RELATIONSHIP BETWEEN DIFFERENT BIOAEROSOL PARAMETERS SAMPLED FROM THE BREATHING ZONE OF WASTE COLLECTORS - IDENTIFICATION OF THE MOST IMPORTANT PARAMETERS*

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Abstract: Measurements of the exposure to bioaerosols are often necessary in studies on the causality of occupational respiratory problems, but controversy exists on the relevance of different bioaerosol exposure parameters as well as on the proper methods for sampling of bioaerosol and analysis of the parameters. The aim of the present study was to elucidate possible correlations between different general microbiological parameters obtained from personal sampling of bioaerosols on filters, and to identify the parameters most useful in the discrimination between exposure levels of waste collectors. Bioaerosol sampling was carried out with the assistance of Danish waste collectors (n = 199) collecting household waste. For a full work shift each waste collector carried two field monitors (25 mm) with filters for the collection of aerosols. The following general exposure parameters were measured: total dust; endotoxin; total counts of microorganisms by epifluorescence microscopy including differential counts of fungal spores, spherical and rod shaped bacteria; culturable bacteria and fungi on agar plates (viable counts). The relationships between the different parameters were investigated by parametric correlation analysis (Pearsons) after logarithmic transformation of the data. Principal component analysis was used to study the contribution of the parameters with respect to the variance between independent samples of bioaerosols. A statistically significant positive correlation (p < 0.05) was observed between a number of different parameters. However, the correlation coefficients were in all cases low (0.2 < r < 0.7). Consequently, the prediction of a bioaerosol parameter based on data of other parameters is expected to have a limited accuracy and validity. Principal component analysis revealed that total counts of fungal spores and rod shaped bacteria can account for 93% of the variance between independent bioaerosol samples. The result is unlikely to be due to poor analytical performance of the method. It is concluded that the total count parameter may provide the best exposure stratification of waste collectors in epidemiological studies and in routine monitoring of workplace exposure.

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INTRODUCTION

Work related exposure to bioaerosols may cause a multitude of different health problems including respiratory diseases (bronchitis, asthma, hypersensitivity pneumonitis), skin problems and possibly gastrointestinal symptoms [3, 6, 7, 11-14]. Evaluation of the exposure to bioaerosols is necessary for the study of the causality of such health

problems, but quantification of the exposure is all but simple. One major problem is the selection of the relevant exposure parameters. Bioaerosols may be very complex in nature containing a multitude of different viable and non-viable microorganisms as well as various biologically active components, such as endotoxin from Gram-negative bacteria [12], (1→3)-β-D-glucan from the cell wall of moulds and yeast [8, 15], and more species specific toxins from fungi and bacteria [5]. Consequently, it is most difficult to assign specific microbiological parameters to particular health problems in work environments with a complex bioaerosol exposure.

In studies on the relationship between bioaerosol exposure and health problems several different parameters are frequently measured to characterize and quantify the bioaerosol exposure. Various types of culturable microorganisms may be enumerated, using selective growth media and growth conditions (e.g. thermophilic microorganisms), to obtain data on specific microorganisms of particular interest, e.g. causing pathological reactions related to specific toxins or allergens. Total or inhalable dust or total microorganisms by microscopy may be determined to study the association between pulmonary symptoms and the general proinflammatoric potential of organic dust or microorganisms respectively. Endotoxin from the cell membrane of Gram-negative bacteria is known to cause pulmonary symptoms particularly in susceptible individuals [3]. In addition, some recent studies have indicated that (1→3)-β-D-glucan from the cell wall of moulds and yeast may also inflict pulmonary inflammation [8, 15].

Perhaps with the exception of endotoxin there are presently no universally accepted standard methods for sampling and analysis of bioaerosols. A multitude of sampling methods exist. Sampling on filters may be particularly useful for personal sampling with subsequent analysis of endotoxin or total counting by microscopy. However, several reports exist on poor survival of some types of microorganisms, e.g. Gram-negative bacteria, during sampling on filters [4, 9, 10]. Alternatively, sampling by impaction onto solid or liquid media may produce more reliable viable counts, but the viable counts will be highly dependent on the ability of the sampled microorganisms to grow on the particular growth media in use. In addition, impactor methods have limited use in personal sampling, and they cannot provide samples for analysis of endotoxin or total count by microscopy. As a consequence, different investigators use different methods for sampling and analysis depending on the primary aim of their study. Obviously, this complicates the comparison of results from different studies, and it may be questionable to compile data obtained with different methods, e.g. in the establishment of tentative acceptance limits for occupational bioaerosol exposure.

The Danish research programme CORE focused on the identification of causes and possible prevention of health problems among workers collecting and recycling domestic waste. In the CORE programme the bioaerosol exposure of waste collectors was determined using personal sampling on filters with subsequent measurements of the following general exposure parameters: Total dust; endotoxin; total microorganisms by microscopy and differential counts of fungal spores (moulds), spherical and rod shaped bacteria; viable counts (plating techniques) of bacteria and fungi. In addition, some studies also included a more specific enumeration of culturable microorganisms such as Gram-negative bacteria, mesophilic and thermophilic actinomycetes, *Aspergillus fumigatus*, etc.

The aim of the present study was to elucidate possible correlations between different general parameters, and to identify the parameters most useful in the discrimination between exposure levels of waste collectors. For this purpose we used principal component analysis.

MATERIAL AND METHODS

Sampling of bioaerosols. Bioaerosols and dust were collected by personal filter sampling techniques with the assistance of Danish waste collectors (n = 199). For a full work shift each waste collector carried two field monitors for collection of total dust (total particulate matter) and microorganisms.

Dust was collected on cellulose acetate/nitrate filters (diameter: 25 mm, pore size: 8.0 µm, Sartorius, Göttingen, Germany) using closed-face Millipore field monitors operated at a flow rate of 1.9 l/min. Microorganisms were collected on polycarbonate filters (diameter: 25 mm, pore size: 0.4 µm) mounted in filter cassettes (Nucleopore, USA) with an inlet diameter of 4.4 mm operating at a flow rate of 1.0 l/min.

Analysis of bioaerosol exposure parameters. Concentrations of dust were determined gravimetrically by weighing of cellulose filters before and after sampling (limit of detection 40 µg).

Endotoxin was extracted from the cellulose filters with 10 ml non-pyrogenic water by orbital shaking (300 rpm, 15 min) and quantified by the kinetic-chromogenic *Limulus* amoebocyte lysate test (limit of detection 0.01 EU/m^3).

Microorganisms collected on polycarbonate filters were suspended in 5 ml sterilized 0.05% Tween-80 by adding the liquid to the filter cassettes followed by orbital shaking (500 rpm, 15 min) at room temperature. Culturable microorganisms were enumerated by plating of serial dilutions on agar plates followed by incubation for up to 7 days at 25°C or higher temperatures (limit of detection 90-1200 cfu/m^3 depending on the sample volume). For counts of the total culturable bacteria, samples were plated on nutrient agar (Oxoid, Basingstoke, UK) supplemented with cycloheximid (50 mg/l). Dichloran glycerol agar (DG18, Oxoid, Basingstoke, UK) was used for the quantification of culturable fungi.

The total numbers of collected microorganisms (bacterial rods and spheres and fungal spores) were determined by epifluorescence microscopy (magnification 1250 ×) after staining with 0.01% acridine orange (bioMérieux, France). The detection limit is dependent on

Table 1. Correlation matrix. For combinations of parameters are given the Pearson correlation coefficient (*helvetica*), the corresponding p-value (**bold**) and the number of observations. Only data sets with both measurement results above the limit of detection are included in the analysis.

	Total counts of rod shaped bacteria	Total counts of spherical bacteria	Culturable bacteria	Culturable fungi	Dust	Endotoxin
Total counts of fungal spores	*0.47* **0.0001** 80	*0.54* **0.0001** 117	*0.29* **0.0003** 146	*0.46* **0.0001** 158	*0.25* **0.001** 159	*0.20* **0.0001** 159
Total counts of rod shaped bacteria		*0.70* **0.0001** 79	*0.19* **0.11** 72	*0.11* **0.31** 79	*-0.02* **0.84** 80	*0.17* **0.84** 80
Total counts of spherical bacteria			*0.22* **0.02** 107	*0.14* **0.13** 116	*0.06* **0.51** 117	*0.21* **0.02** 117
Culturable bacteria				*0.49* **0.0001** 160	*0.22* **0.006** 161	*0.16* **0.05** 158
Culturable fungi					*0.26* **0.0005** 174	*0.11* **0.15** 171
Dust						*0.36* **0.0001** 174

the sampled air volume and the number of fields counted. If it is assumed that only one microorganism is found when counting 40 randomly selected fields, the limit of detection is as high as 190×10^3 cells/m³ at a sampled air volume of 0.043 m³ and 15×10^3 cells/m³ at a sampled air volume of 0.565 m³.

Statistical treatment. The relationship between the different parameters was investigated by calculation of Pearson's correlation coefficient (parametric correlation analysis) after logarithmic transformation of the data. The 'SAS-system' software was used.

Principal component analysis of the data set was performed after normalization of data and exclusion of objects with missing values or values below the limit of detection. The 'UNSCRAMBLER' software was used.
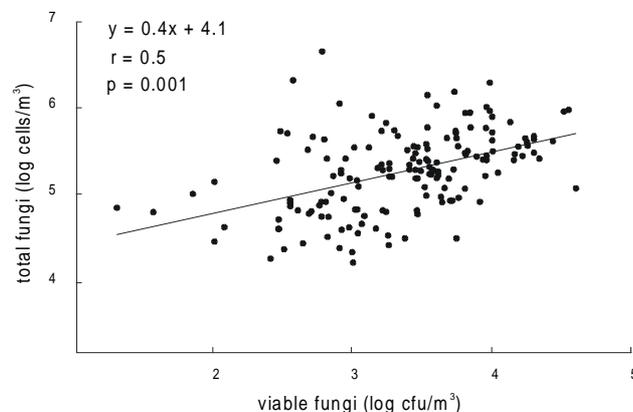
Figure 1. Correlation between viable counts of fungi and total (microscopical) counts of fungal spores. Result of linear regression analysis is included in the figure.

RESULTS AND DISCUSSION

It may *a priori* be assumed that some of the measured parameters will correlate, since they to some extent may reflect the same microbial parameters measured with different methods. The correlation matrix between the parameters, presented in Table 1, shows that several of the parameters actually did correlate significantly ($p < 0.05$). However, the correlation coefficients were in all cases low ($0.2 < r < 0.7$), indicating that prediction of parameters based on measurement results of other parameters will have a limited accuracy and validity.

In the CORE research programme analysis of bioaerosol samples obtained with personal filter techniques during work of waste collectors by epifluorescence microscopy revealed that fungal spores contributed in average to approximately 65% of the total number of microorganisms (unpublished results). A highly significant positive correlation ($p = 0.0001$) was observed between total fungal spores determined by microscopy and viable fungi and the correlation coefficient was fairly high: $r = 0.46$. Figure 1 presents a log-log plot of total spores versus culturable spores. It is observed that, in average, colony forming units determined with plating techniques will only account for a minor fraction of the fungal spores counted by microscopy. This result may be surprising considering that fungal spores are well equipped by nature to withstand high oxygen tension and dehydration when aerosolized. Speculative explanations may be non-viability (old dead spores), accumulation of fungicidal agents on the filter during sampling, low or lacking ability to grow on the media in use or competition between microorganisms. It is noted that the selected growth medium (DG 18) partially suppresses the growth of the
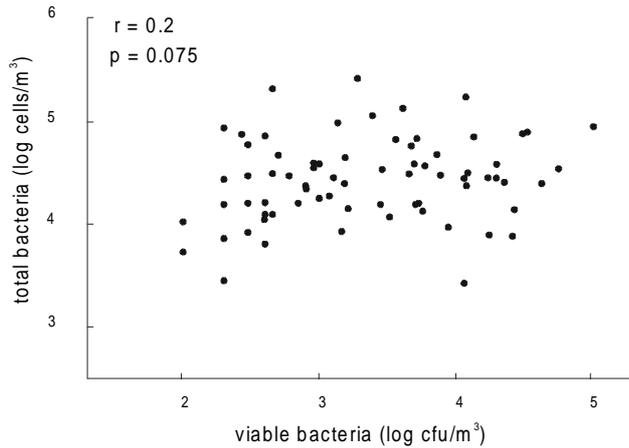
Figure 2. Correlation between viable and total (microscopic) counts of bacteria. Result of correlation analysis is shown. Linear regression analysis was not performed since the correlation was not significant (p>0.05).

fast growing moulds (*Mucor* spp., *Rhizopus* spp. etc.) enabling the enumeration of more slow growing fungi.

Figure 2 shows a similar plot of total bacteria versus culturable bacteria. In this case no correlation exists, and compared to the fungi the culturable bacteria accounted for an even smaller fraction of the total bacteria. Several reports have emphasized that bacteria, particularly the Gram-negative ones, are vulnerable to stress during aerosolization and sample collection on filters (high oxygen tension and dehydration) [4, 9, 10]. It seems likely that the use of bioaerosol sampling on filters has contributed to the loss of viability of bacteria. However, to our knowledge there are no good alternatives to sampling on filters if the personal bioaerosol exposure is
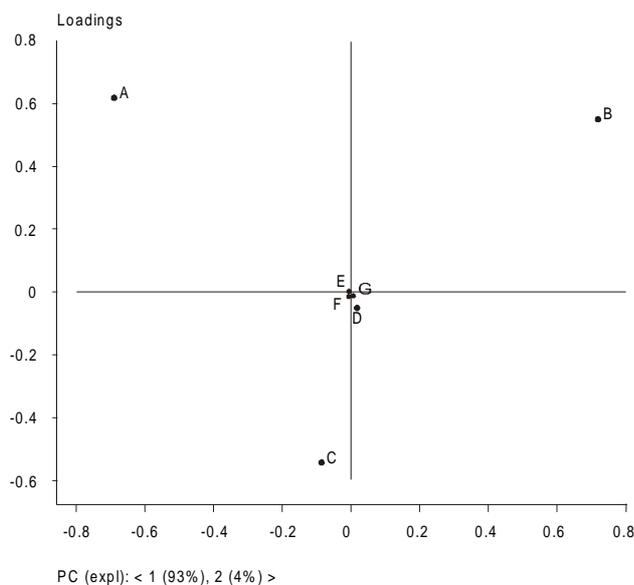

Figure 3. Variable loading plot. Loadings are referred to axes (principal components). The vertical axis accounts for 93% of the variance in the data and the horizontal axis for 4% of the variance. Abbreviations: A, bacterial rods; B, fungal spores; C, cocci; D, viable fungi; E, viable bacteria; F, endotoxin; G, dust.

to be measured. When filters are used, data on culturable microorganisms may still have some qualitative value with respect to characterization of some of the microbial composition of the bioaerosol, e.g. thermophilic actinomycetes and *Aspergillus fumigatus*.

A correlation between endotoxin and bacteria may hypothetically be expected since endotoxin originates from the cell membrane of Gram-negative bacteria. However, this hypothesis is based on the assumption that Gram-negative bacteria contribute significantly to the total counts of bacteria. Due to the very low viability of Gram-negative bacteria during collection on filters the actual contribution of Gram-negative bacteria to the total count of bacteria is unknown. A significant correlation was indeed observed between endotoxin and culturable bacteria (p = 0.05) and endotoxin and total count of spherical bacteria (p = 0.02). However, the correlation coefficients were low indicating a weak relationship. No correlation existed between endotoxin and total counts of bacteria or total count of rod shaped bacteria (Tab. 1). However, it is not possible to distinguish between Gram-negative bacteria, Gram-positive bacteria and spores of actinomycetes in microscopy, and the lack of correlation may merely reflect a high number of e.g. actinomycetes in the samples. In contrast, a far better correlation was found between endotoxin and total dust (p = 0.0001).

In general, the CORE research programme on waste collectors showed low personal total dust exposure levels. A highly significant correlation was observed between total dust and fungal spores (whether cultured or determined by microscopy), between total dust and endotoxin and between total dust and culturable bacteria, whereas the estimated correlation coefficients indicated a weaker or lacking correlation between total dust and total bacteria (Tab. 1).

As emphasized in the introduction, research programmes aiming at elucidating causal relationships between bioaerosol exposures and health problems often tend to include a multitude of different bioaerosol exposure parameters to ensure that the relevant parameters are measured. This was also the case for the CORE programme. However, such large measurement programmes are highly expensive and time consuming, and it is not feasible for local authorities to measure a large series of different parameters in routine surveillance of the working environment. Consequently, we used principal component analysis to identify the bioaerosol exposure parameters which can explain most of the variation in bioaerosol exposure of waste collectors.

The idea of principal component analysis is to calculate a number of new variables called principal components. The principal components are linear combinations of the original variables and may to some extent be compared to the straight line in linear regression. An excellent introduction to principal component analysis has been given by Bye [2].

In this context we will focus on the possibility of using the so-called variable loading plot which illustrates the contribution of each variable to the principal components

explaining most of the variation in the data. Thus, the variable loading plot illustrates the importance of each variable. The further away from the origin (0;0) the more important are the variables in explaining the variance within the data. Furthermore, the variable loading plot illustrates the correlation between variables. Variables that are positively correlated will be grouped closely together in a loading plot [2].

Figure 3 shows the variable loading plot for the two principal components describing 93% and 4% of the variance in the data respectively. The variables fungal spores, spherical and rod shaped bacteria are important to the variance, since they are far from the origin of the loading plot. Particularly fungal spores and rod shaped bacteria can be said to contribute to the overall variance since these two variables are situated far out along the axis of the component explaining 93% of the variance, whereas the contribution from spherical bacteria (cocci) is low. The three parameters contribute equally to the component explaining 4% of the variance.

Consequently, the principal component analysis indicates that total counts of spores and bacteria are the best parameters for detection of differences between independent measurements of bioaerosol exposure among waste collectors. As outlined in 'Materials and Methods' preliminary studies have indicated, that the method of total counting as such has a reasonable low uncertainty. Hence, it is unlikely that the variance in total counts between independent measurements is due to poor analytical performance of the method.

The results of the principal component analysis do not imply that the total count parameters are in any way better correlated to health problems than other microbiological parameters. However, the analysis suggests that these parameters may provide the best stratification of waste collectors with respect to bioaerosol exposure level. Therefore, total counts were included in our first attempt to establish a work condition-exposure matrix for waste collectors [1]. As emphasized in a previous review, both fungal spores and bacteria have been suggested as potential agents of significant importance in this industry as well as other industries with bioaerosol exposures [14]. In conclusion, personal sampling of bioaerosols on filters with subsequent measurement of total counts of fungal spores and bacteria may be suggested as key parameters in routine monitoring of bioaerosol exposures in the waste collection and recycling industries.

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