

Generation of bioaerosols during manual mail unpacking and sorting

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ABSTRACT

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Aims: The dynamics of bioaerosol generation in specific occupational environments where mail is manually unpacked and sorted was investigated.

Methods and Results: Total number of airborne particles was determined in four different size classes (0.3–0.5, 0.5–1, 1–5 and >5 μm) by laser particle counting. Time dependent formation of bioaerosols was monitored by culturing methods and by specific staining followed by flow cytometry. Besides handling of regular mail, specially prepared letters ('spiked letters') were added to the mailbags to deliberately release powdered materials from letters and to simulate high impact loads. These letters contained various dry powdered biological and nonbiological materials such as milk powder, mushrooms, herbs and cat litter. Regarding the four size classes, particulate aerosol composition before mail handling was determined as 83.2 ± 1.0 , 15.2 ± 0.7 , 1.7 ± 0.4 and $0.04 \pm 0.02\%$, respectively, whereas the composition changed during sorting to 66.8 ± 7.9 , 22.3 ± 3.6 , 10.4 ± 4.0 and $0.57 \pm 0.27\%$, respectively. Mail processing resulted in an increase in culturable airborne bacteria and fungi. Maximum concentrations of bacteria reached 450 CFU m^{-3} , whereas 270 CFU of fungi were detected.

Conclusions: Indoor particle concentrations steadily increased during mail handling mostly associated with particles of diameters $>1 \mu\text{m}$. However, it was not possible to distinguish spiked letters from nonspiked by simple particle counting and CFU determinations.

Significance and Impact of Study: The dynamics of bioaerosol generation have to be addressed when monitoring specific occupational environments (such as mail sorting facilities) regarding the occurrence of biological particles.

Keywords: bioaerosol, flow cytometry, impaction, impingement, mail sorting, particle counting.

INTRODUCTION

Bioaerosols are defined as aerosols (solid or liquid particles in a gas) of biological origin. These include viruses, viable organisms such as bacteria and fungi as well as products of organisms such as bacterial or fungal spores, plant parts or pollen. A series of industrial processes such as processing

(e.g. picking, sorting, cleaning, cutting, drying, grinding or packing) of hop (Gora *et al.* 2004), potatoes (Dutkiewicz *et al.* 2002), herbs (Dutkiewicz *et al.* 2001), tobacco (Reiman and Uitti 2000), grain (Dacarro *et al.* 2005), or grapes (Zollinger *et al.* 2005) as well as wastewater treatment (Prazmo *et al.* 2003), handling of municipal solid waste (Lavoie and Guertin 2001), or paper recycling (Breum *et al.* 1999) have been investigated regarding the formation and the presence of bioaerosols. These included mainly particles of microbiological origin composed of bacterial and fungal spores as well as vegetative cells (Lighthart 1997). The study

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on paper recycling (Breum *et al.* 1999) reports also the formation of bioaerols (as comparison to 'dirty paper' from recycling) during mail processing at two different locations (mail unloading, indoor mail sorting). For fungi, CFU per m³ of air were in the range of 200–1400, whereas bacterial aerosols varied between 200–4500 CFU. However, with a few exceptions, publications do not include information on hourly, daily neither seasonal patterns, nor fluctuations and impact loads in relation to time.

Here we report the generation of airborne microorganisms resulting from mail handling (unpacking and sorting of letters) as a function of time. During manual mail processing microorganisms originating from letters or transport containers can be aerosolized and distributed within the occupational environment. The dynamics of bioaerosol generation in specific occupational environments where mail is manually sorted was investigated. Additionally, we added specially prepared letters ('spiked letters') to the regular mail to release biological materials deliberately from letters and to simulate high impact loads of biological and nonbiological particles. These spiked letters contained various dry powdered biological and nonbiological materials such as milk powder, mushrooms, herbs, and cat litter to investigate aerosol generation when letters are handled. In this case, herbs served as surrogate for plant materials, cat litter as reference for clay particles (bentonite), mushrooms as source of chitin as well as fungal spores.

The objectives of the work were (i) to determine quantitatively total particle emission during unpacking and manual sorting of mail as a function of time; (ii) to determine quantitatively airborne culturable bacteria and fungi originating from mail processing; (iii) to correlate bioaerosol emission with specific particle size classes to distinguish between different particles on the basis of fluorescence staining and (iv) to detect specially prepared letters containing biological (powdered) materials during mail handling in order to differentiate them from regular mail.

MATERIALS AND METHODS

Mail processing

Mail was unpacked and manually sorted on a metal sorting table in a separate room of the Sihlpost in Zurich (Switzerland), which was not connected to the ventilation system of the building. During the whole mail handling process, room temperature and relative humidity was continuously monitored. Monitoring equipment (particle counters, air samplers) was placed on tripods as close as possible to the working place, i.e. within a distance of 0.5–1 m.

Specially prepared letters ('spiked letters') were added to the regular mail. An amount of 2 g of milk powder, mushrooms (field mushroom), herbs (mixture of thyme, rosemary and sage), or cat litter were ground to homogeneity in a ball mill and filled into standard envelopes (C5 format). Particle sizes were in the range of approx. 3–20 µm as determined by light microscopy. All materials were obtained from local grocery stores (COOP Milchbuck, Zurich, Switzerland). Single letters were added to mailbags containing regular mail items. After unpacking and sorting, spiked letters were removed from the mailbags.

Particle counting

Three laser particle sizers (MetOne '227B', purchased from SKAN AG (Allschwil, Switzerland) were used to determine particle numbers. Particle size determination is based on optical particle counting by light scattering (refraction, reflection and diffraction) from single particles flowing out of a nozzle. Both the number and the size of particles are simultaneously determined. In total, particles of four different size classes (0.3–0.5, 0.5–1, 1–5 and >5 µm) were measured. Particles with sizes ranging from 0.3 to 0.5 µm were determined as triplicates to assess experimental standard errors. Generally, variations between the three instruments were <4%. One of the particle sizers was equipped with a temperature and humidity sensor. Samples were taken during the whole monitoring period for 21 s (corresponding to 1 l of air) in intervals of 5 min. Two particle size classes can be simultaneously recorded. Single readings were stored in the internal memory, downloaded to a computer and subsequently analysed using the software Particle Vision PortAll 1.2.

Air sampling by impingement

The 'BioSampler' liquid impinger (SKC, Eight Four, PA, USA) was purchased from Blanc-Labo S.A. (Lonay, Switzerland). The BioSampler is an all-glass, swirling aerosol collector consisting of an air inlet, three tangentially arranged nozzles, and a collection vessel (Lin *et al.* 1999). Filtered and sterilized physiological NaCl solution (9 g l⁻¹; 20 ml) was used as collection fluid with the sampler. The sampler was operated at a pump rate of 12.8 l min⁻¹ in irregular intervals depending on activities during mail handling. Background samples (empty room, no mail sorting) were collected for 96 and 114 min, respectively. During mail sorting sampling times of 6–13 min were applied. After ending the sorting process, air was collected for 38 min. All samples were transferred to 50 ml sterile plastic tubes and immediately analysed by flow cytometry after return to the laboratory.

Air sampling by impaction

The 'MAS-100 eco' (MBV, Littau, Switzerland) single-stage impaction sampler was applied for the collection of bioaerosols (Kalogerakis *et al.* 2005). An amount of 100 l of air was collected in time intervals depending on the steps of mail processing. Standard 90 mm Petri dishes containing different growth media were used with the impaction sampler. For determining the total number of culturable bacteria nutrient agar, NA (meat extract, 1 g l⁻¹; yeast extract, 2 g l⁻¹; peptone 5 g l⁻¹; sodium chloride 5 g l⁻¹; agar 15 g l⁻¹) was used. The pH was adjusted to 7.4. After sterilization at 121°C for 20 min, actidion (20 mg l⁻¹) was added through sterile filtration to prevent fungal growth. For the determination of fungi and yeasts malt extract agar, MEA (malt extract, 20 g l⁻¹; yeast extract, 4 g l⁻¹; agar, 20 g l⁻¹) was used. The pH was adjusted to 5.4 with HCl. After air sampling, both NA and MEA plates were incubated at room temperature for 2 days. CFU were counted after visual inspection.

Flow cytometry

Flow cytometric analyses were performed with a portable 'Microcyte' flow cytometer (BioDetect AS, Oslo, Norway) for analysis of microorganisms. Fluorescence is separately determined in 240 channels corresponding to a log particle size distribution of 0.45–13 µm. Calibration was performed with standard kits of monodisperse particles of 1 and 10 µm diameter purchased from the supplier. Dyes SYTO 62, TO-PRO-3, wheat germ agglutinin (WGA Alexa 633) were obtained from Molecular Probes (Eugene, OR, USA). SYTO and TO-PRO were used in final concentrations of 50 nmol l⁻¹, WGA in a final concentration of 2 µg ml⁻¹. Dyes were added to the samples obtained by impingement and incubated in the dark at room temperature for 60 min prior to analysis.

RESULTS

Indoor aerosol generation during manual mail handling was monitored by particle counting during approx. 5.5 h in regular intervals of 2 min starting at 09:38 AM (Fig. 1). During the monitoring, ambient temperature and relative humidity changed only marginally ranging from 23.5 to 26.5°C and 23.0 to 29.5%, respectively (data not shown). Measurements to assess background particle concentrations were taken for 4 h in advance to mail processing. At the start of mail unpacking and sorting distinct changes in airborne particle concentrations are observed by laser particle sizing (Fig. 1). During mail processing a 1.3-fold overall increase in total airborne particles >0.3 µm from approx. 95 000–1 20 000 particles per l was detected (Table 1). However,

regarding the different size classes, particles with diameters of 0.5–1 and 1–5 µm increased by factors of 1.9 and 8.2, respectively, whereas the number of large particles (>5 µm) increased almost 20-fold (Table 1).

During mail processing a significantly increased amount of large particles >1 µm is formed (Fig. 2). Before mail handling (=background), particles with diameters of 0.3–0.5 µm contributed with approx. 83% to the overall aerosol composition whereas during sorting this amount dropped to approx. 66%. Consequently, 11.4% of all airborne particles consisted of particles >1 µm as compared to only 1.7% before mail processing (Fig. 2).

Time dependent formation of bioaerosol was observed during mail unpacking and sorting in relation to processing activities by culturing methods and by specific staining and flow cytometry. Roughly, concentrations of culturable airborne bacteria as well as fungi and yeasts collected by impaction directly onto nutrient plates increased by a factor of five during mail handling (Fig. 3). Maximum values reached 450 CFU m⁻³ for bacteria and 270 CFU m⁻³ for fungi and yeasts. Differences of average concentrations of airborne bacteria and fungi during mail sorting as compared to background concentrations before mail processing are highly significant ($P < 0.0002$) (Table 2).

Flow cytometric analysis of aerosols collected during of mail processing by impingement revealed distinct differences as compared to background measurements (Fig. 4). The time course of the particles sampled in the impinger follows the time course seen with the direct measurements in the air (data not shown). The numbers correspond to roughly 50% of particles with diameters between 0.5 and 5 µm as the flow cytometer does not detect particles below 0.5 µm and particles larger than 5 µm represent only a minor fraction of the total. The two dyes SYTO and TO-PRO, although both staining nucleic acids have different properties. SYTO dyes permeate through membranes of most prokaryotic or eukaryotic cells, both living or dead. They serve as a general DNA stain. TO-PRO dyes are typically impermeable to membranes of living cells (which are therefore not labelled), whereas dead cells with damaged membranes are easily stained. The percentage of stained particles in all three-size classes with SYTO is therefore always larger than the one with TO-PRO (Table 3). In the size class 0.5–0.9 µm the smallest amount of the particles contained nucleic acid (10–14%), followed by the class 0.9–1.5 and 1.5–5.0 µm with 21–32 and 64–76% of the SYTO-stained cells, respectively. Roughly two-third of the number of SYTO stained cells stain also with TO-PRO, indicating that one third of DNA containing cells have membranes impermeable to TO-PRO and are thus still intact. Relative amounts of DNA containing cells increase during the experiment, the percentage of SYTO stained cells becomes greater in all three-size classes.

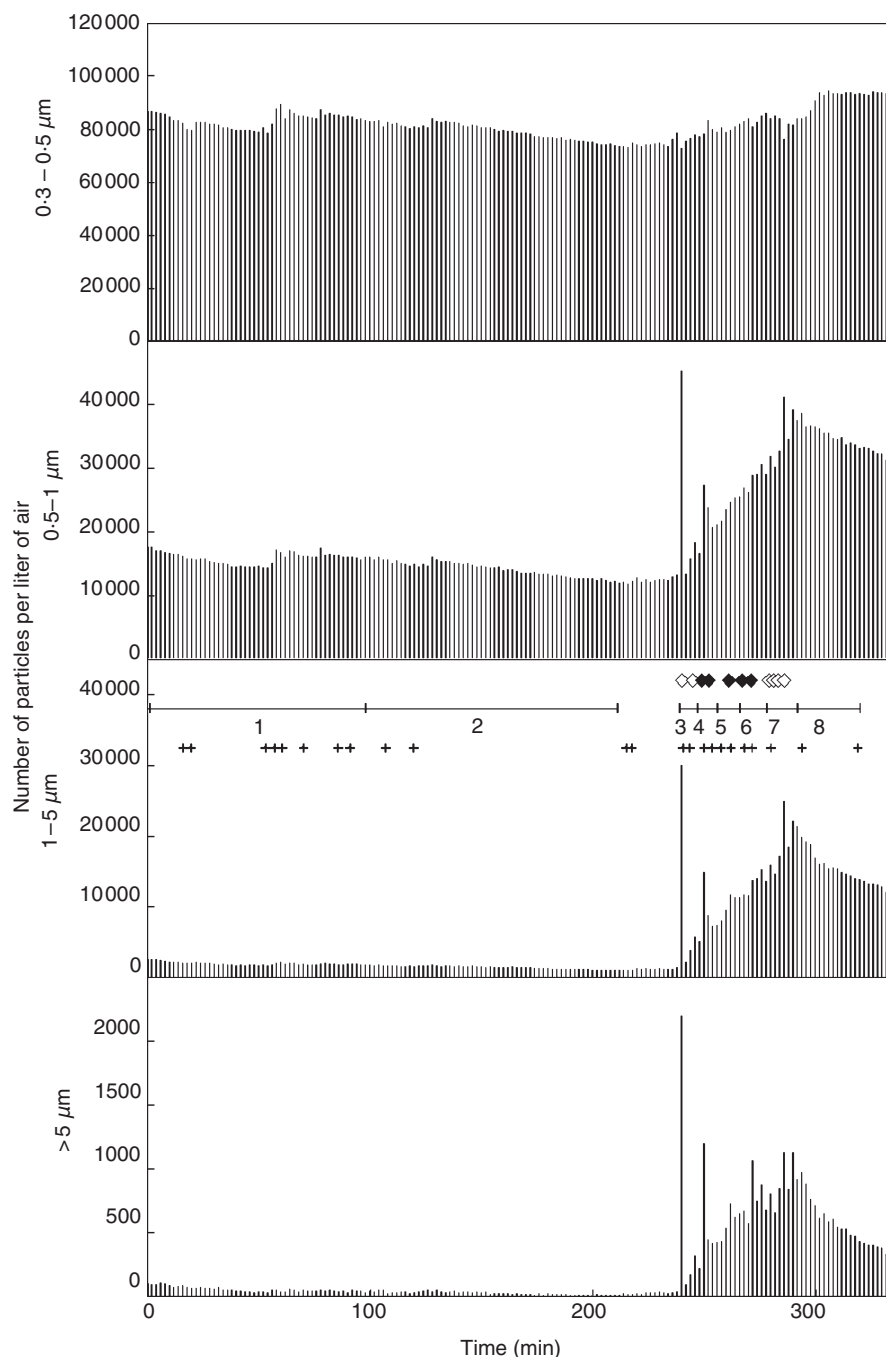


Fig. 1 Formation of particulate aerosols during mail processing (unpacking of mailbags, manual mail sorting) in relation to different particle size classes (0.3–0.5, 0.5–1, 1–5, >5 μm). Measurements took place for 21 s (= 1 l of air collected) in time intervals of 2 min. Open diamond symbols denote single mail handling events (unpacking of mailbags containing regular letters followed by sorting); solid diamonds denote unpacking of mailbags containing 'spiked' letters in addition to regular letters followed by sorting; horizontal bars denote eight time periods of impingement sampling (1–8); crosses denote events of impact sampling

Specifically, Gram-positive bacteria are stained with fluorescent wheat germ agglutinin (WGA) whereas Gram-negative are not (Sizemore *et al.* 1990; Fife *et al.* 2000; Holm and Jespersen 2003). WGA binds to *N*-acetylglucosamine and *N*-acetylgalactosamine residues of the cell wall. As a consequence, vegetative cells of fungi are stained as well with WGA, because of their chitin content of the membranes (Cohen 2001). Cells 0.5–0.9 μm bind clearly less WGA than cells larger than 0.9 μm . After WGA staining the

relative fractions of stained cells decrease slightly during the three phases of the experiment in the two smaller size classes while the fraction of fluorescent particles between 1.5 and 5 μm is increased toward the end of the experiment.

As the size range of the flow cytometer is divided into 240 separate channels it is possible to investigate size changes in more detail. As illustrated in Fig. 4 the signals of nonfluorescent particles peak at approx. 0.7 μm and decline asymmetrically towards larger sizes. In contrast, SYTO

Table 1 Average concentrations (\pm SD) of airborne particles generated during mail unpacking and sorting (29 consecutive measurements in intervals of 2 min) as compared to background particle levels determined before mail handling (120 consecutive measurements in intervals of 2 min)

Size class (μm)	Background ($n = 120$) (counts per l of air)	Unloading and sorting ($n = 29$) (counts per l of air)	Increase factor
0.3–0.5	80 330 \pm 3975	80 786 \pm 3307	1.0
0.5–1	14 688 \pm 1524	27 849 \pm 7905	1.9
1–5	1613 \pm 370	13 284 \pm 6607	8.2
>5	37 \pm 22	728 \pm 411	19.6

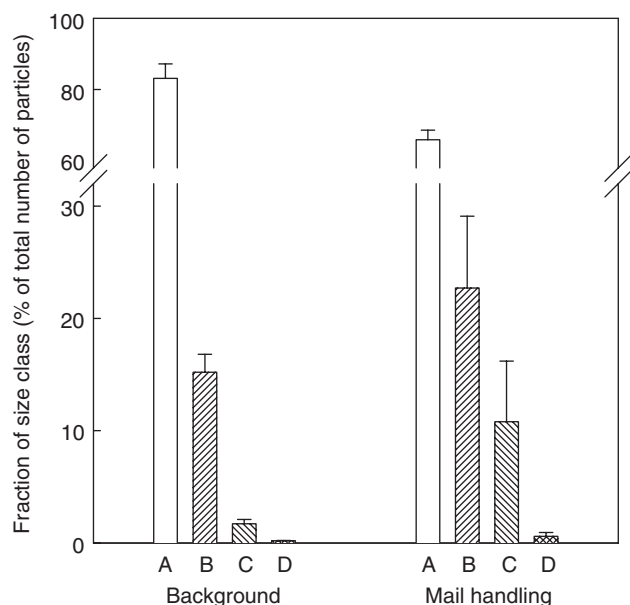


Fig. 2 Composition of aerosols before (=‘background’, $n = 120$) and during mail handling ($n = 29$) in relation to different particle size classes. A: size class 0.3–0.5 μm ; B: 0.5–1 μm ; C: 1–5 μm ; D: >5 μm

stained particles have two maxima, one coinciding with the scatter signals, the second broad one with the maximum at around 2.5 μm . This is different in WGA stained cells. While the nonfluorescent particles peak have the same shape of their size distribution, WGA-fluorescent particles seem to form a single broad population with a maximum at 1.2 μm and a second – however small – maximum of particles >3.5 μm . It is assumed that particles emitted from letters spiked with powdered mushroom material causes this tailing.

DISCUSSION

Particle concentrations steadily increased during mail handling. This is reflected in the time course pattern of

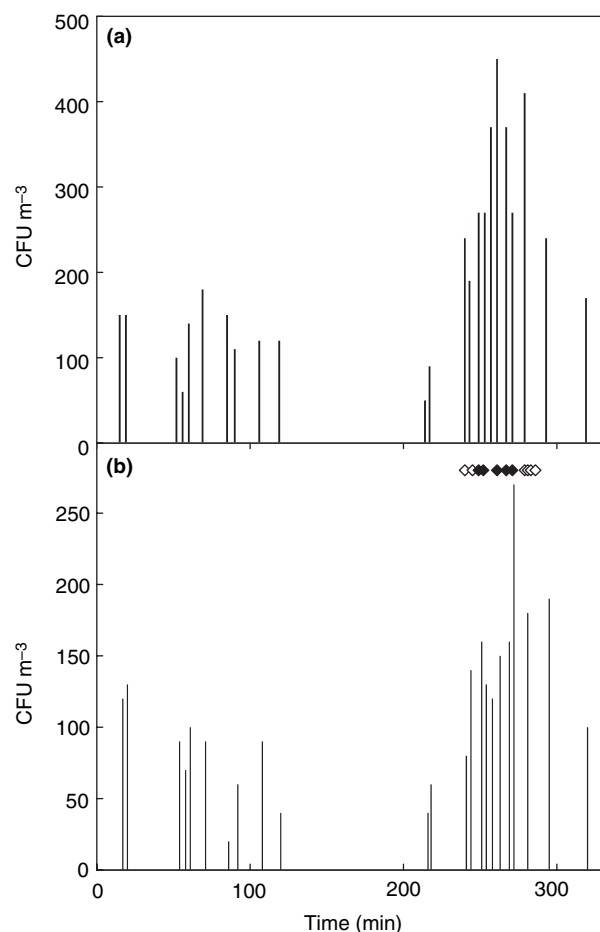


Fig. 3 Bioaerosol formation during mail handling expressed as CFU per m^3 of air. (a) Bacteria; (b) fungi. Open diamond symbols denote single mail handling events (unpacking of mailbags containing regular letters followed by sorting); solid diamonds denote unpacking of mailbags containing ‘spiked’ letters in addition to regular letters followed by sorting

Table 2 Culturable bacteria and fungi (CFU per m^3 of air collected) during mail handling as compared to background concentration before mail processing. Statistical differences (significance levels) are indicated

Time period	n	Bacteria (CFU m^{-3})	Fungi (CFU m^{-3})
Prior to mail handling (background)	12	118 \pm 39	77 \pm 33
Mail unpacking and sorting	10	308 \pm 85	158 \pm 50
Significance level (P)		1.0×10^{-6}	1.8×10^{-4}

each of the three particles size classes determined (0.5–1, 1–5 and >5 μm), except the fraction with the smallest diameters (0.3–0.5 μm), which remained unchanged. It can be concluded that during mail handling airborne particles of

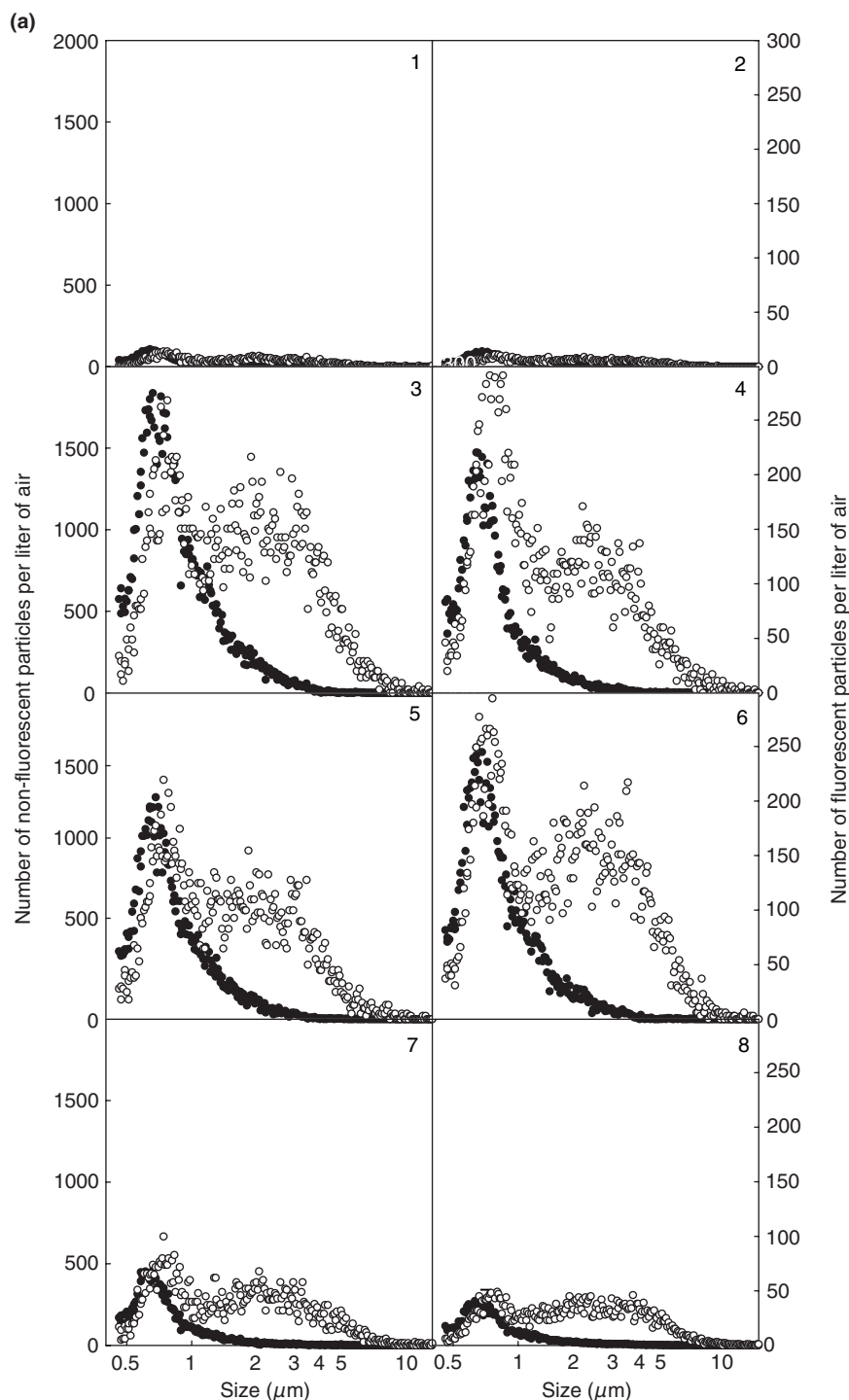


Fig. 4 Flow cytometric analyses of eight air samples collected during mail handling by liquid impingement. Sample numbers (1–8) correspond to sampling periods denoted in Fig. 1. (a) Particles stained with SYTO; (b) particles stained with WGA. Solid symbols represent particles determined by light scattering; open symbols represent particles determined by fluorescence

diameters $>0.5 \mu\text{m}$ are generated. However, it was not possible to detect and identify spiked letters, and distinguish them from nonspiked letters by simple particle counting and CFU determinations. This might be attributed to the time resolution (monitoring intervals of 2 min). Additionally, particles released from the spiked letters are immediately

diluted in the ambient air. It is necessary to perform particle monitoring as close as possible to the location where the mail is unpacked and sorted. Mail sorting might even be carried out in small closed compartments.

In summary, mail handling (unpacking of mailbags, manual mail sorting) resulted in the generation of bacterial

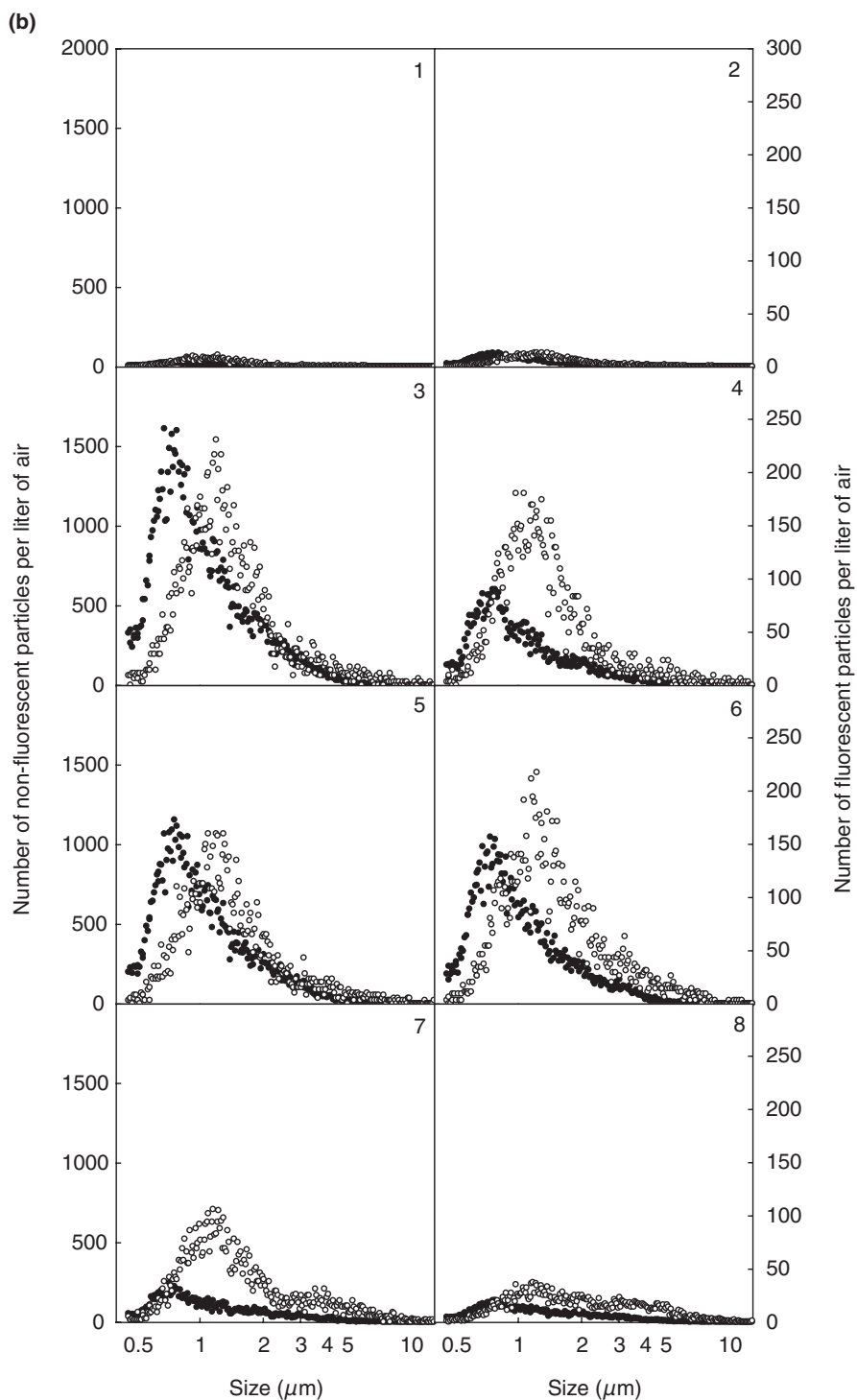


Fig. 4 Continued

and fungal aerosols associated with particles of diameters $>1 \mu\text{m}$ (mainly $1\text{--}5 \mu\text{m}$). These particles were steadily displaced after ending of the sorting process either by passive settling or due to air currents in the occupational indoor environment, but were detectable in the working

environment for a certain time period. Assuming a more or less linear decrease of particles of the size classes $0.5\text{--}1$ or $1\text{--}5 \mu\text{m}$ (as shown in Figs 1 and 3) elevated bioaerosol levels can be observed in our case for several hours until background levels are reached. Bioaerosol generation cannot

Table 3 Average percentage (% stained particles of total number of particles per size class) during mail handling (unpacking and sorting). Particle was stained with three different dyes. Sample numbers 1–8 correspond to impingement sampling shown in Fig. 1

	Dye								
	SYTO			TO-PRO			WGA		
	≤0.9 μm*	0.9–1.5 μm	1.5–5 μm	≤0.9 μm	0.9–1.5 μm	1.5–5 μm	≤0.9 μm	0.9–1.5 μm	1.5–5 μm
Before unpacking, samples 1 and 2	9.6	21.3	64.5	6.7	16.1	46.7	10.1	29.9	28.9
Unpacking, samples 3–7	13.5	25.0	66.5	6.3	12.9	40.0	8.8	26.0	27.0
After unpacking, sample 8	13.9	32.1	76.0	4.7	12.5	53.1	9.5	26.5	34.5

*Detection limit of flow cytometer at 0.45 μm.

be unambiguously assigned to a specific source. Mailbags and letters may both contribute to the formation of bioaerosols.

Manual mail unpacking and sorting resulted in a significant increase of airborne culturable microorganisms. Interestingly, the fraction of biological aerosol particles seems to be in a range between roughly 20–50%, irrespective whether the samples originate from indoor or outdoor and urban or remote areas (Jaenicke 2005). We found that culturable microorganisms comprised only 0.0006% of the total number of airborne particles emitted. Jaenicke included all particles of biological origin (e.g. plant fragments, skin fragments, vegetative cells and spores of bacteria and fungi, proteins) whereas we focused on culturable microorganisms only. Other mail processing locations (Breum *et al.* 1999) showed similar concentrations of culturable microorganisms. However, it is well established that only a small fraction (often less than 0.01% of the total cell counts) can be cultured, depending on the environment the sample originates from (Amann *et al.* 1995).

Furthermore, it can be assumed that bioaerosols as well as other airborne particles are not homogeneously distributed in the indoor workplace and may form clouds. Therefore, local concentrations of bacteria and fungi depend highly on the sampling site, i.e. in our case the number of particles collected is thought to be related to the distance from the mail sorting table.

In comparison to European guidelines (Anonymous 1993), indoor bioaerosol impact loads occurring during mail handling can be considered as low to moderate. However, the guidelines include only residential (apartments) and office environments. Our findings give clues on the dynamics of the generation of biological particles in specific occupational environments such as mail sorting facilities.

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