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Airborne microorganisms associated with waste management and recovery: biomonitoring methodologies

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Summary. This paper presents preliminary results from a year-long indoor bioaerosol monitoring performed in three working environments of a municipal composting facility treating green and organic waste. Composting, whereby organic matter is stabilized through aerobic decomposition, requires aeration, causing the dispersion of microbial particles (microorganisms and associated toxins). Waste can, therefore, become a potential source of biological hazard. Bioaerosol samples were collected on a monthly basis. Through a comparison of results obtained using two samplers – the Surface Air System DUO SAS 360 and the BioSampler – the study aimed at assessing the presence of biological pollutants, and at contributing to the definition of standard sampling methods for bioaerosols leading, eventually, to the establishment of exposure limits for these occupational pollutants.

Key words: bio-aerosol, bacteria, garbage, methods, Limulus test.

Riassunto (*Microrganismi aerodispersi correlati alla gestione e al recupero dei rifiuti: metodologie di biomonitoraggio*). Questo lavoro presenta i risultati di un anno di monitoraggio del bioaerosol *indoor* campionato in tre differenti ambienti di un impianto di compostaggio trattante matrici organiche vegetali. Il compostaggio è un processo aerobico di stabilizzazione della sostanza organica e l'aereazione provoca la dispersione delle particelle microbiche. Ciò costituisce una sorgente di esposizione biologica dovuta al rilascio di microrganismi e prodotti nocivi del metabolismo o delle strutture cellulari dispersi nell'aria durante i processi di lavorazione. I campioni di bioaerosol sono stati prelevati mensilmente utilizzando due differenti campionatori: il Surface Air System (DUO SAS 360) e il BioSampler per confrontare le rispettive capacità di recupero di microrganismi vitali e di endotossine aerodisperse e definire la migliore metodologia di campionamento degli inquinanti di origine biologica per fornire limiti di esposizione.

Parole chiave: bioaerosol, microrganismi, rifiuti, metodi, test del Limulus.

INTRODUCTION

Over the past fifteen years, separate waste collection has become one of the most important components of municipal waste management. In keeping with the principles of industrial ecology, composting is, thus, used to recover the organic fraction by converting it into compost, while reducing biodegradable waste in landfills. Such practices, however, require the formulation of strategic disease and accident prevention proposals, including the optimization of management processes and increased environmental monitoring as a basis for risk analysis and reduction.

Composting is characterized by the generation of heat resulting from aerobic microbial activity. The temperature normally reaches 50-60 °C, destroying most microorganisms. Nevertheless, high temperatures during composting favor the growth of actinomycetes and fungi, and the release of endotoxins resulting from the destruction of the outer membrane of Gram-negative bacteria. Endotoxins have, indeed, been found in many occupational settings in which the presence of organic matter favors bacterial growth [1]. The importance of endotoxins in the assessment of indoor biological contamination derives not only from their usefulness as indicators of biological activity of lipopolysaccharide – the major component of Gram-negative bacteria's outer membrane [2] – but also from the fact that, as inhalable particles, endotoxins contribute to the development of respiratory symptoms such as allergy with sneezing or runny nose, dyspnoea, itchy skin, or itchy or watery eyes non-atopic asthma, airway obstruction, accelerated lung function decline, bronchial hyper responsiveness and fever for organic dust toxic syndrome [1, 3, 4].

Waste, thus, represents a potential source of biological hazard through the release of microorgan-

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isms and toxins associated with their metabolism or cell structure [5]. Such elements may become airborne by adhering to dust particles during waste processing and recovery. The assessment of the degree of contamination and associated level of risk is complicated by the presence of both biological and chemical contaminants. While exposure limit values for chemical substances have been established and regulated for, similarly valid limits for biological exposures would be more difficult to obtain. Here, individual immune responses must be taken into account, which may be correlated to the intensity and duration of exposure to the biological agents in question, as well as to their infectivity, pathogenicity and virulence, all factors on which information is still limited. In fact, epidemiological studies exploring associations between exposures to microorganisms and specific pathologies, in themselves, are limited in their ability to establish cause-effect relations between exposure and disease, or defining biological risk thresholds [6].

The scientific literature on bioaerosol contamination describes many different sampling and analysis methodologies [7-11]. This is partly due to the fact that no regulations exist as to the methodologies to be used in order to sample, assess and control biological pollution.

The objectives of this study were to assess the level of indoor airborne microbial and endotoxin pollution at a municipal composting facility, and to contribute to the definition of standard, practicable sampling methods for bioaerosols, by comparing the results obtained using two different samplers.

MATERIALS AND METHODS

Indoor bioaerosol sampling

The composting facility studied is located in the vicinity of Rome. The facility in question is designed to process about 30 000 tons/year of biodegradable organic market, household and green waste into high quality compost for agriculture and floriculture. Indoor bioaerosol monitoring was carried out in three different working environments: the sorting, composting and office areas. Sampling was performed on a monthly basis for an entire year. Each environment was repeatedly monitored during working hours. As negative control, an empty room outside of the composting facility was also monitored. This room was assumed to have been either less contaminated or uncontaminated with airborne microorganisms.

Two different samplers were used: the Surface Air System DUO SAS 360 (PB International), and the BioSampler (SKC). Both were placed 150 cm above the floor, which is the average breathing height for humans.

The DUO SAS 360 is a dual head "active" impaction air sampler. It aspirates a known volume of air at a constant flow rate of 180 L/min, allowing the direct quantification of viable bacteria. Its main advantage lies in that air samples are simultaneously collected through its two aspirating heads. Air samples, then, impact 55 mm contact Rodac plates equipped with selective and/or specific agar media for the growth of the microorganisms of interest as described below (microbiological detection). This allows the calculation of average values for a more representative result.

The BioSampler is a swirling sampler. Bioaerosol and biologically inert airborne particles can be collected into a swirling liquid at a flow rate of 12.5 L/min by a portable Air Cube flow pump. The advantage of this sampler is that microorganisms are not damaged by collision with the impinger base plate as happens for the other standard impingers where the collection liquid tends to bubble violently, causing the collected particles to re-enter ambient air. BioSampler, in fact, consists of three parts: inlet, nozzle section with three tangential sonic nozzles and collection vessel. Nozzles eject particles at an angle to the sampler's inner wall to reduce particle bounce and preserve aggregates of organisms because the airflow through the three tangential nozzles causes the collection liquid to gently swirl moving particles into the collection liquid without re-aerosolization.

An additional advantage is that the collection liquid lends itself to many different kinds of tests. Indeed, in the present study, one of the investigated parameters was the concentration of airborne endotoxin produced by Gram-negative bacteria. The collection vessel was, thus, filled with 10 ml of sterile, pyrogen-free solution so as to sample 200 liters of air.

To quantify the presence of microorganisms, multiple serial dilutions were subsequently performed and solutions spread on specific agar media as described below (microbiological detection). Bioaerosol endotoxin concentrations were assessed by the Limulus Amebocyte Lysate (LAL) test.

Endotoxin detection

Following the European Committee for Standardization (CEN) draft protocol for the measurement of endotoxins, these were evaluated by the gel-clot LAL *in vitro* assay according to the manufacturer's instructions [12]. Due to an enzymatic chain reaction lysate, prepared from horseshoe crab *Limulus polyphemus* amebocytes, gels in the presence of endotoxins, even in minute amounts. The Limulus assay, based on the above phenomenon, is the most sensitive test available for the rapid detection of endotoxins. This assay is able to detect endotoxins both when dissolved and when associated with the intact cell walls of viable Gram-negative bacteria.

Control standard endotoxin (CSE) series in concentrations ranging from 0.250 Endotoxin Unit per milliliter (EU/mL) to 0.03 EU/mL, as well as endotoxin-free water, were used as positive and negative controls, respectively [12]. Following one hour of incubation at 37 °C ± 1 °C, all tubes were visually evaluated for gel-clot formation, indicating the presence of endotoxin.

The result, expressed as Endotoxin Units per cubic meter of air sampled (EU/m^3), was obtained by determining the range between the last standard dilution yielding a positive result and the first yielding a negative result, considering the volume of pyrogen-free water used in the BioSampler, the sensitivity of the kit employed and the quantity of air sampled.

Microbiological detection

All microbiological analysis – on samples obtained using either sampler in each of the working environments – were carried out in triplicate.

Microbial cultures and counts were performed on certified agar media.

The following microbiological parameters were tested for: total telluric bacteria (total bacterial count at 22 °C); total mesophilic bacteria (total bacterial count at 37 °C); total fungi (moulds, yeasts and *Aspergillus fumigatus*), Actinomycetes, Enterococci and Staphylococci (Gram-positive bacteria), total coliforms, fecal coliforms, *Esherichia coli*, *Pseudomonas* spp, *Aeromonas* spp and *Vibrio* spp (Gram-negative bacteria).

Specific or selective standardized media were used for the microbial groups to be counted. The following culture media, temperatures and incubation times were used: Plate Count Agar (Oxoid) for total bacterial count at 22 °C and at 37 °C, with incubation at 21 ± 1 °C, up to 7 days and at 36 ± 1 °C for 48-72 hours, respectively; Rose Bengal Chloramphenicol Agar (Oxoid) to enumerate mould and yeast colonies at 21 ± 1°C and Czapek-Dox Agar (Oxoid) for *Aspergillus fumigatus* at 50 °C, both up to 7 days. Actinomycetes were recovered using Actinomycete Isolation Agar (Difco) incubated at 27 ± 1 °C for 7-14 days. Streptococci were counted on Slanetz Bartley Medium (Oxoid) at 36 ± 1 °C for 24 hours; Baird Parker Agar (Oxoid), incubated at 36 ± 1 °C for 72 hours, were used for Staphylococci. Count *E. coli* Isolation Agar (Biolife) were employed for total coliforms, fecal coliforms and *E. coli*, with incubation at 36 ± 1 °C for 24 hours to enumerate Total coliforms and at 43 ± 1 °C for 24 hours to enumerate fecal coliforms and *E. coli*. *Pseudomonas* species were recovered by *Pseudomonas* Isolation Agar (Difco), incubated at 27 ± 1 °C for 24-48 hours. The medium *Aeromonas* Selective Agar Havelaar (Biolife) were used for *Aeromonas* spp with incubation at 27 ± 1 °C for 24-48 hours and TCBS cholera medium (Oxoid) was employed for *Vibrio* spp incubated at 27 ± 1 °C for 24-48 hours.

Results were expressed as colony forming units per 1cubic meter of air sampled (CFU/m^3).

RESULTS AND DISCUSSIONS

Tables 1 and *2* present maximum and mean concentrations of microbial and endotoxin contaminations as measured in samples collected by both samplers in the three working environments as well as in the negative control.

Table 1 lists the concentration of microbial flora isolated from samples collected using the DUO SAS 360, while *Table 2* shows the concentrations of microorganisms and endotoxins as recovered by the BioSampler.

Table 1 | *Mean and maximum microbial concentrations in bioaerosol samples collected by DUO SAS 360 in sorting, composting and office areas as compared to a negative control area*

Parameters	Sorting		Composting		Office		Negative control	
	Microbial concentration (CFU/m^3)							
	Mean	Max	Mean	Max	Mean	Max	Mean	Max
TBC 22 °C	7.5 x 10^2	1.5 x 10^3	8.4 x 10^1	2.7 x 10^2	1.2 x 10^2	2.7 x 10^2	1.7 x 10^1	2.5 x 10^1
TBC 37 °C	6.4 x 10^2	2.2 x 10^3	3.1 x 10^1	8.3 x 10^1	5.4 x 10^1	7.5 x 10^1	4.0 x 10^0	5.0 x 10^0
Moulds	6.1 x 10^2	9.0 x 10^2	4.6 x 10^2	9.0 x 10^2	2.6 x 10^2	3.6 x 10^2	2.8 x 10^1	3.2 x 10^1
A. fumigatus	0	0	0	0	0	0	0	0
Yeasts	1.2 x 10^2	2.2 x 10^2	2.3 x 10^2	4.3 x 10^2	1.8 x 10^1	4.4 x 10^1	1.0x10^0	3.0 x 10^0
Actinomycetes	1.0 x 10^2	1.9 x 10^2	1.4 x 10^2	3.3 x 10^2	3.6 x 10^1	8.0 x 10^1	0	0
Enterococci	1.9 x 10^1	4.0 x 10^1	0	0	0	0	0	0
Staphylococci	1.8 x 10^2	3.4 x 10^2	0	0	3.0 x 10^1	8.4 x 10^1	1.1 x 10^0	2.0 x 10^0
Total coliforms	6.0 x 10^0	1.2 x 10^1	0	0	0	0	0	0
Fecal coliforms	3.0 x 10^0	7.0 x 10^0	0	0	0	0	0	0
Escherichia coli	0	0	0	0	0	0	0	0
Pseudomonas spp	8.0 x 10^0	1.9 x 10^1	1.1 x 10^1	1.9 x 10^1	4.0 x 10^0	7.0 x 10^0	0	0
Aeromonas spp	1.0 x 10^0	7.0 x 10^0	0	0	0	0	0	0
Vibrio spp	0	0	0	0	0	0	0	0

CFU = colony forming unit; TBC = total bacterial count; A. fumigatus = Aspergillus fumigatus.

Table 2 | *Mean and maximum microbial and endotoxin concentrations in bioaerosol samples collected by BioSampler in sorting, composting and office areas as compared to a negative control area*

Parameters	Sorting		Composting		Office		Negative control	
	Microbial concentration (CFU/m^3)							
	Mean	Max	Mean	Max	Mean	Max	Mean	Max
TBC 22 °C	3.1×10^3	4.8×10^3	4.0×10^2	7.3×10^2	5.3×10^2	9.0×10^2	2.8×10^1	4.3×10^1
TBC 37 °C	1.4×10^3	2.4×10^3	1.4×10^2	5.7×10^2	1.6×10^2	5.2×10^2	1.2×10^1	2.2×10^1
Moulds	3.1×10^2	5.3×10^2	3.4×10^2	5.2×10^2	1.3×10^2	1.8×10^2	3.5×10^1	4.1×10^1
A. fumigatus	0	0	0	0	0	0	0	0
Yeasts	7.4×10^1	1.4×10^2	6.5×10^1	1.4×10^2	9.0×10^0	2.4×10^1	1.1×10^0	4.0×10^0
Actinomycetes	2.4×10^2	4.3×10^2	1.9×10^2	3.2×10^2	5.7×10^1	1.1×10^2	0	0
Enterococci	2.4×10^1	4.7×10^1	2.8×10^1	3.9×10^1	0	0	0	0
Staphylococci	4.2×10^2	7.1×10^2	3.0×10^2	4.6×10^2	4.7×10^1	1.2×10^2	1.3×10^0	3.0×10^0
Total coliforms	1.2×10^1	2.2×10^1	5.0×10^0	1.8×10^1	0	0	0	0
Fecal coliforms	4.0×10^0	1.0×10^1	0.5×10^0	2.0×10^0	0	0	0	0
Escherichia coli	0	0	0	0	0	0	0	0
Pseudomonas spp	1.8×10^1	4.2×10^1	2.6×10^1	4.6×10^1	1.4×10^1	8.0×10^1	0	0
Aeromonas spp	2.0×10^0	9.0×10^0	0	0	0	0	0	0
Vibrio *spp*	0	0	0	0	0	0	0	0
Endotoxin (EU/m^3)	7.81	12.50	6.25	12.50	nd	nd	nd	nd

CFU = colony forming unit; TBC = total bacterial count; A. fumigatus = Aspergillus fumigatus*; EU = endotoxin unit; nd= not determining*

Generally speaking, microbial concentrations, using both samplers, were low in all three working environments monitored (sorting, composting and office) throughout the year.

Comparing the two samplers one finds that, for most parameters, samples collected using the BioSampler yielded higher concentrations (by one order of magnitude). The only exception to this rule were the moulds, probably due to the hydrophobic characteristic of spores and in light of the BioSampler's collection system, which is based on liquid medium.

In one year of monitoring, opportunistic and/or pathogenic species such as *Aspergillus fumigatus*, *Escherichia coli* and *Vibrio* spp have never been detected in any of the indoor environments studied. *Aeromonas* spp was found only in the sorting area, which was also generally the most polluted.

Average microbial concentrations found in the present study are in agreement with those reported in the literature, despite the use of different methodologies [5, 11, 13, 14]. One exception is the absence, in our samples, of *Aspergillus fumigatus*, found by other authors [15]. This may be attributed to differences in the composition of wastes treated, in the composting processes or in the sampling methodologies used.

Under normal conditions, microorganisms are not evenly distributed in indoor air. This is due to the fact that they are attached to floating dust particles. Other factors, such as moisture, temperature, electrostatic charge, U.V. light, air movement and human presence, also affect their distribution.

Airborne microorganisms are normally injured due to lack of nutrients and dehydration. Gram-positive bacteria are relatively resistant and are, therefore, more likely to be isolated directly from air. Gram-negative bacteria are very sensitive to these conditions, and thus more difficult to recover. Even when present, stressed Gram-negative bacteria may fail to be recovered due to the lack of define detection methods. The use of selective agar alone, for example, greatly underestimates contamination with Gram-negative bacteria. This should be taken into account when assessing airborne microbial contamination. The concomitant use of different kinds of samplers and/or the use of dual head air samplers (such as Duo SAS 360), as in the case of the present study, is thus necessary to obtain a more representative estimate of the microbial population present. For the same reason, tests were performed in triplicate to allow the calculation of means, in accordance with the standards for good microbiological practice.

Endotoxins, detected using the gel-clot method, have probably been underestimated in the present study, because this method allowed to quantify concentrations of higher or equal 0.03 EU/mL (maximum limit lysate sensitivity available). It has, therefore, not been possible to assess potentially harmful chronic exposures to lower doses of endotoxin. Pathological manifestations of exposure to endotoxins, which can be present both in the inhalable and respirable dust fractions [1, 3, 4], consist of fever, skin and respiratory symptoms, including a decline in lung function. The individual immune response to endotoxins, however, is the result of a complex interaction between dose and timing of exposure,

additive or synergistic effects and genetic predisposition [16]. The modulation between tolerance or activation of inflammatory mediators and the immune system by endotoxins remains unclear in occupationally exposed humans.

CONCLUSIONS

Microbial concentrations found in the present study, using both DUO SAS 360 and BioSampler, were low in all three working environments monitored (sorting, composting and office) throughout the year. Nevertheless, the BioSampler tended to yield higher concentrations than the DUO SAS 360, with moulds being the only exception.

The use of BioSamplers allowed us to assess the concentration of endotoxins, which cannot be measured using "active" impaction samplers such as the DUO SAS 360. The gel-clot method used, however, probably resulted in an underestimate of the concentrations of endotoxins.

Considering the concentrations of contaminants measured, the virulence and infectious doses of the microorganisms analyzed and the exposure time, the occupational risk seems fairly low.

Infection requires an exposure to a specific pathogen, but also depends on a number of variables such as the pathogen's minimal infectious dose, the level and duration of exposure and more. Guidelines for the sampling, standardized analytical procedure, monitoring and analysis of microbial pollution in bioaerosol are, thus, necessary for a better assessment of risk. More research is needed, however, to establish which are the best exposure assessment tools and to validate newly developed methods. The lack of standard exposure assessment procedures has, thus far, hampered direct comparisons across studies. For the time being, any comparison between studies should be considered exclusively indicative rather than absolute.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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References

1. Rylander R. Review: Endotoxin in the environment − exposure and effects. *J Endotox Res* 2002;8(4):241-52.
2. Flak TA, Heiss LN, Engle JT, Goldman WE. Synergistic epithelial responses to endotoxin and a naturally occurring muramyl peptide. *Infect Immun* 2000;68:1235-42.
3. Liebers V, Raulf-Heimsoth M, Brüning T. Health effects due to endotoxin inhalation (review). *Arch Toxicol* 2008; 82:203-10.
4. Smit LAM, Heederik D, Doekes G, Blom C, van Zweden I, Wouters IM. Exposure – response analysis of allergy and respiratory symptoms in endotoxin-exposed adults. *Eur Respir J* 2008;31:1241-8.
5. Nadala M, Inza I, Schuhmacher M, Figueras MJ, Domingo JL. Health risks of the occupational exposure to microbiological and chemical pollutants in a municipal waste organic fraction treatment plant. *Int J Hyg Environ* 2009;212:661-9.
6. Douwes J, Thorne PS, Pearhce N, Heederik D. Bioaerosol health effects and exposure assessment: progress and prospects. *Ann Occup Hyg* 2003;47(3):187-200.
7. Henningson EW, Ahlberg MS. Evaluation of microbiological aerosol samplers: a review. *J Aerosol Sci* 1994;25(8): 1459-92.
8. Duchaine C, Thorne PS, Meriaux A, Grimard Y, Whitten P, Cormier Y. Comparison of endotoxin exposure assessment by bioaerosol impinger and filter-sampling methods. *Appl Environ Microb* 2001;67(6):2775-80.
9. UNI EN 13098. *Linee guida per la misurazione di microrganismi e di endotossine aerodispersi.* Milano: Ente Italiano di Unificazione; 2002.
10. Chun DTW, Chew V, Bartlett K, Gordon T, Jacobs RR, Larsson BM, Lewis DM, Liesivuori J, Michel O, Rylander R, Thorne PS, White EM, Gunn VC, Würstz H. Second inter-laboratory study comparing endotoxin assay results from cotton dust. *Ann Agric Environ Med* 2002;9:49-53.
11. Albrecht A, Witzenberger R, Bernzen U, Jackel U. Detection of airborne microbes in a composting facility by cultivation based and cultivation-independent methods. *Ann Agric Environ Med* 2007;14:81-5.
12. CEN/TC137/WG5. *Workplace atmosphere-determination airborne endotoxin, a draft report of the European Standardization Committee*. Brussels: CEN; 2001.
13. Nielsen BH, Würtz H, Holst E, Breum NO. Microorganisms and endotoxin in stored biowaste percolate and aerosols. *Waste Manage Res* 1998;16(2):150-9.
14. Durand KTH, Muilenberg ML, Burge HA, Seixas NS. Effect of sampling time on the colturability of airborne fungi and bacteria sampled by filtration. *Ann Occup Hyg* 2002;46(1):113-8.
15. Chiang CF, Yang HH, Chi TW. Monitoring of bioaerosol emission from sludge composting facility. *Int J Appl Sci Eng* 2003;1(2):148-59.
16. Vandenbulcke L, Bachert C, Van Cauwenberge P, Claeys S. Small non specialized farming as a protective factor against immediate-type occupational respiratory allergy? *Allergy* 2005;59:1294-300.