REVIEW ARTICLES AAEM

Ann Agric Environ Med 2002, 9, 17–23

BACTERIAL AND FUNGAL AEROSOLS IN INDOOR ENVIRONMENT IN CENTRAL AND EASTERN EUROPEAN COUNTRIES*

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Górny RL, Dutkiewicz J: Bacterial and fungal aerosols in indoor environment in Central and Eastern European countries. *Ann Agric Environ Med* 2002, **9**, 17–23.

Abstract: Studies of indoor bioaerosols conducted in Central and Eastern European countries, as a result of the scarcity of funding, mostly do not attain the level presented by similar studies in Northern America and Western Europe. For socio-economic reasons, most of the intense studies on indoor bioaerosols in Central and Eastern European countries were carried out in industrial facilities and have contributed significantly to occupational health science. In contrast, until recently, insufficient of studies have been conducted on bioaerosols of residential and communal premises (dwellings, offices, schools, etc.) and no network for monitoring the microbiological quality of air in such premises exists. In Poland, in the mid-1990s complex bioaerosol investigations were carried out by the Bioaerosol Group at the Institute of Occupational Medicine and Environmental Health in Sosnowiec. The concentrations of airborne bacteria and fungi in dwellings without mold problems were between 88-4297 cfu/m^3 and 0-1997 cfu/m^3, while in moldy homes they were 178–4751 cfu/m^3 and 49-16968 cfu/m^3, respectively. As many as 167 microbial species were isolated from the air of examined dwellings. Most frequently occurred Gram-positive cocci (*Micrococcus*/*Kocuria* spp., *Staphylococcus* spp.), endospore-forming bacilli (*Bacillus* spp.), Gram-negative bacteria (*Pseudomonadaceae*, *Aeromonas* spp.), filamentous fungi (*Penicillium* spp., *Aspergillus* spp.), and yeasts. Notable studies of indoor bioaerosols have also been performed in the other Central and Eastern European countries: Lithuania, Latvia, Estonia, Russian Federation, Ukraine, Czech Republic, Slovakia, Bulgaria and Hungary, are reviewed in this article. The lack of reference limit values for bioaerosols seriously hinders interpretation of results obtained in various countries. The following residential limit values (RLV) for dwellings and communal premises are proposed for the concentration of airborne bacteria, fungi and bacterial endotoxin: 5×10^3 cfu/m^3, 5×10^3 cfu/m^3 and 5 ng/m^3 (50 EU), respectively. The proposed values of occupational exposure limit (OEL) for industrial settings contaminated by organic dust are 100×10^3 cfu/m^3, 50×10^3 cfu/m^3 and 200 ng/m^3 (2000 EU), respectively. It is also proposed that the presence in indoor air of microorganisms from risk groups 3 and 4 of European Community Directive 2000/54/EC (e.g., *Mycobacterium tuberculosis*, *Bacillus anthracis*, *Coxiella burnetii*), independently of the concentration, should always be inadmissible and result in preventive actions.

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Key words: bioaerosols, indoor air, bacteria, fungi, Central and Eastern Europe, exposure limits.

INDOOR BIOAEROSOLS - CURRENT PROBLEMS

The last decade has been characterized by a significant increase of the worldwide scientific database on bioaerosols in indoor environment. Development of new sampling techniques and analytical methods, as well as advances in human exposure determination, have been allowed more precise identification of the sources of microbial contamination, evaluation of the quality of indoor air and the assessment of potential hazards.

In spite of this tremendous scientific progress, which has been taken place mainly in developed countries

Received: 10 May 2002
*Report presented during the Joint WHO-NIOSH-USEPA Expert Meeting on WHO Guidelines for Biological Agents in the Indoor Environment, Berlin, Germany, 22-25 April 2002.

(Western Europe and USA), the state of knowledge about biologically originated indoor air pollution in Central and Eastern European countries seems to be still relatively narrow and insufficient. The reasons for these limited resources should be perceived mainly in:

- lack of modern sampling instrumentation,
- common use of old methods (e.g., sedimentation) to evaluate microbiological quality of the air,
- relatively high costs of instrumental analyses (e.g., for bacterial and fungal toxins and their markers – 3-OH fatty acids and ergosterol),
- lack of commonly approved criteria for assessing exposure to biological factors,
- very low number of institutions/organizations interested in (or obligated to perform) comprehensive environmental monitoring of bioaerosols.

To date in Central and Eastern European countries, there is no existing network of sanitary-epidemiological stations responsible for microbiological indoor air measurements/control. Indoor bioaerosol measurements are carried out only as scientific projects and hence their frequency is low. The expansion of this type of measurement is still limited by a relatively small technical staff. If bioaerosols appear at the center of interest, it is usually related to the occupational matters and/or to cases of health complaint. Such a passive attitude towards bioaerosols is increased by a lack of internationally recognized criteria for assessing exposure to biological factors.

In Poland, as in many Central and Eastern European countries, the presence of elevated levels of bioaerosols is still a hidden problem. Limited studies performed on indoor air indicated that microbiological quality of the air could be a potential causative agent of health complaints of inhabitants. Currently, such cases usually attract public attention when they appear as relatively numerous (mold problems in old buildings) or after big environmental disasters (such as the 1997 flood in Poland) [32].

For socio-economic reasons, most of the intense studies on indoor bioaerosols in Central and Eastern European countries were carried out in industrial facilities [1, 2, 4, 6, 8, 19, 23], some even in environments scarcely studied in Western countries (herb industry, fur and wool processing, fiberboard and chipboard factories) [9, 10, 13, 22, 26]. These studies have made a significant contribution to occupational health science.

Below, is presented a short review of recent research on indoor bioaerosols conducted in Poland, largely with the participation of the authors, and in some other Central and Eastern European countries. The authors apologize that because of the limited volume of this paper could not present many other valuable papers on indoor bioaerosols that have been performed in this region of the world.

BIOAEROSOL MEASUREMENTS IN POLAND

In the mid-1990s, the first modern complex bioaerosol investigations of dwellings were initiated in Poland. The Bioaerosol Group at the Institute of Occupational Medicine and Environmental Health, Sosnowiec, in co-operation with the Department of Occupational Biohazards at the Institute of Agricultural Medicine, Lublin, as a first research unit initiated comprehensive measurements in dwellings. The investigations were carried out on a group of more than 100 flats located in 15 towns of the Upper Silesia conurbation [14, 15, 16, 24, 25, 27]. Upper Silesia is the highly industrialized, urbanized, and heavily polluted region of southern Poland (2.7 thousand km^2) inhabited by ca. 4 million people (10% of the population of Poland). The measurements were carried out in flats in multi-family buildings, homes, and offices, with and without mold problems. Most of the examined dwellings were in buildings erected from large panels (72% of the measurements). The remainder were flats of bricks and/or air bricks buildings. All the investigated premises were equipped with a water-based central heating system and ventilated in naturally, without the use of any ventilating or air-conditioning devices. The bacterial and fungal residential bioaerosols were investigated in rooms where the inhabitants spent most of their time. Sampling was performed at the height of 1.4 m above floor level to simulate the human breathing zone. Measurements of bioaerosols concentrations were carried out using the six-stage Graseby-Andersen impactor.

The microbial taxa (167 different species) isolated from indoor air and their percentage in the total examined microflora is presented in Table 1. A short summary of the findings is presented below:

- 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% of flats;
- in the group of Gram-negative mesophilic bacteria: species of the 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 singularity of investigated residential apartments was the high frequency of the occurrence of *Aeromonas* bacteria. These rod-shaped Gram-negative bacteria are associated with outdoor environments (water, sewage) and have not yet been reported as common in indoor air. Besides, the surprisingly high occurrence of yeasts in the air of dwellings was comparable with levels characteristic of subtropical climate apartments.

The concentrations (mean, median, standard deviation, and range) of different groups of microorganisms in the air of the examined dwellings, offices, and moldy homes of the Upper Silesian conurbation are presented in Table 2. All mean concentrations for healthy (i.e. no health complains) dwellings and offices were below the 10^4 cfu/m^3 level and may be treated as normal values for these types of premises. Average bacterial and fungal concentrations in moldy homes always exceeded their levels recorded in the healthy dwellings.

Endotoxin concentration in the air of Upper Silesia conurbation dwellings is presented in Table 3. The mean values measured using gel-clot and kinetic chromogenic methods were below 1 ng/m^3. Only in one sample out of 40 examined, was observed a concentration above 5 ng/m^3.

In contrast to dwellings, Dutkiewicz *et al.* [6, 7, 8, 9, 10] found in Polish agricultural and wood industry settings high concentrations of airborne microorganisms and bacterial endotoxin, in many cases exceeding the levels of 10^5 cfu/m^3 and 100 ng/m^3, respectively.

BIOAEROSOL MEASUREMENTS IN OTHER CENTRAL AND EASTERN EUROPEAN COUNTRIES

The situation in other Central and Eastern European countries, in principle, seems to resemble the Polish example. The available indoor bioaerosol measurement data are usually related to the occupational environment. Very little is known about the microflora of dwellings. Below is the short summary of indoor bioaerosol data available from scientific literature.

Lithuania. Krikštaponis presented very comprehensive studies on fungal species in residential and occupational environments [22]. Airborne fungi were collected using a slit-to-agar single stage Krotov 818 impactor. The investigated premises included 14 dwellings, as well as individual rooms in occupational premises. In 86%, the investigated dwellings were recognized as having a mold problem (visible mold growth). Maximal fungal concentration exceeded 10^4 cfu/m^3, whereas the mean concentration in reference dwellings (without mold growth) did not reach 200 cfu/m^3. Fungi dominating in dwellings belonged to the following genera: *Penicillium*, *Aspergillus*, *Cladosporium*, *Alternaria* (all present in 100% of dwellings), *Mucor* (93%), *Rhizopus* (86%), *Ulocladium* (79%), *Mortierella* (71%), *Aureobasidium* (71%), *Oidiodendron* (57%), *Geotrichum* (57%), and *Trichoderma* (36%).

Concentrations of fungi (and their dominant genera) in the examined occupational environments were as follows: hospital rooms 26-78 cfu/m^3 (*Penicillium* and *Aspergillus*), sanatorium rooms 156-720 cfu/m^3 (*Penicillium*, *Cladosporium*, *Chrysosporium*, and *Aspergillus*), medicine packing company 80-9040 cfu/m^3 (*Penicillium*, *Aspergillus*, and *Alternaria*), dairy 600-15169 cfu/m^3 (*Penicillium* and *Geotrichum*), shoe-making company 47-293 cfu/m^3 (*Aspergillus* and *Penicillium*), paper producing company 240-360 cfu/m^3 (*Penicillium*, *Cladosporium*, *Aspergillus*, *Mortierella*, *Aureobasidium*, *Botrytis*), buffet and café 921-7735 cfu/m^3 (*Penicillium*, *Aspergillus*, and *Cladosporium*), library 28-4100 cfu/m^3 (*Aspergillus*, *Penicillium*, *Cladosporium*, *Mortierella*, *Trichoderma*, *Geotrichum*, *Botrytis*, and *Paecilomyces*) [22].

It was ascertained that enzymatic (proteolytic, lipolytic, cellulolytic) activity was characteristic of the majority of isolated fungal strains. The production of fungal toxins (*Aspergillus flavus* aflatoxins, *Penicillium cyclopium* and *Penicillium notatum* penicillic acid, and *Penicillium islandicum* emodin) was also demonstrated. A correlation was confirmed between a fungal species diversity, high concentration of particular aerosols, high relative humidity and temperature [22].

Lugauskas *et al.* [26] performed in Vilnius a study on concentration and species composition of airborne fungi in a fur processing facility. In 7 examined premises, fungal concentrations ranged within 2.5×10^2 - 3.3×10^3 cfu/m^3. The highest degree of pollution (2.1 - 3.3×10^3 cfu/m^3) was found in cutting-assembling and round-rolling shops where active technological processes were performed. As many as 123 fungal species, ascribed to 41 genera, were isolated and identified in the examined facility, including numerous allergenic, toxin-producing and infectious species. Dominating genera were as follows: *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Mucor*, *Trichophyton*, and *Microsporum*.

Latvia. Erman *et al.* [12] found large concentrations of microorganisms (of the order 10^4-10^6 cfu/m^3) in the air of animal farms housing cows, pigs and poultry. The authors stated that the animal farm workers steadily exposed to microbial concentrations above 5.0×10^4 cfu/m^3 showed a significant increase in work-related respiratory and skin diseases, and in allergic reactions.

Estonia. Indermitte [20] reported an average level of microorganisms in 4 office buildings (total 45 rooms) with natural and mechanical ventilation systems. The bioaerosols were sampled using FH3 impactor during the heating season (October–April). The average level for airborne bacteria was 384 cfu/m^3, and for airborne fungi 165 cfu/m^3, both of which were interpreted as "low values". Eleven different fungal species were identified. In some samples, actinomycetes were detected but their content remained at "low level". Rooms with reported previous moisture damages did not show any elevated concentration of microorganisms.

Russian Federation. Petushkova and Kandyba [28] studied airborne microflora of the Moscow Kremlin Cathedrals. Gravitational (sedimentation) methodology was used in this study. Concentration of bacterial flora ranged from 0.13–0.43 cfu/cm^2 for the central part of the cathedral and from 0.38–1.28 viable cfu/cm^2 for the crypt. The highest number of bacteria was found near the air-conditioning system. Among the isolated bacteria, *Micrococcus* spp. and *Rhodococcus* spp. were predominant, but species from *Pseudomonas*, *Xanthomonas*, *Alcaligenes*, *Arthrobacter*, *Flavobacterium*, *Corynebacterium*, *Cellulomonas*, *Bacillus*, *Streptomyces*, *Spirillum*, *Cytophaga*, and myxo-bacteria genera were also isolated. Fungi concentration ranged from 0.04–0.25 cfu/cm^2 in the central part of building and from 0.2–0.67 cfu/cm^2 in the crypt. The species from genera *Acremonium*, *Penicillium*, *Chrysosporium*, *Verticillium*, *Aspergillus*, *Gilmaniella*, *Geotrichum*, *Cladosporium*, and yeasts were the most frequently isolated.

Table 1. Microbial taxa isolated from the air of Upper Silesia conurbation dwellings, Poland [14, 16, 17]. Percentage presence of identified species in the total number of examined flats is shown in parentheses.

I.	Gram-positive mesophilic bacteria
I.1	Gram-positive cocci
I.1.1	Species of genus *Micrococcus (Kocuria)*: *M. kristinae* (13.3), *M. varians/roseus* (45.0), *M.* spp. (100.0);
I.1.2	Species of genus *Staphylococcus*: *S. aureus* (30.0), *S. capitis* (5.0), *S. chromogenes* (syn. *S. hyicus* ssp. *chromogenes*) (5.0), *S. cohnii* (28.3), *S. epidermidis* (83.3), *S. haemolyticus* (26.7), *S. hominis* (20.0), *S. intermedius* (syn. *S. aureus* biotyp E) (3.3), *S. lentus* (syn. *S. sciuri* ssp. *lentus*) (28.3), *S. lugdunensis* (33.3), *S. saprophyticus* (61.7), *S. schleiferi* (1.7), *S. sciuri* (syn. *S. sciuri* ssp. *sciuri*) (25.0), *S. simulans* (3.3), *S. warneri* (6.7), *S. xylosus* (36.7), *S.* spp.(18.3);
I.1.3	Species of genus *Streptococcus*: *S. adjacens* (syn. *Abiotrophia adiacens*) (1.7), *S. equinus* (1.7), *S. mitis* (syn. *S. mitior*) (1.7), *S.* spp. (5.0);
I.1.4	Species of genus *Enterococcus*: *E. durans* (syn. *Streptococcus durans, Streptococcus faecium* ssp. *durans*) (3.3), *E.* spp. (1.7);
I.1.5	Species of genus *Aerococcus*: *A. viridans* (20.0), *A.* spp. (3.3);
I.2	Endospore-forming Gram-positive rods
I.2.1	Species of genus *Bacillus*: *B. alvei* (1.7), *B. amyloliquefaciens* (1.7), *B. brevis* (31.7), *B. cereus* (5.0), *B. circulans* (18.3), *B. firmus* (5.0), *B. lentus* (8.3), *B. megaterium* (13.3), *B. pumilus* (13.3), *B. sphaericus* (21.7), *B. stearothermophilus* (1.7), *B. subtilis* (1.7), *B.* spp. (53.3);
I.3	Irregular, non-sporing Gram-positive rods
I.3.1	Species of genus *Arthrobacter* spp. (5.0);
I.3.2	Species of genus *Corynebacterium* spp. (1.7);
I.4	Mycobacteria
I.4.1	Species of genus *Mycobacterium* spp. (3.3);
I.5	Aerobic actinomycetes
I.5.1	Species of genus *Nocardia* spp. (31.7);
I.5.2	Species of genus *Rhodococcus* spp. (6.7);
I.5.3	Species of genus *Streptomyces* spp. (10.0);
II.	Gram-negative mesophilic bacteria
II.1	Gram-negative aerobic rods
II.1.1	Species of genus *Pseudomonas*: *P. aeruginosa* (6.7), *P. alcaligenes* (8.3), *P. chlororaphis* (syn. *P. lemonnieri, P. fluorescens*) (3.3), *P. diminuta* (syn. *Brevundimonas diminuta*, CDC gr. Ia) (1.7), *P. fluorescens* (13.3), *P. putida* (syn. *P. ovalis*) (15.0), *P. stutzeri* (syn. *P. stanieri*, CDC gr. Vb-1, Vb-3) (33.3), *P. vesicularis* (syn. *Brevundimonas vesicularis*, *Corynebacterium vesiculare*) (1.7), *P.* spp. (10.0);
II.1.2	Species of genus *Xanthomonas*: *X. maltophilia* (syn. *Stenotrophomonas maltophilia*, *Pseudomonas melanogena*, *Pseudomonas maltophilia*/CDC gr.1) (5.0);
II.1.3	Species of genus *Burkholderia*: *B. cepacia* (syn. *Pseudomonas cepacia*, *Pseudomonas multivorans*, *Pseudomonas kingae*, CDC gr. EO-1) (16.7);
II.1.4	Species of genus *Sphingomonas*: *S. paucimobilis* (syn. *Pseudomonas paucimobilis*) (18.3);
II.1.5	Species of genus *Flavimonas*: *F. oryzihabitans* (syn. *Pseudomonas oryzihabitans*, CDC gr. Ve-2) (15.0);
II.1.6	Species of genus *Chryseomonas*: *C. luteola* (syn. *Pseudomonas luteola*, CDC gr. Ve-1) (30.0);
II.1.7	Species of genus *Agrobacterium*: *A. radiobacter* (syn. *A. tumefaciens*, CDC gr. Vd-3) (3.3);
II.1.8	Species of genus *Ochrobactrum*: *O. anthropi* (syn. *Achromobacter*, CDC gr.Vd) (15.0);
II.1.9	Species of genus *Moraxella*: *M. lacunata* (syn. *Bacillus lacunatus, M. liquefaciens*) (6.7), *M.* spp. (6.7);
II.1.10	Species of genus *Acinetobacter*: *A. junii/johnsonii* (syn. *A. calcoaceticus*) (1.7), *A. lwoffii* (syn. *A. calcoaceticus* var. *lwoffii*) (1.7), *A.* spp. (6.7);
II.1.11	Species of genus *Flavobacterium*: *F. breve* (syn. *Bacillus brevis*) (1.7), *F. indologenes* (syn. *Chryseobacterium indologenes*, *F. aureum*, CDC gr. IIb) (6.7), *F. odoratum* (syn. CDC gr. M-4f) (1.7);
II.1.12	Species of genus *Weeksella*: *W. zoohelcum* (syn. *Bergeyella zoohelcum*, CDC gr.IIj) (1.7);
II.1.13	Species of genus *Alcaligenes*: *A. faecalis* (syn. *A. odorans*, *Achromobacter xylosoxidans*) (3.3);
II.2	Facultatively anaerobic Gram-negative rods
II.2.1	Species of genus *Leclercia*: *L. adecarboxylata* (syn. *Escherichia adecarboxylata*) (1.7);
II.2.2	Species of genus *Klebsiella*: *K. pneumoniae* ssp. *ozaenae* (syn. *K. ozaenae*) (1.7);
II.2.3	Species of genus *Enterobacter* spp. (3.3);
II.2.4	Species of genus *Pantoea*: *P. agglomerans* (syn. *Enterobacter agglomerans*, *Erwinia herbicola*, *Erwinia milletiae*) (10.0);
II.2.5	Species of genus *Erwinia* spp. (10.0);
II.2.6	Species of genus *Serratia*: *S. plymuthica* (1.7), *S. rubidaea* (syn. *S. marinorubra*) (3.3), *S.* spp. (5.0);
II.2.7	Species of genus *Proteus* spp. (1.7);
II.2.8	Species of genus *Tatumella*: *T. ptyseos* (1.7);
II.2.9	Species of genus *Listonella*: *L. damsela* (syn. *Photobacterium damsela*, *Vibrio damsela*) (1.7);
II.2.10	Species of genus *Aeromonas*: *A. hydrophila/caviae* (25.0), *A. salmonicida* ssp. *masoucida/achromogenes* (6.7), *A. salmonicida* ssp. *salmonicida* (6.7), *A. sobria* (3.3);
II.2.11	Species of genus *Pasteurella*: *P. haemolytica* (8.3), *P. multocida* ssp. *multocida* (syn. *P. multocida*) (1.7), *P.* spp. (10.0);
II.2.12	Species of genus *Haemophilus* spp. (1.7);
II.2.13	Species of genus *Chromobacterium*: *C. violaceum* (1.7);
III.	Thermophilic actinomycetes
III.1	Species of genus *Saccharopolyspora*: *S. rectivirgula* (syn. *Micropolyspora faeni, Faenia rectivirgula*) (8.3);
III.2	Species of genus *Streptomyces*: *S.* spp. (11.7);
III.3	Species of genus *Thermoactinomyces*: *T. vulgaris* (11.7);

IV.	Fungi
IV.1	Yeasts
IV.1.1	Species of genus *Candida*: *C. famata* (syn. *Torula candida, Torulopsis candida, Cryptococcus candida*) (1.7), *C. lambica* (5.0), *C. lipolytica* (syn. *Mycotorula lipolytica*) (1.7), *C. lusitaniae* (1.7), *C. maris* (1.7), *C. parapsilosis* (syn. *C. parakrusei, Monilia parapsilosis*) (6.7), *C. zeylanoides* (1.7), *C.* spp. (21.7);
IV.1.2	Species of genus *Cryptococcus* spp. (1.7);
IV.1.3	Species of genus *Geotrichum*: *G. candidum* (15.0), *G. flavo-brunneum* (5.0);
IV.1.4	Species of genus *Rhodotorula*: *R. glutinis* (syn. *Rhodosporidium toruloides, Rhodosporidium diobovatum*) (8.3), *R. rubra* (syn. *R. pilimanae, R. mucilaginosa*) (10.0), *R.* spp. (15.0);
IV.1.5	Species of genus *Trichosporon*: *T. cutaneum* (syn. *T. beigelii*) (1.7);
IV.1.6	Species of genus *Saccharomyces*: *S. cerevisiae* (1.7);
IV.2	Filamentous fungi
IV.2.1	Species of genus *Absidia*: *A. corymbifera* (5.0);
IV.2.2	Species of genus *Alternaria*: *A. alternata* (syn. *A. tenuis*) (23.3), *A. fasciculata* (5.0), *A.* spp. (1.7);
IV.2.3	Species of genus *Aspergillus*: *A. candidus* (5.0), *A. flavus* (1.7), *A. fumigatus* (21.7), *A. niger* (syn. *A. awamori, A. ficcum, A. foetidus, A. intermedius, A. nanus, A. phoenicis, A. pulverulentus, A. tubingensis, A. usamii*) (10.0), *A. ochraceus* (8.3), *A. repens* (5.0), *A. restrictus* (1.7), *A. terreus* (10.0), *A. wentii* (1.7), *A.* spp. (11.7);
IV.2.4	Species of genus *Cephalosporium*: *C. charticola* (6.7), *C. terricola* (6.7);
IV.2.5	Species of genus *Cladosporium*: *C. atroseptum* (3.3), *C. cladosporioides* (syn. *Hormodendrum cladosporioides*) (1.7), *C.* spp. (1.7);
IV.2.6	Species of genus *Fusarium* spp. (3.3);
IV.2.7	Species of genus *Gilmaniella*: *G. humicola* (5.0);
IV.2.8	Species of genus *Gliocladium* spp. (5.0);
IV.2.9	Species of genus *Humicola*: *H. brevis* (3.3), *H. grisea* (3.3);
IV.2.10	Species of genus *Mucor*: *M. mucedo* (6.7), *M.* spp. (15.0);
IV.2.11	Species of genus *Oidiodendron*: *O. citrinum* (1.7), *O. flavum* (3.3), *O. rhodogenum* (3.3), *O. tenuissimum* (1.7);
IV.2.12	Species of genus *Penicillium*: *P. atro-sanguineum* (3.3), *P. aurantio-violaceum* (1.7), *P. brasilianum* (6.7), *P. chrysogenum* (syn. *P. cyaneofulvum, P. griseoroseum, P. meleagrinum, P. notatum*) (3.3), *P. commune* (*P. album, P. camemberti, P. candidum*) (8.3), *P. echinulatum* (6.7), *P. fuscum* (10.0), *P. goldweskii* (6.7), *P. italicum* (13.3), *P. janthinellum* (5.0), *P. kazachstanicum* (5.0), *P. mirabile* (8.3), *P. onobense* (8.3), *P. roqueforti* (*P. gorgonzolae, P. stilton*) (6.7), *P. sclerotiorum* (5.0), *P. spinulosum* (8.3), *P.* spp. (46.7);
IV.2.13	Species of genus *Rhinocladiopsis*: *R. vesiculosa* (10.0);
IV.2.14	Species of genus *Rhinocladium*: *R. sporotrichoides* (5.0);
IV.2.15	Species of genus *Rhizopus* spp. (1.7);
IV.2.16	Species of genus *Sporotrichum*: *S. salmonicolor* (1.7);
IV.2.17	Species of genus *Trichoderma*: *T. album* (5.0), *T. lignorum* (3.3), *T. sympodianum* (11.7);
IV.2.18	Species of genus *Tritirachium* sp. (5.0).

Garasko *et al.* [13] found the microbial concentration of 2.7×10^4 cfu/m^3 in the air at initial stages of the production cycle in wool weaving mills, and a significant decrease of pollution in further stages of the cycle.

Bukharin *et al.* [1] stated that performing drilling operations caused a significant decrease in the total number of microorganisms and increase in the number of staphylococci in the ambient air.

Ukraine. Tsapko *et al.* [33] and Chudnovets [2, 3], on the basis of the studies performed in the Kiev region, found that bioaerosols, particularly mycotoxin-producing fungi, present a significant risk for workers of the animal feed industry. Chudnovets [3] stated in this environment large concentrations of microorganisms varied within 1.5×10^3-2.6×10^9 cfu/m^3. The most common organisms were fungi, spore-forming bacilli and Gram-negative bacteria. Out of 39 identified fungal species, 11 possessed the ability to produce mycotoxins, which should be considered as a potential occupational risk factor in the animal feed industry. Kuchuk *et al.* [23] found that the concentration of airborne endotoxin in facilities of the Ukrainian animal feed industry was within the range of 0.031–240.0 ng/m^3.

Czech Republic and Slovakia. Klanova and Drahonovska [21] investigated fungal flora in 68 rooms located in Prague, Czech Republic. Air samples were taken using RCS Plus aeroscope. The investigated rooms were divided twice: according to their service appropriation into 3 categories (dwellings, classrooms, offices) and into 4 categories taking into account the absence or presence of mold spots on walls, as well as absence or presence of health complaints of the occupants. It was found that higher concentrations of fungi were detected mainly in the dwellings rather than in the classrooms and offices, as well as in rooms with a low temperature and high humidity. Based on correlation analysis between health complaints and fungi concentrations, the authors suggest that a concentration of fungi in indoor air above 2000 cfu/m^3 is a serious risk factor for the health of the occupants.

Piecková and Jesenská [29] reported that among the most important species of fungi in indoor air of dwellings *Aspergillus niger* was isolated from 1-2% of homes in Plzen, Czech Republic. From the 16 strains of *Aspergillus versicolor* isolated in the Czech and Slovak Republics sterigmatocystin was produced by 69% of strains [34].

Holcatova *et al.* [18] studied microbial contamination in the operating theatre and intensive care units of the surgery clinic in Prague. Microbial contamination of the air was determined using an aeroscope. Saprophytic flora, mostly *Micrococcus albus* and *Sarcina lutea*, were present in the investigated rooms. Presence of *Staphylococcus epidermidis*, *Corynebacterium* spp., *Streptococcus faecalis*, *Pseudomonas* spp., and fungi (including yeasts) were also recorded. Average airborne concentration of microorganisms was between 150-250 cfu/m^3.

Table 2. Concentration (cfu/m^3) of bacterial and fungal indoor aerosols in Upper Silesia conurbation, Poland.

Type of premises/microorganisms	Range	Median	Mean	Standard deviation
Dwellings [14, 17]				
Gram-positive mesophilic bacteria	88–3442	409	602	563
Gram-negative mesophilic bacteria	0–228	35	51	53
Thermophilic actinomycetes	0–627	0	12	81
Fungi	2–1997	78	189	351
Total indoor microflora	90–3445	647	854	682
Offices [25, 27]				
Bacteria	112–956	272	295	N/A
Fungi (summer)	50–1689	136	245	N/A
Fungi (winter)	18–109	53	49	N/A
Moldy homes [24, 27]				
Bacteria	178–4751	1100	980	N/A
Fungi (summer)	103–16968	504	834	N/A
Fungi (winter)	49–3852	239	256	N/A

N/A - not available.

Table 3. Endotoxin concentration (ng/m^3) in the air of Upper Silesia conurbation dwellings [15].

Method	Range	Median	Mean	Standard deviation
Classic Limulus test (gel-clot procedure)	0.028–0.868	0.087	0.191	0.211
Kinetic chromogenic LAL method	0.03–5.417	0.162	0.531	1.032

Bulgaria. In Bulgaria, Ikonomova *et al.* [19] found in the air of the tricot knitting mills concentrations of airborne microbes of the order 10^2-10^3 cfu/m^3. Among bacteria, the *Bacillus* species prevailed, followed by Gram-positive cocci, whereas *Cladosporium* spp., *Penicillium* spp., *Alternaria* spp. and *Fusarium* spp. were most common among fungal isolates. Levels of airborne microbes were significantly higher during the warm period of the year compared to the cold one.

Other Bulgarian authors, Dimitrov *et al.* [4], reported high concentrations of potentially allergenic and toxinogenic fungi (*Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium*, *Fusarium*) in the air of plants processing cotton and hemp, posing a risk of respiratory disease for the exposed workers.

Table 4. Proposal for occupational exposure limits (OEL) [11] and residential limit values (RLV) for various bioaerosol components measured as inhalable fraction in industrial settings and residential dwellings.

Type of setting, bioaerosol component	Proposal of OEL/RLV
Industrial settings contaminated by organic dust	OEL
Fungi	50×10^3 cfu/m^{3*}
Total mesophilic bacteria	100×10^3 cfu/m^{3*}
Gram-negative bacteria	20×10^3 cfu/m^{3*}
Thermophilic actinomycetes	20×10^3 cfu/m^{3*}
Endotoxin	200 ng/m^3 (2000 EU)
Residential dwellings	RLV
Fungi	5×10^3 cfu/m^3
Total mesophilic bacteria	5×10^3 cfu/m^3
Endotoxin	5 ng/m^3 (50 EU)

* For respirable fraction the proposed limits should be twice as low, i.e., 25×10^3 cfu/m^3 for fungi, 50×10^3 cfu/m^3 for total mesophilic bacteria, 10×10^3 cfu/m^3 for Gram-negative bacteria, and 10×10^3 cfu/m^3 for thermophilic actinomycetes. EU - endotoxin unit.

Hungary. Szam *et al.* [30, 31] characterized the factors influencing the microflora of indoor air in the metro stations and in the highest tower building in the Hungarian capital of Budapest.

VALUES FOR BIOAEROSOL REFERENCE LIMITS

To ensure the reliability of bioaerosol measurement methods and their proper interpretation, in the first place it is necessary to unify the methodology, i.e., to recommend the use of volumetric methods. The active air sampling should allow the measurement of a concentration of indoor air microorganisms and describe their taxonomical origin. For easier interpretation of the results, the reference limit values for bacterial, fungal, and endotoxins concentrations in indoor air should be recognized by the international

scientific community. Such categorization in indoor bioaerosol studies should describe the parameters for interpretation of the investigated events (particularly important for medical statements on occupational diseases). The proposed occupational exposure limits (OEL) [11] and residential limit values (RLV) of various bioaerosol components in industrial settings and residential dwellings are presented in Table 4. Independently of these reference values, in an assessment of indoor exposure the general assumption should be that in certain circumstances the microbial pathogen may be a cause of health problems, even at concentrations below the reference limit. The presence in indoor air of microorganisms from the risk groups 3 and 4 of the European Community Directive 2000/54/EC (e.g., *Mycobacterium tuberculosis*, *Bacillus anthracis*, *Coxiella burnetii*) [5], independently of the concentration, should always be inadmissible and result in preventive actions.

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