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SIZE DISTRIBUTION OF BACTERIAL AND FUNGAL BIOAEROSOLS IN INDOOR AIR

Rafał L. Górny[1], Jacek Dutkiewicz[2], Ewa Krysińska-Traczyk[2]

[1]Department of Indoor Exposure Assessment, Institute of Occupational Medicine and Environmental Health, Sosnowiec, Poland
[2]Department of Occupational Biohazards, Institute of Agricultural Medicine, Lublin, Poland

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Abstract: The aim of this study was to determine the size distribution of bacteria and fungi occurring in the air of human dwellings. The concentration and size distribution of particulate aerosol, Gram-positive mesophilic bacteria, Gram-negative mesophilic bacteria and fungi were examined in 60 flats situated in the Upper Silesia conurbation, southern Poland. The investigated flats comprised three quantitatively equal (20 flats each) groups: flats without additional emission sources of particulate aerosol and microorganisms (Group I), flats with persons who smoke at least one packet of cigarettes per day (Group II), and flats located near steelworks (Group III). The concentrations of four fractions of particulate aerosol were measured by Harvard impactors (PM 2.5 and PM 10) as well as by cyclone HD and 37 mm filter disc holder (PM 5 and TSP). The concentrations of bacteria and fungi were measured by a particle-sizing six-stage Graseby-Andersen impactor. It was found that the concentrations of particulate aerosol in examined flats were below 0.6 mg/m^3 and the concentrations of microorganisms were below the level of 10^4 cfu/m^3. The dominant bacteria present in the air of examined dwellings (*Micrococcus*/*Kocuria* spp., *Staphylococcus* spp., *Bacillus* spp., *Pseudomonadaceae*, *Aeromonas* spp., *Nocardia* spp.) occurred mostly as single particles in the dwellings without additional emission sources, while in the air of dwellings inhabited by tobacco smokers, they often formed aggregates composed of bacterial and dust particles. The fungi dominant in the air of examined dwellings (*Penicillium* spp., *Aspergillus* spp., yeasts) occurred mostly as single particles.

Address for correspondence: Rafał L. Górny, Ph.D., Department of Indoor Exposure Assessment, Institute of Occupational Medicine and Environmental Health, Kościelna 13, 41-200 Sosnowiec, Poland. E-mail: r-gorny@imp.sosnowiec.pl

Key words: indoor air, bacteria, fungi, size distribution, particulate aerosol.

INTRODUCTION

Biological aerosol (or bioaerosol) is defined as an artificially generated or naturally occurring collection (gathering) of biological particles diffused in the air or in another gaseous phase [15, 27]. According to Hirst, the bioaerosol is a kind of aerosol containing small parts of biological origin or biologically active substances [43], which in living organisms can cause reactions such as infections, allergies, toxic reactions and others [12].

The microorganisms can occur in the air:

- as single cells or aggregates of cells, as well as fragments of bacterial cells, spores of bacilli, actinomycetes and fungi, parts of actinomycetal and fungal hyphae, endotoxins, exotoxins, enzymes, glucans, mycotoxins [8, 17, 27];
- as conglomerations (usually in great numbers) with small dust particles as well as with water or saliva droplets as so-called “nuclei droplets” (seldom more than one microbe for particle) [23].

For biological particles, whether naturally occurring or artificially generated, dusts often act as their carriers [8, 16, 17]. In this way, both pathogenic and allergenic microbes are transferred as well as allergens themselves (e.g. in the form of dust from cotton and other plants). Inorganic dusts can serve as an excellent transporting

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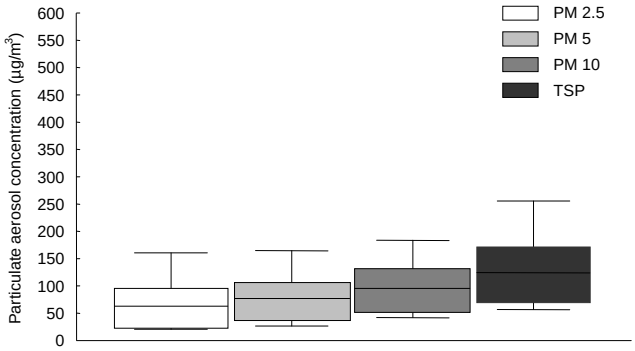
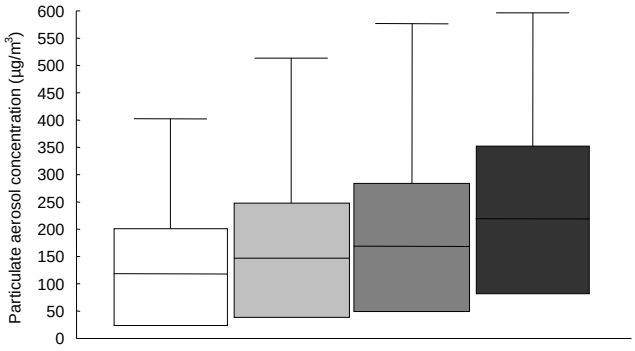
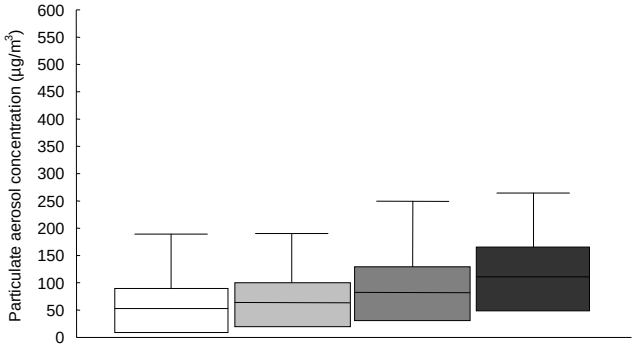


A. Flats without indoor bioaerosol emission sources



B. Flats with tobacco smokers



C. Flats located near steelworks

Figure 1. The concentration (arithmetic mean, standard deviation and range) of particulate aerosol in the examined flats of Upper Silesian conurbation.

medium for organic particles, such as bacterial cells dispersed in the air [17]. The effectiveness of this transfer process depends (despite certain physicochemical factors) on the interdependence of sizes of transporting and transported particles. So far, investigations carried out in the indoor environment have shown that the particles of the small aerodynamic diameters (e.g. particles from tobacco smoke) cannot transport bacteria and fungi which, as a rule, are several times greater than dust particles, but can become a good conveyor for other kinds of bioaerosols, e.g. bacterial endotoxins [21, 42].

The reactivity of aerosols is highly conditioned not only by their potential ability for penetration the human respiratory system but also by biological composition of particles. The exposure to bioaerosols in the indoor environment has significant influence on the intensity of appearance of sick building syndrome symptoms, such as nasal and pharyngeal mucous membrane irritations, skin dryness, itchy eyes, breathlessness, wheezing, headache, concentration problems or fatigue [7, 12, 19, 27, 28, 40, 43, 51].

The aim of this study was to determine the size distribution of bacteria and fungi occurring in the air of human dwellings.

MATERIALS AND METHODS

The investigations were carried out on a group of 60 flats selected at random, situated in the area of 15 towns of the Upper Silesian conurbation, i.e. Sosnowiec, Katowice, Będzin, Bytom, Chorzów, Dąbrowa Górnicza, Gliwice, Jaworzno, Knurów, Mikołów, Mysłowice, Ruda Śląska, Siemianowice Śląskie, Tychy and Zabrze. The total of investigated flats was divided into three quantitatively equal groups (20 flats each): flats without additional indoor emission sources of investigated bioaerosol (Group I - control group), flats with persons who smoke at least one packet of cigarettes per day (Group II) and flats located near steelworks, without tobacco smokers (Group III). The sampling points of the two examined groups of flats (i.e. the control group and the group of flats with tobacco smokers) were not in the vicinity of large (point and/or lineal) sources of aerosol emission. The third group of investigated dwellings embraced flats situated in the vicinity of steelworks (8 flats in the area of Sosnowiec, 5 in the area of Katowice, 2 in the area of Będzin and 1 flat each in the area of Dąbrowa Górnicza, Gliwice, Ruda Śląska, Siemianowice Śląskie and Zabrze), situated 50 m to 700 m from the border of the area occupied by a metallurgical factory.

All measurements were carried out in flats in multi-family buildings whose total surface exceeded 200 m^2. The mean area of investigated apartments was 58.7 m^2 (range 25-94 m^2). The flats were situated on different storeys of buildings, from the ground floor to the 9th storey. Most of the examined flats were located in buildings erected from large panels (72% of the measurements). The remaining part were flats in buildings erected from bricks and/or air bricks (28% of the measurements). All of the investigated flats were equipped with a central heating system and were ventilated in a natural manner, without the use of any additional ventilating or air-conditioning devices. Only "healthy" flats were selected for the measurements, i.e. the flats in which occupants did not complain of indisposition provoked by the sanitary status of their flats and in which no apparent sources of investigated aerosols (e.g. fungi on the wall, etc.) were discovered.

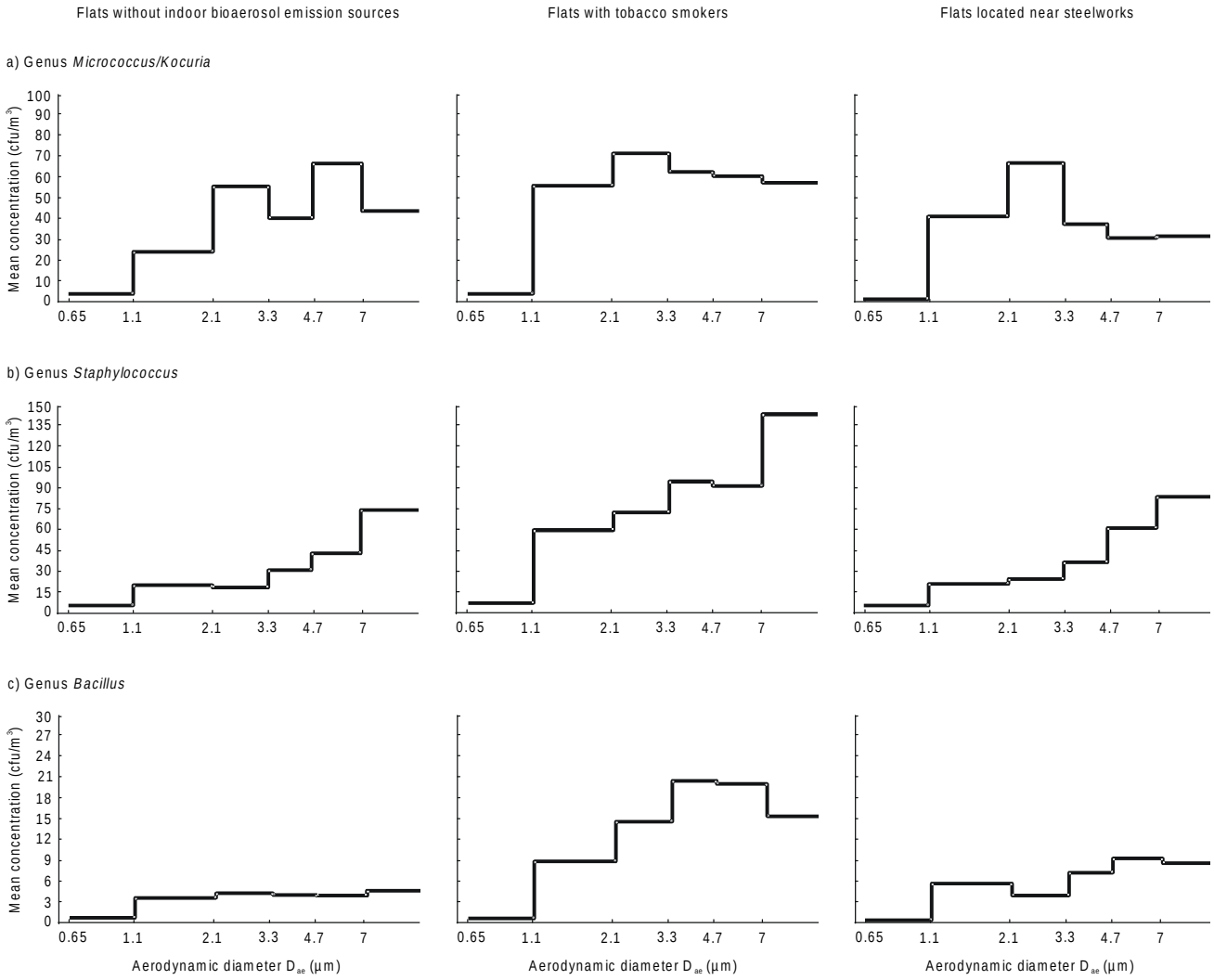


Figure 2. Size distribution of the most common Gram-positive bacteria in air of the three groups of investigated flats.

In each apartment, the data about numbers of inhabitants (including number of smokers in flats of Group II), pets and ornamental plants were also gathered. For the three groups of flats these data, i.e. median (range) for numbers of inhabitants, pets and plants respectively, were as follows: Group I – 3 (1-6), 0.5 (0-1) and 2.5 (1-9), Group II – 3 (1-4) (among them smokers 1 (1-2)), 0 (0-1) and 2.5 (0-7), Group III – 3 (1-6), 0 (0-2) and 3 (0-6).

In all of the 60 flats, the concentrations of particulate aerosol as well as of bacterial and fungal bioaerosols were examined. The particulate aerosol as well as bacterial and fungal bioaerosols were investigated in the rooms where inhabitants spent most of their time ("living rooms") at the height of 1.4 m above floor level to simulate the human breathing zone [11, 20, 31]. The measurements of particulate aerosol concentrations embraced four fractions: particles with aerodynamic diameter (D_{ae}) up to 2.5 µm (PM 2.5), with D_{ae} up to 5 µm (PM 5), with D_{ae} up to 10 µm (PM 10) and total suspended particles (TSP). For the concentration assessment of these four dust fractions, two different samplers were used, i.e.: for evaluation of PM 2.5 and PM 10 Harvard impactors with a pump (Air Diagnostic and Engineering Inc., Naples, USA) [20, 37]; for measurements of PM 5 and TSP cyclone HD [3, 20] and filter disc holder for 37 mm disc, respectively, both equipped with their own AFC 123 pumps (Casella Ltd., London, UK). In all cases, teflon filters type SA240PR100 (Andersen Instruments Inc., Smyrna, USA) were used. The flow rates were as follows: 10 lpm for Harvard impactors, 1.9 lpm for cyclone HD and 2.0 lpm for filter disc holder for 37 mm disc. In the particulate aerosol investigations, the sampling time was selected so that the volume of filtered air amounted to 3 m^3.

The measurements of bioaerosols concentrations were carried out simultaneously with measurements of particulate aerosol using the six-stage Graseby-Andersen impactor with a pump (Graseby-Andersen, Atlanta, USA) [20, 24]. The use of this instrument made it possible to

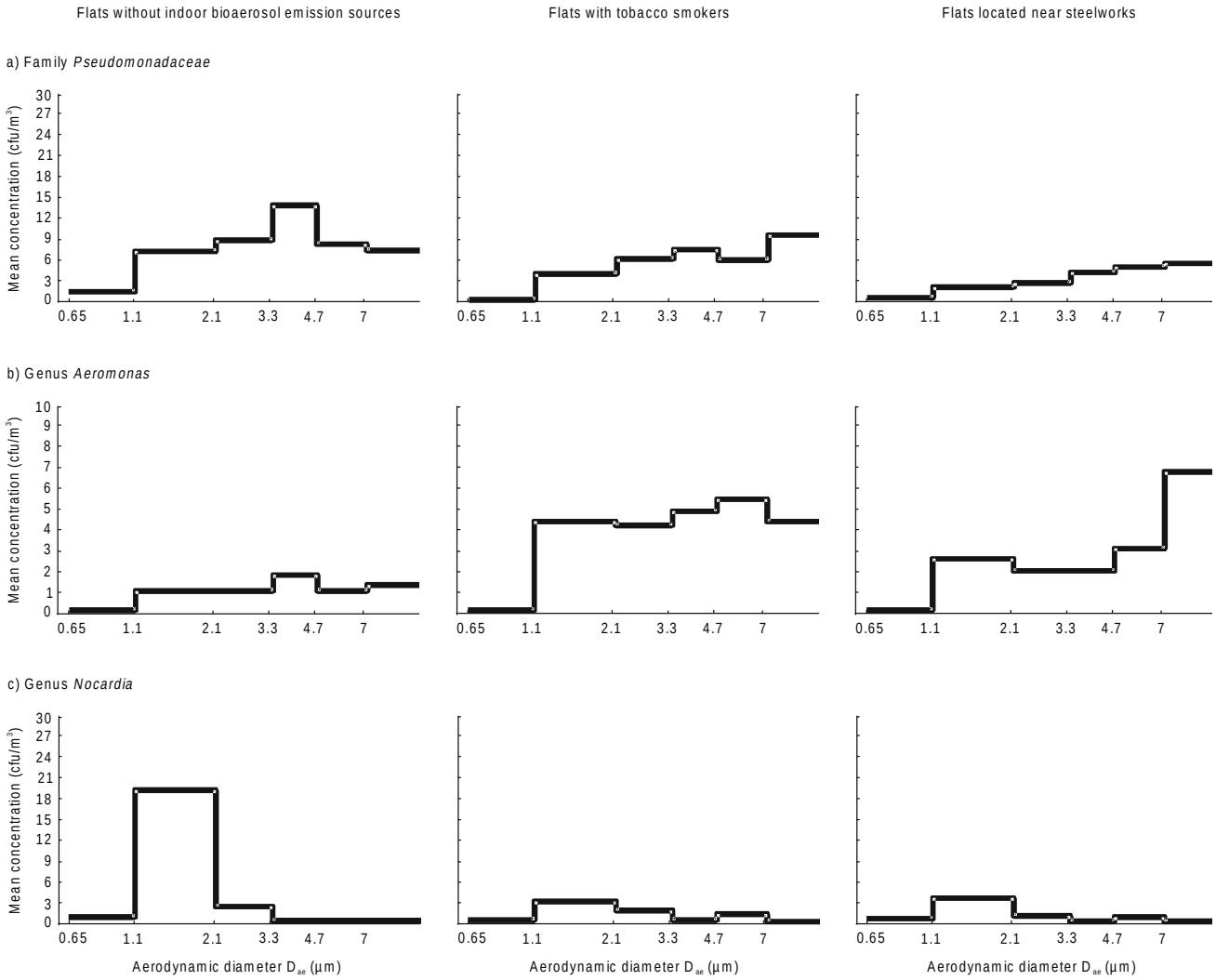


Figure 3. Size distribution of the most common Gram-negative bacteria and actinomycetes in air of the three groups of investigated flats.

divide bioaerosol particles into six fractions according to their aerodynamic diameters: above 7.0 µm, 7.0-4.7 µm, 4.7-3.3 µm, 3.3-2.1 µm, 2.1-1.1 µm and 1.1-0.65 µm [1]. The flow rate was 28.3 lpm and sampling time 20 minutes. The use of three kinds of media, i.e.: blood TSA (tryptic soya) agar, eosin methylene blue (EMB) agar and malt extract agar (MEA) made it possible to determine the concentrations and to identify the most common microorganisms in the groups of Gram-positive mesophilic bacteria, Gram-negative mesophilic bacteria and fungi, both moulds and yeasts.

Because these studies were conducted during a 20 month period, during the four seasons, simultaneously with aerosol investigations, the measurements of relative humidity and temperature of the air were carried out.

The obtained results were subjected to statistical analysis using Mann-Whitney test and Spearman correlation test (with the aid of software package: STATISTICA for Windows, release 4.5, StatSoft©, Inc. 1993).

RESULTS AND DISCUSSION

The physical analysis of particulate aerosol in the three examined groups of flats revealed the differences between them in concentrations of PM 2.5, PM 5, PM 10 fractions and TSP. The arithmetic means, standard deviations and ranges are presented in Figure 1. The data showed that all the measured concentrations of particulate aerosol in examined flats were below 600 µg/m^3 (0.6 mg/m^3). The obtained values were of medium height, compared to those reported by other authors from indoor air. They approximated the concentrations of airborne dust observed in many regions of the USA [9, 10, 25, 29, 35, 47] and south-east Asia [31, 32, 39], were slightly higher than in Western-European countries [4, 6, 41], but significantly lower than in some locations in Mexico, India and China [2, 5, 46].

The mean concentrations for all of the investigated dust fractions in the flats inhabited by tobacco smokers were

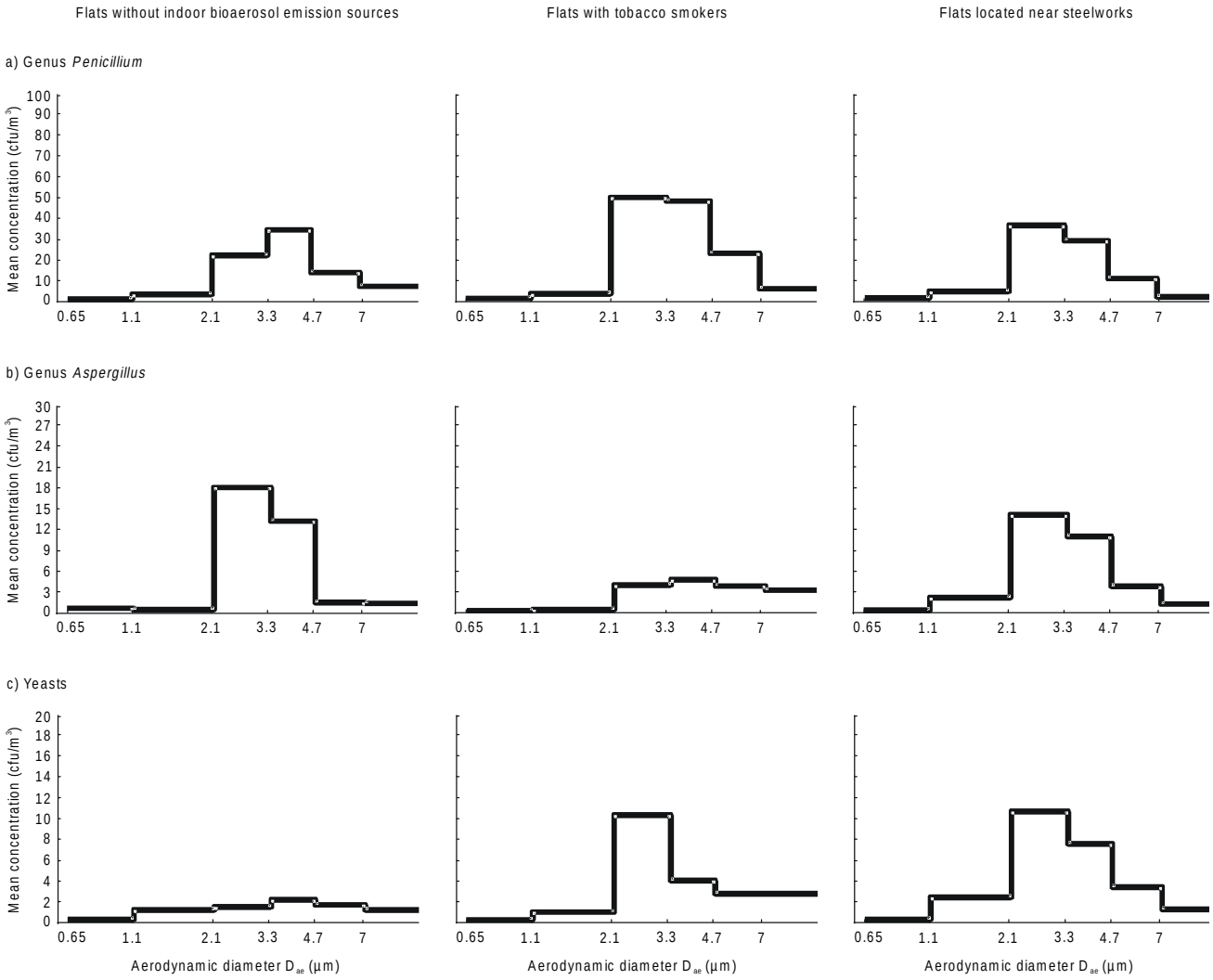


Figure 4. Size distribution of the most common fungi in air of the three groups of investigated flats.

1.7-2.5 times higher than in the flats without additional indoor emission sources and in the flats located near steelworks. Besides, it can be concluded that the fine particles, i.e. particles with aerodynamic diameter D_{ae} below 2.5 µm, formed from 41% (in the flats near steelworks) to 51% (in the flats with tobacco smokers) of the total suspended particles (TSP). In the air of dwellings inhabited by tobacco smokers, the percentage contribution of all investigated fractions (PM 2.5, PM 5 and PM 10) in relation to TSP was higher than in the two remaining groups of flats.

Statistical analysis by Mann-Whitney test confirmed the significant differences between the investigated groups of flats in relation to all of the examined particulate aerosol fractions. The greatest differences were observed between the flats with tobacco smokers (Group II) and flats located near steelworks (Group III) where the values of probabilities p were lower than 0.000001 for all fractions, i.e. PM 2.5, PM 5, PM 10 and TSP. For the other relationships, i.e. for Group II/Group I and for Group I/Group III, the values of p were 0.05–0.01 and 0.05–0.001, respectively.

The following microorganisms were most often present in the indoor air:

- in the group of Gram-positive mesophilic bacteria: *Micrococcus*/*Kocuria* spp. and *Staphylococcus* spp. occurred in 100% of examined flats, *Bacillus* spp. in 90%, and *Nocardia* spp. in 33%;
- in the group of Gram-negative mesophilic bacteria: species of family *Pseudomonadaceae* occurred in 80% of examined flats, and *Aeromonas* spp. in 40%;
- in the group of fungi: *Penicillium* spp. occurred in 97% of investigated dwellings, *Aspergillus* spp. in 62%, and yeasts in 52%.

The species composition of bacterial and fungal bioaerosol found in the air of Upper Silesia flats was similar to that reported by other authors from European and American dwellings [13, 14, 18, 22, 30, 34, 36, 38, 44, 45]. The

Table 1. Concentrations (arithmetic mean, median, standard deviation and range) of the most common microorganisms in the air of examined flats of Upper Silesian conurbation, expressed as cfu/m^3.

Group I	Mean	Median	Min.	Max.	SD
Micrococcus spp.	228.70	110.5	19	1187	305.96
Staphylococcus spp.	197.95	166.0	41	569	144.86
Bacillus spp.	19.65	12.0	0	113	26.64
Pseudomonadaceae	34.55	18.5	0	163	48.97
Aeromonas spp.	2.15	0.0	0	12	3.66
Nocardia spp.	8.50	0.0	0	118	26.27
Penicillium spp.	92.10	19.0	2	746	170.19
Aspergillus spp.	18.00	3.0	0	272	60.20
Yeasts	2.45	0.0	0	12	4.24
Group II					
Micrococcus spp.	306.70	132.5	12	2039	444.36
Staphylococcus spp.	462.95	269.5	42	1686	461.45
Bacillus spp.	71.35	10.5	0	392	131.82
Pseudomonadaceae	26.10	7.0	0	87	29.97
Aeromonas spp.	9.95	0.0	0	124	27.79
Nocardia spp.	1.45	0.0	0	21	4.72
Penicillium spp.	116.05	23.0	0	896	264.76
Aspergillus spp.	9.45	3.0	0	76	18.63
Yeasts	12.90	2.0	0	164	36.47
Group III					
Micrococcus spp.	212.60	127.5	21	1314	288.95
Staphylococcus spp.	222.35	207.5	69	627	125.30
Bacillus spp.	35.00	11.5	0	148	41.68
Pseudomonadaceae	16.50	9.0	0	71	21.42
Aeromonas spp.	7.45	0.0	0	53	14.72
Nocardia spp.	2.90	0.0	0	14	4.27
Penicillium spp.	80.90	32.0	2	599	142.97
Aspergillus spp.	22.80	2.0	0	357	79.07
Yeasts	15.70	3.0	0	120	33.92

singularity of investigated apartments was high frequency of the occurrence of *Aeromonas* bacteria. These rod-shaped Gram-negative bacteria are associated with outdoor environments (water, sewage) and so far have not been reported as common in the indoor air. They may produce protein enterotoxin and are suspected to cause gastroenteritis in the workers of a sewage treatment plant exposed to the inhalation of large quantities of *Aeromonas* [49].

Besides, the surprisingly high occurrence of yeasts in the air of Silesian dwellings was comparable with the levels characteristic of subtropical climate apartments [26, 33]. Recent investigations have shown that yeasts may induce inflammatory reactions in lungs by non-specific, immunotoxic stimulation of alveolar macrophages [50]. The other fungi dominant in the air of examined dwellings, e.g. *Aspergillus* and *Penicillium* species, are widely known as causative agents of allergic and immunotoxic diseases [12, 17, 27, 43].

The concentrations (arithmetic mean, median, standard deviation and range) of the most common microorganisms in the air of the examined flats of Upper Silesian conurbation are presented in Table 1. The concentrations of all microbial genera were below the level of 10^4 cfu/m^3. The species of genera: *Micrococcus*/*Kocuria*, *Staphylococcus*, *Bacillus*, *Aeromonas* and *Penicillium* attained the highest mean concentrations in the flats inhabited by tobacco smokers, the species of family *Pseudomonadaceae* and genus *Nocardia* reached their maximum levels in the flats without emission sources of investigated aerosols, and the species of fungi, i.e. genus *Aspergillus* and yeasts, attained highest level in the air of dwellings near steelworks. However, the statistical analysis confirmed only two significant differences, i.e. for *Staphylococcus* spp. between Group II and Group I ($p < 0.05$) and for *Nocardia* spp. between Group II and Group III ($p < 0.01$).

The analyses of the size distribution of the nine most common microorganisms in the air of the examined dwellings (Figs. 2-4) showed that:

- for the species from genera *Micrococcus*/*Kocuria* (Fig. 2a): in the flats of Group I these bacteria were observed as naturally dispersed particles or as bacterial cells aggregates. This may be seen as two distinct peaks corresponding to the size range of single cells (D_{ae} from 2.1-3.3 µm) or clusters of several cells (D_{ae} from 4.7-7 µm). In the flats of Group II these microbes appeared in the form of aggregates of bacterial and dust particles. This may be seen as the uniformly high concentration of the particles in fractions above 1.1 µm ($D_{ae} > 1.1$ µm). In the flats near steelworks, the size distribution was similar to that for Group I, but the aggregates consisting only of bacterial cells rather did not appear;
- for the species from genus *Staphylococcus* (Fig. 2b): in all kinds of flats staphylococci were present as the aggregates. While in the indoor air of the control group and Group III they appeared almost solely as aggregates of biological particles, in the flats with tobacco smokers they posed an additional, very distinct pool of aggregates consisted of bacterial and dust particles;
- for the species from genus *Bacillus* (Fig. 2c): in indoor environment without additional emission of aerosols (Group I), these bacteria occurred as separate single cells. If such emission took place, these bacteria commonly created conglomerations with dust particles (see size distribution for the flats from Groups II and III);
- for the species from family *Pseudomonadaceae* (Fig. 3a): in the group of flats without emission sources, the bacteria belonging to this family were mainly present as single cells. In the flats of tobacco smokers, these Gram-negative mesophilic bacteria occurred simultaneously as

separate cells and as aggregates of bacterial and dust particles. In the air of the third group of dwellings, these rod-shaped bacteria created, as a rule, aggregates with particulate aerosol, but they were less frequent than in the flats of Group II;

- for the species from genus *Aeromonas* (Fig. 3b): in the indoor air of Group I these rods occurred singly or as biological cells aggregates. In the flats of Group II there occurred characteristic aggregates of bacterial and dust particles. A similar situation was observed in the Group III, but in this case the numbers of pure biological as well as biological and dust aggregates seem to be just as large;
- for the species from genus *Nocardia* (Fig. 3c): irrespective of the kind of flats, these representatives of actinomycetes appeared in the air mainly as single cells. If the additional emission of particulate aerosol occurred, a small number of biological-dust aggregates was formed;
- for the species from genus *Penicillium* (Fig. 4a): in all kinds of dwellings, these moulds occurred mostly as naturally dispersed spores or hyphae. The rare conglomerations of fungal and dust particles were found only in the flats of tobacco smokers;
- for the species from genus *Aspergillus* (Fig. 4b): in the flats from Groups I and III, these fungi occurred as naturally dispersed particles and as aggregates of biological cells. In contrast to other microorganisms, in the flats of Group II the biological aggregates were less common;
- for the yeasts species (Fig. 4c): in the air of Group I flats the yeasts appeared as single cells. In the air of dwellings belonging to Groups II and III, these micro-organisms created the connections with dust particles much more readily, especially in the flats near steelworks.

In the scientific literature there are only a few data about size distribution of particular genera of microorganisms in indoor air. The trends of size distribution of fungal particles in Upper Silesian flats conform to those reported by Reponen *et al.* [48]. By contrast, the analysis of size distribution of bacterial particles made by Nevalainen [38] showed trends that differed from the findings presented in this work. The fact that the allergenic fungi of the genera *Penicillium* spp. and *Aspergillus* spp. occurred in the air of examined dwellings mostly as single, readily respirable particles, indicates a possibility of fungal respiratory allergy in the domestic environment. In the Upper Silesian dwellings this risk was decreased by a low concentrations of fungi in the air which, on average was 10^2 cfu/m^3 and rarely reached 10^3 cfu/m^3.

The correlation analysis between particulate aerosol concentrations and the concentrations of particular groups of examined microorganisms showed only two negative but statistically significant ($p < 0.05$) relationships, both between *Penicillium* levels and PM 2.5 and PM 5 concentrations. However, though in the present study the statistically significant positive correlations between particulate and biological aerosols were not found, the size distribution analyses revealed that almost all genera of investigated microorganisms were able to form aggregates with dust particles. Such conglomerations of particles can provoke noxious respiratory effects as a result of synergistic action of both viable and inorganic components [17].

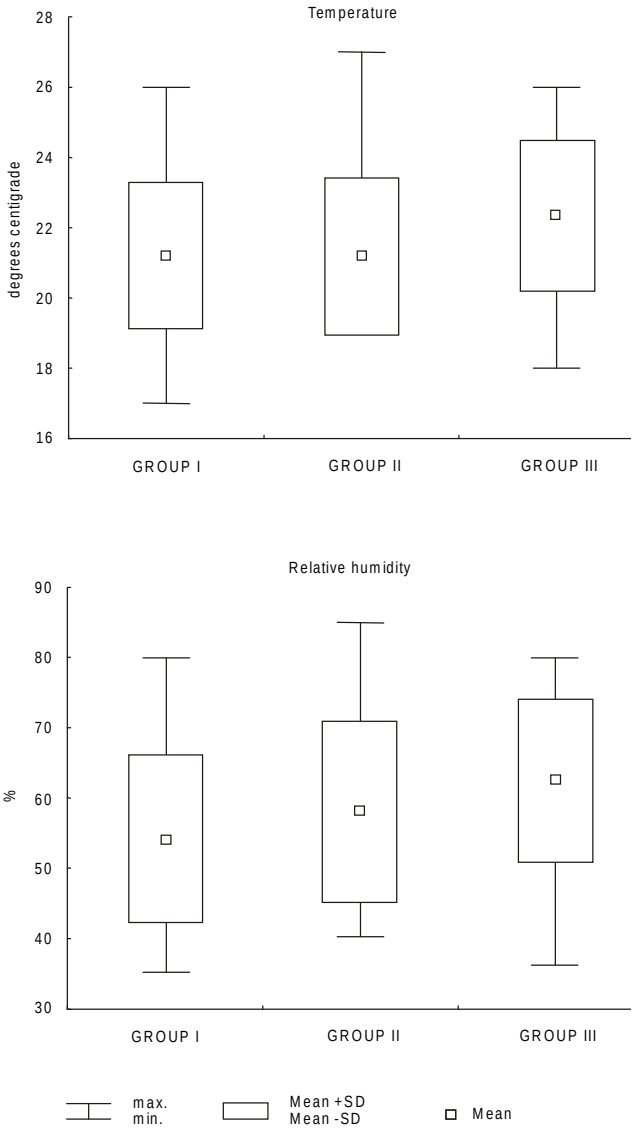


Figure 5. Values (arithmetic mean, standard deviation and range) of temperature and relative humidity of air in the examined flats of Upper Silesian conurbation.

The values (arithmetic mean, standard deviation and range) of temperature and relative humidity of the air in the examined flats of Upper Silesia conurbation are shown in Figure 5. The relative humidity had statistically significant ($p < 0.05$) negative influence on concentration of PM 5 fraction in the air of Group II and Group III flats and positive influence on the level of yeasts in the air of flats without emission of measured aerosols (Group I) and flats located near steelworks (Group III). The temperature had statistically significant ($p < 0.05$) negative influence

on the concentrations of yeasts in the indoor air of Group I. Although the differences between temperature and humidity in the examined groups of flats were statistically significant (Group III/Group I: $p < 0.05$ for temperature and $p < 0.01$ for humidity; Group III/Group II: $p < 0.001$ for temperature and $p < 0.05$ for humidity), their influence on the quantity of the examined kinds of microorganisms seems to be unimportant.

In relation to remaining parameters, e.g. area of flats, numbers of inhabitants, pets and ornamental plants, the investigated dwellings formed a statistically homogenous group. The values of these parameters had no statistically significant influence on size distribution of analysed microbial genera.

CONCLUSIONS

The dominant bacteria present in the air of dwellings of the Upper Silesian conurbation (*Micrococcus*/*Kocuria* spp., *Staphylococcus* spp., *Bacillus* spp., *Pseudomonadaceae*, *Aeromonas* spp., *Nocardia* spp.) occurred mostly as single particles in the dwellings without additional emission sources, while in the air of dwellings inhabited by tobacco smokers they often formed aggregates composed of bacterial and dust particles.

The fungi dominant in the above described dwellings (*Penicillium* spp., *Aspergillus* spp., yeasts) occurred mostly as single particles.

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