-independent methods. Reference dust samples were taken outside of the composting plants (Table 1).

Storage and transportation of filters

While the filters for total cell counting were kept cool (+6°C), all other loaded filters were transported at ambient temperature, not exceeding 25°C, to the laboratory. With the exception of filters for quantification of endotoxin/pyrogentic activity (risk of contamination) and those for measurement of cfu of moulds and bacteria, filters were conditioned for 24 h before the dust load was weighed and filters were extracted.

Extraction of filters and measurement

Total cell counting

Investigation of total cell counting after DAPI staining (DAPI = 4′,6-Diamidin-2′-phenylindoldihydrochloride) was performed as described previously (Martin and Jäckel 2011). In brief, cells were detached from the filter surface using a Stomacher (Stomacher, 80 Biomaster; Seward, Worthing, UK) before cells were fixed and stabilized. After the addition of 10 µl DAPI solution to 1 ml fixed cells, the stained cells were quantitatively filtrated on a black polycarbonate filter. Fluorescence labelled cells were counted with an epifluorescence microscope. The counted area and the amount of cells allowed the calculation of cells per cubic metre sampled air.

Inhalable dust and antigen quantification

The sampling was performed with pre-weighed and conditioned filters. After sampling and reconditioning for 24 h, the dust load of filters was weighed, and filters were extracted for antigen quantification. Extraction of filters was performed as described elsewhere (Sander *et al.* 2012b). The amounts of antigens were measured by sandwich ELISAs using antibodies against the different antigens. While the assay for β-(1–3)-glucans was based on monoclonal mouse antibodies, for the other ELISAs polyclonal rabbit antibodies have been used. Quantification of antigens has been described previously for *A. fumigatus, Penicillium chrysogenum, Cladosporium* spp. (Sander *et al.* 2012b), β-(1–3)-glucans (Sander *et al.* 2008), domestic mites (Sander *et al.* 2012a), and *Aspergillus versicolor* (Zahradnik *et al.* 2013). Dust and antigen levels below the detection

limit of assays were assigned a value of two thirds of the detection limit.

Particle measurement (LungVision), endotoxin, and pyrogenic activity

Extraction of filters for particle measurement as well as for the determination of endotoxin and pyrogenic activity was performed as described elsewhere (Liebers *et al.* 2012).

Particles were measured with the software 'Eyetech' and the LungVision analyzer (DIPA 2000, Donner Technologies, Or Aqiva, Israel). The measurement is based on the principle of light obscuration, also known as the Laser Obscuration Time (LOT) technology (Fireman *et al.* 2008). Measurements were performed with 1.5 ml of the undiluted filter extract introduced into a one-way optical clear polystyrene cuvette and stirred with a magnetic stirrer.

Endotoxin activity was determined with the LAL test (LAL test; Charles River, Sulzfeld, Germany). All samples were measured in a dilution of 1:100. Pyrogenic activity was measured with a whole blood assay (WBA) using cryopreserved blood measuring interleukin (IL-)1β release with a specific ELISA in the cell-free supernatant. Both methods have been described before (Liebers *et al.* 2009, 2012).

Colony forming units of moulds and bacteria

Measurement of colony forming units (cfu) of moulds (total mesophilic moulds, *A. fumigatus*) and bacteria (thermophilic and mesophilic actinomycetes) was done by the indirect method according to TRBA 405 following the standard protocol of the 'Institute for Occupational Safety and Health of the German Social Accident Insurance' [IFA-Arbeitsmappe no. 9420 (moulds), IFA-Arbeitsmappe no. 9430 (bacteria)].

For the measurement of cfu of moulds, filters were suspended being vibrated mechanically for 4 min in 10 ml of 0.9% NaCl and 0.01% Tween 80 and suspensions were serially 10-fold diluted with 0.9% NaCl and 0.01% Tween 80. For the measurement of total fungal count, solutions were inoculated on dichloraneglycerol agar (DG 18, Oxoid, Wesel, Germany) and on malt extract agar for detection of *A. fumigatus.* The plates were incubated at 25°C for determination of the total fungal count and at 42°C for *A. fumigatus* detection. Colonies were counted after 48 h and then every 24 h up to 7 days.

The bacteria-contaminated filters were placed in physiological saline solution immediately after the sampling, transported, and then vibrated mechanically in the laboratory prior to dilution plating. The agar used to determine number of actinomycetes was glycerol arginine agar with cycloheximide and nystatin to suppress fungal growth. The incubation temperature was 28°C for mesophilic and 52°C for thermophilic actinomycetes, and the incubation time was 2 weeks.

Fungal and actinomyete colonies were identified and distinguished on the basis of colony-morphological characters, e.g. structure of mycelia and colour.

Statistical analysis

For correlations, the Spearman rank correlation method was used because data were not normally distributed. According to the correlation coefficient (r), the correlations were defined as weak (r > 0–0.4), moderate (r > 0.4–0.7), and strong (r > 0.7). The Mann–Whitney *U*-test or ANOVA with multiple comparisons (Kruskal–Wallis test) was used for analyses of statistical differences between groups. A P value of below 0.05 was considered statistically significant for all analyses, and all tests were performed two sided. Data were analysed and visualized by using GraphPad Prism version 5.01 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

In each of the 31 composting plants at four different workplaces, inhalable dust was collected, resulting in 3 × 124 dust samples. While particle numbers, total cell counts, β-(1–3)-glucans, endotoxin, and pyrogenic activity were detectable in all dust samples from the composting plants, only a part of samples were positive for antigens of *A. fumigatus, P. chrysogenum*, *Cladosporium* spp., *A. versicolor,* and domestic mites (Table 2). With the exception of particle numbers, the concentrations of all measured parameters (inhalable dust, total cell number, antigens of *A. fumigatus*, *P. chrysogenum*, *Cladosporium* spp., *A. versicolor*, domestic mites, endotoxin, and pyrogenic activity) were significantly correlated with each other (P < 0.0001; $r_{Spearman}$: 0.40–0.80) (see Supplementary Table S1, available at *Annals of Occupational Hygiene* online). Out of these correlations, 84% were moderate and 16% strong.

According to LungVision analysis, the particles smaller than 5 μm amounted in most of the 124

Table 2. Results of inhalable dust and particles measurement, total cell counting, antigen determination, measurement of endotoxin, and pyrogenic activity in 124 inhalable dust samples at 31 composting plants

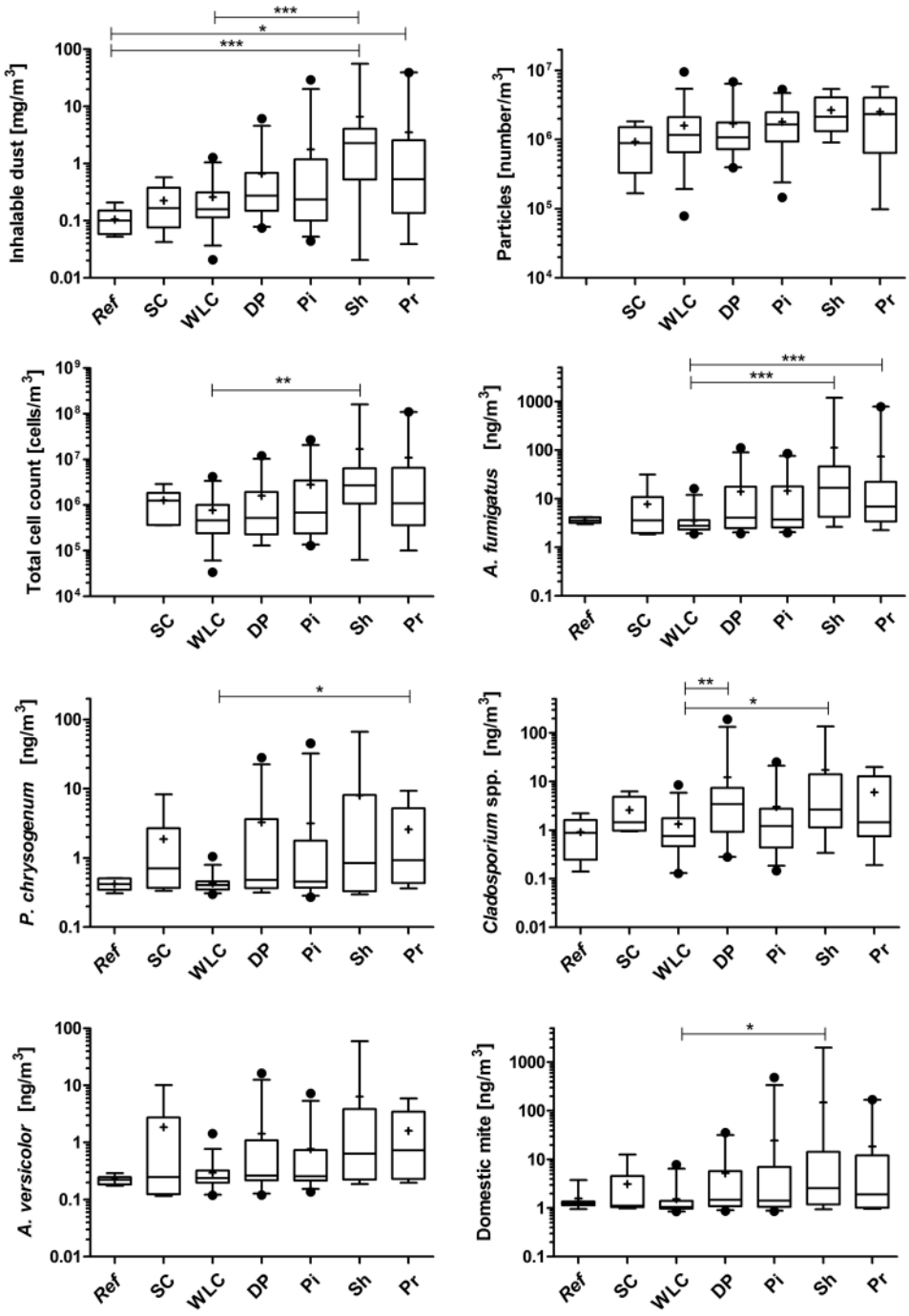
Parameter	Samples positive [*n*, (%)]	Median	Maximum	Geometric mean
Inhalable dust	87 (70%)	0.29 mg m^{-3}	56.14 mg m^{-3}	0.37 mg m^{-3}
Particles	124 (100%)	1.4×10^6 particles m^{-3}	9.5×10^6 particles m^{-3}	1.3×10^6 particles m^{-3}
Total cell count	124 (100%)	7.2×10^5 cells m^{-3}	1.6×10^8 cells m^{-3}	9.0×10^5 cells m^{-3}
Aspergillus fumigatus antigen	53 (43%)	3.69 ng m^{-3}	1196 ng m^{-3}	6.51 ng m^{-3}
Penicillium chrysogenum antigen	46 (37%)	0.45 ng m^{-3}	66.67 ng m^{-3}	0.86 ng m^{-3}
Cladosporium spp. Antigen	117 (94%)	1.45 ng m^{-3}	191.8 ng m^{-3}	1.80 ng m^{-3}
Aspergillus versicolor antigen	53 (43%)	0.26 ng m^{-3}	59.5 ng m^{-3}	0.46 ng m^{-3}
Domestic mite antigen	50 (40%)	1.37 ng m^{-3}	1976 ng m^{-3}	2.52 ng m^{-3}
β-(1–3)-Glucans	124 (100%)	13.64 ng m^{-3}	3400 ng m^{-3}	17.62 ng m^{-3}
Endotoxin	124 (100%)	11.74 EU m^{-3}	5428 EU m^{-3}	20.92 EU m^{-3}
Pyrogenic activity	124 (100%)	3831 PU IL1ß m^{-3}	1.2×10^5 PU IL1ß m^{-3}	5678 PU IL1ß m^{-3}

samples to more than 90% of total particle numbers (median: 98.6%, range: 68.1–99.9%).

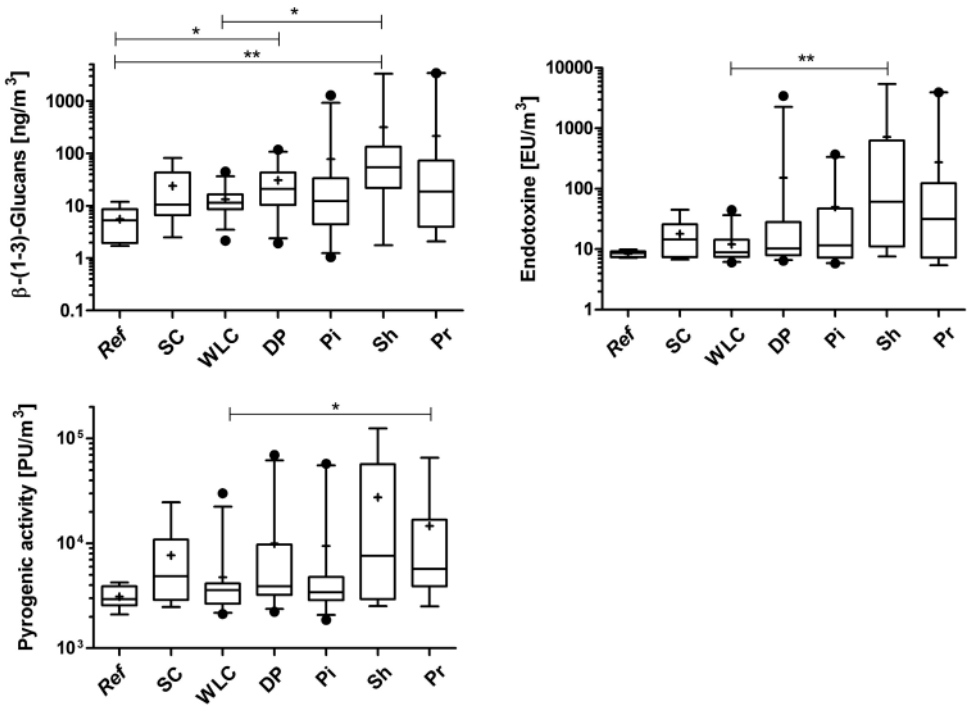
As described before, places of sampling were grouped in six working areas: sorting cabin ($n = 6$), WLC ($n = 33$), delivery place ($n = 26$), piles ($n = 26$), shredding ($n = 14$), and processing ($n = 19$). Depending on the working area, antigens of *A. fumigatus* were above the detection limit in 9% (WLC) up to 86% (shredding) of samples, antigens of *P. chrysogenum* in 9% (WLC) up to 67% (sorting cabin), antigens of *Cladosporium* spp. in 85% (WLC) up to 100% (sorting cabin, delivery place, and shredding), antigens of *A. versicolor* in 30% (WLC) up to 64% (shredding), and domestic mites antigens in 15% (WLC) up to 57% (shredding) (data not shown).

Distribution of measured values and significant differences are shown in Fig. 1. For inhalable dust, total cell number, *A. fumigatus* antigens, *Cladosporium* spp. antigens, domestic mites antigens, β-(1–3)-glucans, and endotoxin activity, significant differences in concentrations were detectable between the WLC and the workplace 'shredding'. In addition, significantly different concentrations were found for *A. fumigatus* antigens, *P. chrysogenum* antigens, and pyrogenic activity between WLC and the working area 'processing'. Only for *Cladosporium* spp. antigens, the highest amount were found at the delivery places.

At 36 sampling places in nine composting plants, inhalable dust samples were collected for a second time. In these and in nine references from outside of composting plants, cultivation of moulds and bacteria was performed. Results are shown in Table 3. While for mesophilic moulds the references were between 700 and 5080 cfu m^{-3}, inside the composting plants the lowest value was measured in a WLC (238 cfu m^{-3}) and the highest amount was found in a sorting cabin (1.3×10^7 cfu m^{-3}). The German control value TKW for mesophilic moulds (50 000 cfu m^{-3}) was exceeded in 2 out of 3 sorting cabins and in 2 out of 10 (20%) WLCs. However, repeated measurements



1 Measurement of inhalable dust, particle numbers, total cells, antigens of different moulds (*Aspergillus fumigatus, Penicillium chrysogenum, Cladosporium* spp., *Aspergillus versicolor*), and domestic mites, as well as levels of (1–3)–β–glucans, endotoxin, and pyrogenic activities at different working places in composting plants. Ref, references ($n = 7$); SC, sorting cabins ($n = 6$); WLC, wheel loader cabins ($n = 33$); DP, delivery place ($n = 26$); Pi, piles ($n = 26$); Sh, shredding ($n = 14$); Pr, processing ($n = 19$). Box plots display the 25th and 75th percentile including the median (solid line) and mean values (+). Whiskers represent 5–95 percentiles and dots the outliers. *P* value was determined according to Mann–Whitney test (*P* values: *<0.05; **<0.01; ***<0.001).



1 *Continued*

in the sorting cabin with primary 1.4 × 10E5 cfu m^{-3} resulted in 17800 cfu m^{-3} and 19800 cfu m^{-3} after adjustment of the ventilation system.

To investigate the linkage between cultivation-based and cultivation-independent methods, results of quantification of dust, antigens, and pyrogenic parameters were correlated to cfu of mesophilic moulds, *A. fumigatus,* and actinomycetes (Table 4). Colony numbers of mesophilic moulds and mesophilic actinomycetes correlated significantly with data of all cultivation-independent methods. In the case of mesophilic actinomycetes, 80% of these correlations were strong. Resulting scatter plots for the different mould antigens versus cultivated moulds are shown in Fig. 2.

Because of the expected high variability in the concentrations of antigens and pyrogenic substances, 36 samples were taken twice in intervals of some days to determine the levels of bioaerosols in replicate air samples using identical methods and sampling points. In all cases with the exception of particle numbers, the measured values of the two samples taken at different days correlated significantly (see Supplementary Table S2, available at *Annals of Occupational Hygiene* online).

DISCUSSION

In this study, we were able to quantify different parameters of bioaerosols in inhalable dust samples from German composting plants by using different, mainly cultivation-independent, methods. The application of cultivation-independent methods is meaningful, because Albrecht *et al.* (2007) reported that inactive or even dead cells can also have the potential to cause adverse health effects. Therefore, in composting facilities, a risk assessment based only on measuring colony-forming units may, in some cases, not be sufficient.

Inhalable dust and the two known pro-inflammatory components endotoxins and β-(1–3)-glucans have been quantified in composting plants by other groups before. In four large-scale composting facilities in UK, the overall geometric mean (GM) exposure was 0.99 mg m^{-3} for inhalable dust, 35.1 EU m^{-3} for endotoxin activity, and 0.98 ng m^{-3} for β-(1–3)-glucans measured by a LAL assay variant (Sykes *et al.*

Table 3. Results of colony forming units (cfu) of moulds and bacteria in inhalable dust samples from different workplaces at composting plants and in reference samples from outside composting plants

Sampling place	Mesophilic moulds [cfu m^{-3}] Median (Range)	*Aspergillus fumigatus* [cfu m^{-3}] Median (Range)	Mesophilic actinomycetes [cfu m^{-3}] Median (Range)	Thermophilic actinomycetes [cfu m^{-3}] Median (Range)
References	3053	187	540	68
	(700–5080)	(<79–500)	(100–3300)	(<32–<80)
	$n = 9$	$n = 9$	$n = 5$	$n = 4$
Sorting cabin[a]	1.4×10^5	238	4.4×10^4	4212
	$(2.4 \times 10^4–1.3 \times 10^7)$	$(238–5.5 \times 10^6)$		(3315–5110)
	$n = 3$	$n = 3$	$n = 1$	$n = 2$
Wheel loader cabin (WLC)[a]	6900	649	1.5×10^4	318
	$(238–1.8 \times 10^5)$	$(159–6.1 \times 10^4)$	$(1367–6.6 \times 10^5)$	(32–776)
	$n = 10$	$n = 10$	$n = 6$	$n = 4$
Delivery place	3.4×10^5	1.4×10^4	5.5×10^4	3810
	$(6585–4.1 \times 10^6)$	$(238–3.1 \times 10^6)$	$(1.2 \times 10^4–1.3 \times 10^5)$	$(953–5.7 \times 10^4)$
	$n = 8$	$n = 8$	$n = 4$	$n = 4$
Piles	5733	300	1.8×10^4	5227
	$(2380–5.3 \times 10^5)$	$(159–1.2 \times 10^5)$	$(1.0 \times 10^4–1.0 \times 10^6)$	(159–8259)
	$n = 7$	$n = 7$	$n = 3$	$n = 4$
Shredding	5600	794	7.9×10^4	635
	$(300–4.2 \times 10^6)$	$(100–2.9 \times 10^6)$	$(9533–1.8 \times 10^6)$	
	$n = 5$	$n = 5$	$n = 4$	$n = 1$
Processing	1.6×10^6	5.9×10^5	4.9×10^6	4.1×10^4
	$(5.8 \times 10^5–2.6 \times 10^5)$	$(2.7 \times 10^4–2.2 \times 10^5)$	$(1.2 \times 10^5–9.7 \times 10^6)$	
	$n = 3$	$n = 3$	$n = 2$	$n = 1$

[a]The German 'Technical Control Value' (TKW) for mesophilic moulds (50 000 cfu m^{-3}) was exceeded in two sorting cabins and in two WLCs (one with and one without protective ventilation).

Table 4. Spearman correlation coefficients (r) between colony forming units (cfu) of moulds and bacteria and different antigens and pyrogenic substances in inhalable dust samples from composting plants taken on identical dates and places

Parameter	Mesophilic moulds [cfu m^{-3}] ($n = 36$)	*Aspergillus fumigatus* [cfu m^{-3}] ($n = 36$)	Mesophilic actinomycetes [cfu m^{-3}] ($n = 20$)	Thermophilic actinomycetes [cfu m^{-3}] ($n = 16$)
Inhalable dust	0.48	*0.31*[#]	**0.73**	0.63
Particles	*0.38*	*0.34*	0.42[#]	*0.39*[#]
Aspergillus fumigatus antigen	0.67	0.44	**0.77**	0.60
Penicillium chrysogenum antigen	**0.73**	0.48	**0.80**	0.67
Cladosporium spp. antigen	**0.73**	0.53	**0.77**	0.63
Aspergillus versicolor antigen	0.59	0.50	**0.74**	0.46[#]
Domestic mite antigen	0.60	0.45	**0.81**	0.63
β-(1–3)-Glucans	0.56	0.46	0.49	0.59
Endotoxin	0.65	0.47	**0.81**	0.61
Pyrogenic activity	0.60	0.50	**0.77**	*0.19*[#]

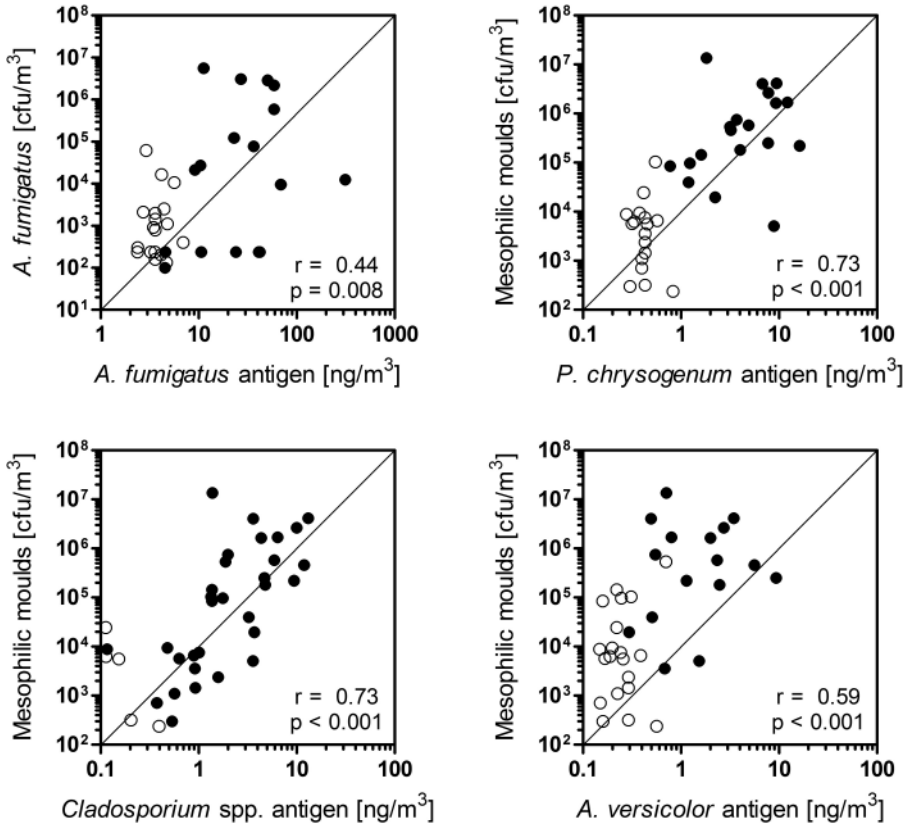
With exception of marked values (*), all correlations were significant ($P < 0.05$).
Correlations were defined as weak ($r > 0$–0.4, italic font), moderate ($r > 0.4$–0.7, normal font), and strong ($r > 0.7$, bold font).

2011). Our study showed in generally lower concentrations of inhalable dust (GM: 0.37 mg m^{-3}) and endotoxin activities (GM: 20.9 EU m^{-3}). Only the GM for β-(1–3)-glucans (17.6 ng m^{-3}) was higher than in the study of Sykes *et al.* (2011). However, the detected concentrations of β-(1–3)-glucans were clearly below those found by an inhibition ELISA in 13 Dutch composting facilities, which varied between 360 and 4930 ng m^{-3} (Wouters *et al.* 2006). Although results of different methods for detection of β-glucans were mostly correlated, significant high differences in the levels were observed (Brooks *et al.* 2013). Similar to the findings in our study, other authors reported a high correlation between the concentrations of endotoxin activity and β-(1–3)-glucans (Krajewski *et al.* 2004).

Schlosser *et al.* (2009) reported that the median of indicated endotoxin values in different composting facilities ranged from 200 to 500 EU m^{-3}, which is higher than the mean concentrations found in our study. But also other authors reported endotoxin activity in composting plants up to 3 orders of magnitude lower than those reported by Schlosser *et al.* (2009) and Pankhurst *et al.* (2011). Bünger *et al.* (2007) stated that endotoxin exposure of compost workers did generally not exceed 200 EU m^{-3}. In this context, it has to be considered that results depend on sampling strategies and the applied extraction and test methods (Liebers *et al.* 2007). This is also true for β-glucans. While many studies use the glucan-specific LAL assay, in our study a β-(1–3)-glucan-specific sandwich immunoassay based on monoclonal antibodies was used.

Further reasons why in nearly all studies, including our own, exposure levels show large variations may be meteorological conditions, source of composting material, waste biodegradation stage, and sampling location in relation to the emission sources. However, it was the aim of our study to evaluate different quantification methods and to compare the exposure levels in different working areas and not to assess the exact bioaerosol exposures in the different plants.

Due to the fact that in our study filters for the measurement of endotoxin activity were extracted without detergents, the same samples could be used to measure the pyrogenic activity using WBA. This test offers the opportunity to measure IL-1β release of stimulated monocytes reflecting whole pyrogenic and not only endotoxin activity. It has been used to quantify



2 Correlation between cultivation-based measurement of moulds and antigen quantification. The open dots represent antigen values below detection limit.

pyrogenic activity after passive airborne dust sampling using electrostatic dustfall collectors (EDCs) in social rooms of the same composting plants as in our study (Liebers *et al.* 2012). A similar significant correlation between pyrogenic activity and endotoxin levels was found in the inhalable dust samples from the workplaces.

Measurement of total cells after DAPI staining has been performed before in a German municipal composting plant and resulted in 10^6–10^9 cells m^{-3} (Albrecht *et al.* 2007), which is in a higher range than the values measured in our study ($3.4x10^4$-$1.6x10^8$). While we measured 124 samples and many of them derived from WLCs with protective ventilation system, data from Albrecht *et al.* (2007) were based on only 13 dust samples.

The newly developed Sandwich-ELISAs for *A. fumigatus, A. versicolor, P. chrysogenum,* and *Cladosporium* spp. were usable to quantify the antigens in inhalable dust samples in composting plants and to differentiate between distinct fungal genera. Due to the fact that ELISAs were based on polyclonal antibodies directed to homogenized extracts containing spores and mycelia, it could be expected that various fungal fragments were detected with this method. In the meantime, methodology as well as first results have been published (Sander *et al.* 2012b).

In a previous study, using an ELISA based on polyclonal rabbit IgG against extracellular polysaccharides from *Aspergillus* and *Penicillium* (EPS-Asp/Pen) in 49% of samples of the compost industry EPS was detectable (Wouters *et al.* 2006). However, with this test it was not possible to differentiate between distinct fungal genera. Not in the composting industry but in poultry farms an ELISA based on monoclonal antibodies to the single allergen Asp f 1 was used to quantify *A. fumigatus* antigen levels in settled dust samples (Prester *et al.* 2010). However, Asp f 1 seems

to be rarely detectable by that assay in airborne samples from composting plants as out of 159 measured samples from our study only the sample with the highest *A. fumigatus* antigen concentration was above the detection limit of that assay (data not shown).

Since it is known that for example humidity supports the proliferation of both moulds and mites, dust samples in our study have also been tested for domestic mites using an ELISA. High mite antigen concentrations have been measured in airborne dust samples from working places in textile recycling (median 159 ng m^{-3}, maximum 377 ng m^{-3}) (Sander *et al.* 2012a). While the mean domestic mite antigen value measured in our study was much lower (median 1.37 ng m^{-3}), very high maximal values (maximum 1976 ng m^{-3}) were observed next to shredders and sieving machines.

With the exception of particle numbers, all measured parameters showed a significant correlation indicating a close association between them. Overall, high dust levels led to high levels of total cell count, endotoxin, pyrogenic activity, and the above-mentioned antigens in the air of composting plants which seems to be plausible. The missing correlation between the particle numbers and other parameters could probably be based on principal differences of methods. Using dynamic laser technology, physical particles were measured whether of biological origin or not. Although during this measurement a magnetic stirrer drives particles in motion, solid aggregates would not be disrupted. Thus, particle aggregation could influence the results. Additionally, although the number of particles below 5 µm amounted to mostly more than 90% of the total particle numbers, the weight of this particle fraction is usually only a minor part of inhalable dust. For example, in personal samples from large-scale composting facilities, the median respirable dust concentration was 0.05 mg m^{-3}, whereas the median inhalable dust fraction was 1.08 mg m^{-3} (Sykes *et al.* 2009).

The material is often turned during the composting process. Consequently similar to results from other authors (Schlosser *et al.* 2009; Persoons *et al.* 2010; Sykes *et al.* 2011), we detected highest concentrations of dust, particles, microorganisms, mould and mite antigens, and pro-inflammatory substances next to the shredding machines and during processing, while concentrations were mostly low during periods when no activities were carried out. Only for *Cladosporium* the highest amounts of antigens were found at delivery places. This could be explained by the fact that *Cladosporium* is a ubiquitous fungus and one of the most common environmental moulds (Göttlich *et al.* 1994). Thus, the high amount of *Cladosporium* in the input material seems to be plausible.

The concentrations of cultivable mesophilic moulds in 36 inhalable dust samples (up to 1.3×10^7 cfu m^{-3}) were in the same range as measured by other authors in composting plants (Schlosser *et al.* 2009). As described above for the cultivation-independent methods, we found the highest values for cultivable microorganisms during shredding, processing, and in the sorting cabins. Nadal and coworkers (2009) reported that workers in the sorting cabins seemed to be the most exposed. However, in 31 German composting plants, we only found six sorting cabins in operating state because due to technical innovation manual sorting is becoming rare. Very low values for nearly all parameters were measured in WLCs. The German TKW for mesophilic moulds (50 000 cfu m^{-3}) was exceeded in 2 out of 10 WLCs. Nine of them had a protective ventilation system; so only in one of the protective ventilated loaders concentrations of moulds were in excess of the TKW. This could be explained by the observation that the driver opened the cabin door several times during the working process. Significant reductions in the levels of dust and microorganisms inside of vehicle cabins compared to workers located outside were reported also by other authors (Sykes *et al.* 2011, Schlosser *et al.* 2012).

Although it is apparent that the data collected in our study will provide only a 'snapshot' of bioaerosol concentrations at different working areas in German composting plants, we were able to detect diverse determinants of bioaerosol exposures using different quantification methods. In the light of the close correlation between these methods themselves and also between cultivation-independent and culture-based methods, combining these techniques analyzing bioaerosol compounds such as mould antigens or pyrogenic activity might be useful for a more detailed analysis of exposure.

Concentrations of airborne microorganisms and their products in dusty working areas might rise to levels hazardous to health during prolonged exposure. Thus, while working in these areas, suitable respirator

masks should be used during operations with expected high exposures. Bioaerosol exposures in WLCs were low in most cases. However, regular cleaning of the WLCs as well as regular inspection of the protective ventilation system can provide safe working conditions inside loaders with regard to bioaerosol exposure.

CONCLUSIONS

A panel of cultivation-independent methods was developed or adapted for the enumeration of biological agents at workplaces and in the environment. Methods were evaluated for their practical use. Correlations to each other and the 'classical' measurement of colony forming units were determined. The measurements show a good correlation between these methods and with the cultivation of microorganisms. In addition, these methods may provide more detailed information about bioaerosol composition and should be evaluated in further workplace studies according to their predictive value for health effects in humans.

SUPPLEMENTARY DATA

Supplementary data can be found at http://annhyg.oxfordjournals.org/.

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