

BACTERIA AND FUNGI IN ORGANIC DUST AS POTENTIAL HEALTH HAZARD*

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Abstract: People engaged in agriculture, waste collection and other professions are exposed to large quantities of bioaerosols associated with organic dusts, ranging from 10^3 - 10^{10} cfu/m³. These bioaerosols are composed of Gram-negative rod-shaped bacteria, Gram-positive, non-branching bacteria (corynebacteria, cocci and endospore-forming bacilli), actinomycetes and fungi (moulds and yeasts). Endotoxin produced by Gram-negative bacteria causes non-specific, inflammatory reaction in the lungs of exposed people, while thermophilic actinomycetes and moulds are a common cause of allergy. So far only little is known about health effects of Gram-positive bacteria, which in many cases form a dominant fraction of dust-borne bioaerosols.

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MICROORGANISMS ASSOCIATED WITH ORGANIC DUSTS

People engaged in agriculture, waste collection and other professions are exposed to large quantities of bioaerosols, which are released into air with organic dust particles or droplets during handling of different materials or are excreted by farm animals. The dusts contain bacteria and fungi of plant and animal origin in the concentrations usually ranging from 10^4 - 10^{12} cfu g⁻¹ [6, 14]. The concentration of bacteria and fungi in dusts and their potential pathogenicity depend on a variety of environmental factors including kind of source materials, climatic conditions and technology of the storage and processing of materials.

Among microorganisms occurring in organic dusts, four major groups could be distinguished:

1) Gram-negative, rod-shaped bacteria producing endotoxin, which are mostly epiphytic species developing abundantly on plant surfaces as saprobionts. The most common is fermentative species *Pantoea agglomerans*

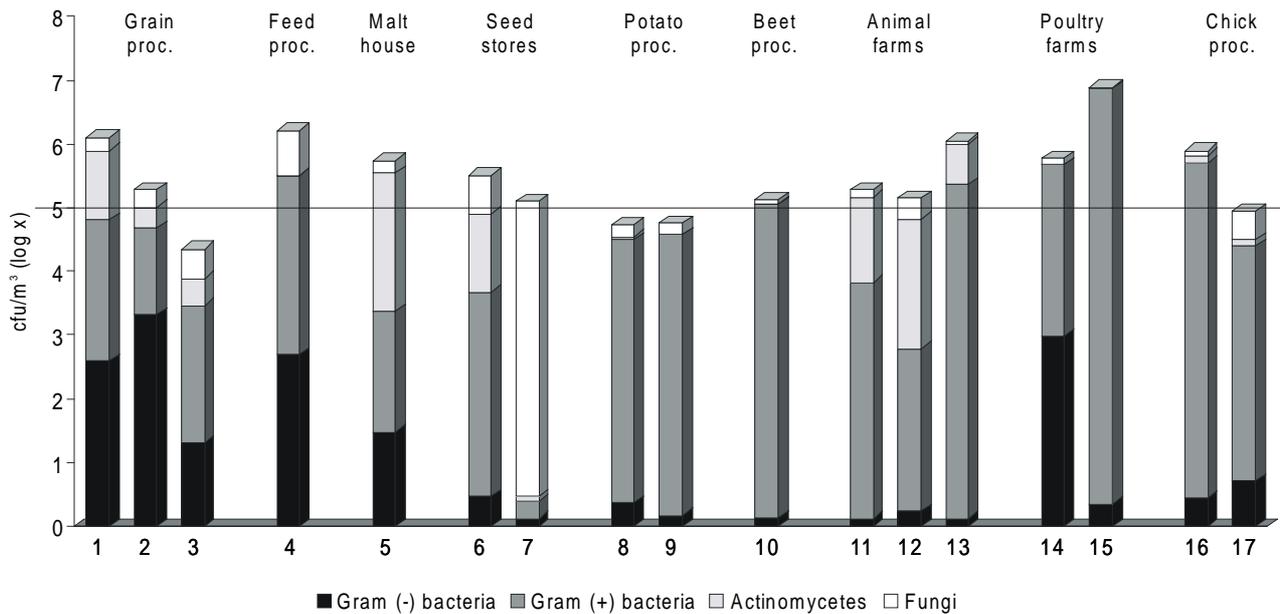
(synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*) occurring in large quantities on freshly harvested grain and cotton bract, present also in wood, hay and other plant materials [6, 7]. Dusts of plant origin may commonly contain also *Pseudomonas* spp., *Klebsiella* spp., *Rahnella* spp. and *Alcaligenes faecalis*. In dusts of animal origin prevails coccoid, non-fermentative species *Acinetobacter calcoaceticus*, less common are enterobacteria (*Klebsiella* spp., *Escherichia coli*) and *Alcaligenes faecalis*. Droplet aerosols from water, sewage or metalworking fluids may contain *Aeromonas hydrophila*, *Pseudomonas* spp., *Flavobacterium* spp. and *Cytophaga allerginae* [6, 24, 25, 29].

2) Gram-positive, non-branching bacteria which are predominant organisms in dusts of animal origin and may be also very common in dusts from stored plant materials. The most abundant are two morphological types: corynebacteria (*Arthrobacter* spp., *Corynebacterium* spp., *Brevibacterium* spp., *Microbacterium* spp.) and cocci (*Staphylococcus saprophyticus*, *S. epidermidis*, *Micrococcus* spp., *Streptococcus* spp.). Aerobic endospore-forming bacilli (*Bacillus subtilis*, *B. megaterium*, *B. cereus*) are

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Horizontal line indicates proposed threshold limit value 10^5 cfu/m³

Figure 1. Concentration and composition of microflora in the air of various agricultural settings. The heights of the bars show the concentration of cfu in 1 m³ air in logarithmic values. The segmentation of the bars presents the proportional composition of the microflora. Numbers under bars indicate following industrial settings or processes (source references in square brackets): 1) granaries [5], 2) mills: grain-cleaning rooms [5], 3) mills: milling and sacking flour and bran [5], 4) feed mixture processing plant [7], 5) malt house [5], 6) seed store: cleaning parsley [7], 7) seed store: packing carrot [7], 8) potato processing: unloading [7], 9) potato processing: blanching [7], 10) sugar beet processing [4], 11) cow barns [8], 12) horse stables [8], 13) piggeries [8], 14) hatchery: ducklings [5], 15) broiler house: chickens, after 10-week fattening [5], 16) chicken processing: slaughtering [7], 17) chicken processing: sorting [7].

always present in organic dust but usually not in large numbers. Other Gram-positive bacteria (e.g. *Lactobacillus* spp.) are rare.

3) Actinomycetes, the filamentous Gram-positive bacteria, may commonly occur in dusts from stored plant materials. The most abundant could be thermophilic species, developing in wet stacked materials of plant origin (e.g. hay, silage or compost containing 30-45% water) which heat spontaneously up to 50-65°C. The most common species are *Saccharopolyspora rectivirgula* (synonyms: *Faenia rectivirgula*, *Micropolyspora faeni*), *Thermoactinomyces vulgaris*, *Thermoactinomyces thalophilus*, *Saccharomonospora viridis* and *Thermomonospora* spp. [15, 16]. Mesophilic actinomycetes, developing in soil and vegetable materials are mainly members of the genera *Streptomyces*, *Rhodococcus* and *Agromyces* [5, 17].

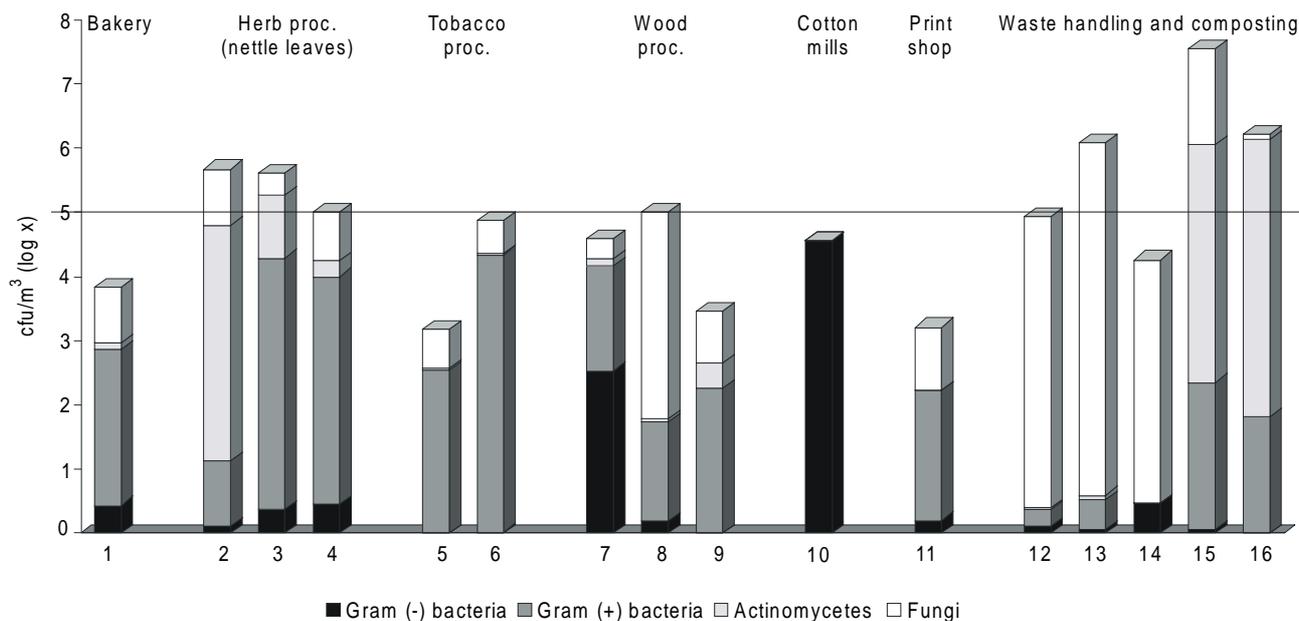
4) Fungi, comprising multicellular filamentous fungi (described as moulds) and unicellular yeasts, are common in organic dusts. Moulds may be divided into field fungi (*Alternaria* spp., *Cladosporium* spp., *Didymella* spp.) developing on grasses and other living plants, and storage fungi which decompose stored organic products, mostly at temperatures above 30°C, water content above 20% and water activity above 0.90 a_w [16]. The storage fungi mostly belong to the genera: *Aspergillus* (*A. fumigatus*, *A. flavus*, *A. candidus*, *A. terreus*, *A. clavatus*, *A. niger*), *Penicillium*, *Eurotium*, *Trichoderma*, *Absidia*, *Mucor* and *Rhizopus* [6, 9, 15, 16, 28]. Yeasts (*Candida* spp.,

Rhodotorula spp. *Endomycopsis* spp.) enter organic dusts as organisms colonizing stored grain and other plant matter or as constituents of animal feeds.

EXPOSURE

In facilities polluted with organic dusts, the concentrations of microorganisms in the air of breathing zone may range from 10^3 - 10^{10} cfu/m³ [6, 17]. Mostly it is within 10^4 - 10^7 cfu/m³ of which usually 40-50% may penetrate into deeper parts of the lungs [7]. Using the methods based on microscopic scanning of air samples and reporting results in single cells per m³ usually gives values higher by 1-3 orders of magnitude compared to routine cultivation methods, where the results are expressed as cfu per m³ [9, 14]. The advantage of microscopic methods is an exact estimation of the total microbial load of the air, while their drawback is inability of the determination of species composition.

As shown in Figures 1 and 2, the proportion of the four major groups of microorganisms may vary to a marked extent in the particular working environments. Gram-positive bacteria are overall most common organisms, being prevalent in the animal breeding and processing facilities (Fig. 1) and in many settings where plant materials are stored and processed, except for grain-based and cotton industry where Gram-negative bacteria are numerous (Fig. 1-2). In wood industry, Gram-negative bacteria are common in early stages of production while



Horizontal line indicates proposed threshold limit value 10^5 cfu/m³

Figure 2. Concentration and composition of microflora in the air of various industrial and municipal settings. The heights of the bars show the concentration of cfu in 1 m³ air in logarithmic values. The segmentation of the bars presents the proportional composition of the microflora. Numbers under bars indicate following industrial settings or processes (source references in square brackets): 1) bakery [7], 2) herb industry: cleaning of nettle leaves [7], 3) herb industry: cutting of nettle leaves [7], 4) herb industry: packing of nettle leaves [7], 5) tobacco industry: production of cigarettes [12], 6) tobacco industry: production of cigars [12], 7) sawmills: frame sawing pine [7], 8) chipboard factory: chipper [7], 9) furniture factory: polishing [7], 10) cotton mills [11], 11) print shop [25], 12) waste collection [22], 13) waste loading [4], 14) waste sorting [19], 15) waste composting [4], 16) production of the compost for mushroom growing [4].

fungi at later stages (Fig. 2). People handling waste are exposed mostly to fungi (Fig. 2) which form over 85% of total microflora [1, 4, 19, 22]. Fungi are very common also at composting [3] but in this environment they are commonly outnumbered by actinomycetes [4].

Based on the presented data and other sources [6, 17, 18, 20, 24, 29] the exposure of the particular professions could be characterized as follows:

1) Crop farmers are exposed at harvesting and threshing to Gram-negative bacteria and field fungi and during haymaking and silo unloading to thermophilic actinomycetes and storage fungi [6, 15, 18, 20].

2) Animal breeders are exposed to large quantities of Gram-positive bacteria, occasionally also to endotoxin and thermophilic actinomycetes [8].

3) Workers of food industry could be exposed to large quantities of Gram-negative and Gram-positive bacteria, occasionally to endotoxin and fungi [6, 7].

4) Workers of wood industry could be exposed at early stages of production (frame sawing in the sawmills) to Gram-negative bacteria and endotoxin and at later stages (trimming, processing of wood chips) to filamentous fungi (*Rhizopus microsporus*, *Aspergillus fumigatus*, *Penicillium* spp.) [7, 9].

5) Workers of metalworking industry are exposed to oil mist from coolant fluids containing Gram-negative bacteria (mostly *Pseudomonas testosteronilcaligenes*) and endotoxin [29].

6) Biotechnology workers could be exposed to various immunotoxic products of fungi and bacteria as enzymes and endotoxin [17].

7) Workers of print shops are exposed to humidifier droplet aerosols containing Gram-negative bacteria producing endotoxin (mostly *Pseudomonas*, *Flavobacterium* and *Cytophaga*) [25].

8) Sewage workers are exposed to Gram-negative bacteria producing endotoxin and/or protein enterotoxin [24].

9) Workers collecting and recycling waste are exposed to filamentous fungi (mostly *Penicillium* spp. and *Aspergillus* spp.) [22]. The exposure level of workers collecting green waste is 35-55 times greater compared to those collecting other kinds of waste [1].

HEALTH EFFECTS

Bacteria and fungi occurring in organic dusts are mainly non-infectious, but may, however, exert adverse effects on respiratory tract of exposed persons causing:

- 1) Mucous membrane irritation (MMI);
- 2) Immunotoxic diseases:
 - organic dust toxic syndrome (synonyms: ODTS, inhalation fever, grain fever, silo unloader's disease, toxic pneumonitis);
 - byssinosis;
 - humidifier syndrome;
 - mycotoxicoses;

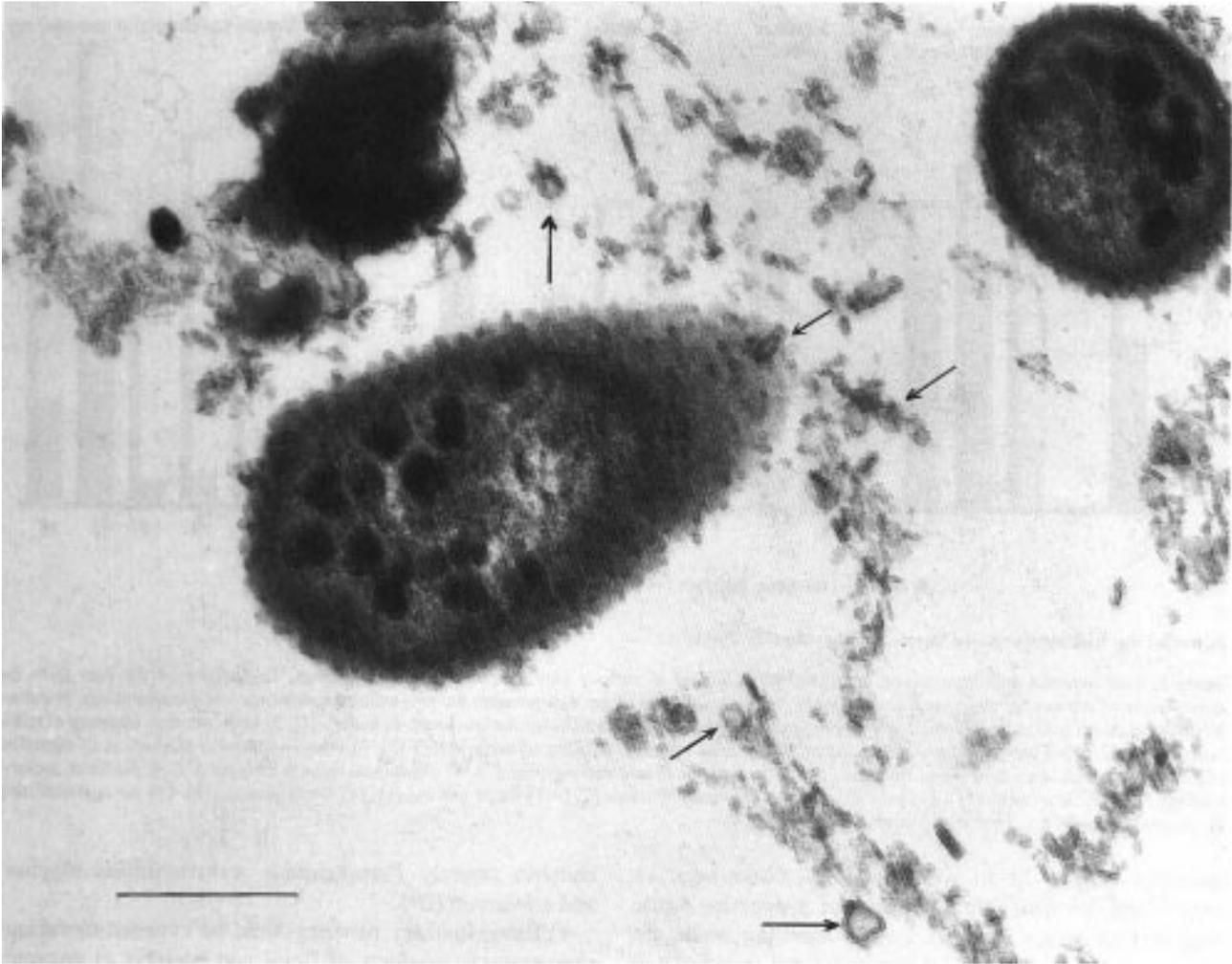


Figure 3. Gram-negative bacteria in the lumen of a wood cell of American basswood (*Tilia americana*) peeling the endotoxin-containing microvesicles (some of them marked with arrows). EM, thin-sectioned sample of pulverized wood, bar represents 0.2 μm [7].

- probably sick building syndrome or building related disease.
- 3) Allergic diseases:
 - allergic alveolitis (synonyms: hypersensitivity pneumonitis, granulomatous pneumonitis);
 - asthma;
 - allergic rhinitis [2, 17, 25].

The particular groups of microorganisms occurring in organic dusts differ in their pathogenic properties.

Gram-negative bacteria are important as the producers of endotoxins which are the main pulmonary immunotoxicants. Endotoxins are high-molecular-weight lipopolysaccharides (LPS) which occur in the outer membrane of the cell walls of Gram-negative bacteria as heteropolymers with proteins and phospholipids, and can easily be released in large quantities into organic dusts in the form of discoid particles (microvesicles) 30–50 nm in diameter with a characteristic triple-tracked membrane [7] (Fig. 3). They occur in the air of different occupational environments in the concentration range of 10^1 – 10^6 ng/m^3 [6, 23]. When inhaled, endotoxins cause acute inflammatory response in respiratory tract and toxic pneumonitis by

non-specific activation of alveolar macrophages and the subsequent release of cytokines and other mediators [2, 17, 26]. A significant dose-response relationship has been demonstrated between the level of endotoxin in the air and impairment of lung function in workers exposed to various organic dusts [2, 17, 23].

Other immunotoxicants produced by Gram-negative bacteria are less known. Protein enterotoxin produced by *Aeromonas hydrophila* was suggested as a cause of gastrointestinal symptoms (diarrhoea, tiredness) observed in workers exposed to droplet aerosol from sewage sludge [24]. Some Gram-negative species (*Pantoea agglomerans*, *Alcaligenes faecalis*, *Cytophaga allerginae*) were reported as causative agents of allergic alveolitis [6, 21].

Gram-positive bacteria, despite their abundance in airborne dusts, were only little studied in respect to their potential pathogenicity and are generally regarded as relatively less harmful. Nevertheless, some of their constituents such as enzymes and other proteins, or cell wall peptidoglycan should be regarded as potential immunotoxicants [2], considering a huge load of these bacteria in the air of some working environments.

Respiratory disease was described in the workers of factories producing enzymatic washing powders who are exposed to the airborne proteolytic enzymes of *Bacillus subtilis* (subtilisins) ranging from 10^1 – 10^2 ng/m³ and in the workers of bioinsecticide factory exposed to the crystalline protein toxin of *Bacillus thuringiensis* [15, 17]. Among corynebacteria, *Arthrobacter* sp. and *Brevibacterium linens* were reported as causative agents of allergic alveolitis in Poland [21].

Actinomycetes. Thermophilic species produce strong allergens causing allergic alveolitis [15] and mesophilic species (*Streptomyces* spp.) are also known to evoke this disease [6, 17].

Fungi. Filamentous fungi (moulds) represent a major hazard for people exposed to organic dusts. Inhalation of mould spores and hyphal fragments commonly leads to allergy. Many species were identified as a cause of allergic alveolitis: *Aspergillus fumigatus*, *A. flavus*, *A. clavatus*, *A. terreus*, *A. versicolor*, *Cryptosporium corticale*, *Eurotium rubrum* (synonym: *Aspergillus umbrosus*), *Penicillium glabrum*, *P. casei*, *P. roqueforti*, *Penicillium* spp., *Trichoderma viridis* [6, 17]. Asthma and allergic rhinitis may be caused both by field fungi (*Alternaria* spp., *Cladosporium* spp., *Didymella* spp.) and storage fungi (*Aspergillus* spp., *Penicillium* spp. and others) [15, 16].

There is a growing evidence that fungi may be also an important cause of immunotoxic diseases. Developing on stored timber *Rhizopus microsporus* causes ODS symptoms among wood trimmers [9]. The inhalation of large amounts of the *Aspergillus candidus* spores caused the ODS symptoms in groups of Polish students after shovelling grain [13]. The (1→3)-β-D-glucans, basic polyglucose constituents of fungal cell wall occurring in the air in the concentrations of 10^2 – 10^3 ng/m³, have been suggested to cause chronic lung inflammation and evoke diseases, such as chronic byssinosis or sick house syndrome (building-related disease). A significant correlation was reported between concentrations of (1→3)-β-D-glucan in the air and the presence of respiratory symptoms in exposed workers [27].

A great potential threat pose mycotoxins, the low molecular cyclic metabolites produced by filamentous fungi (aflatoxins produced by *Aspergillus flavus* and *A. parasiticus*, ochratoxins produced by *Aspergillus* spp. and *Penicillium* spp., trichothecens produced by *Fusarium* spp. and others) [16, 28]. Mycotoxin levels in the air polluted with organic dusts are low (0– 10^2 ng/m³) and their role in evoking respiratory symptoms is under discussion [17]. In contrast to bacterial endotoxins, mycotoxins suppress the activity of alveolar macrophages and pulmonary defence system, increasing the risk of respiratory cancer [28].

Other substances of fungal origin suspected to be involved in the etiology of immunotoxic diseases are enzymes and volatile organic compounds (VOCs), comprising aldehydes, alcohols and other low molecular products [28].

The pathogenic significance of yeasts has not yet been definitely proven but certainly needs further studies as zymosan and other yeast glucans are strong immunomodulators and potential immunotoxicants [28].

PERSPECTIVES FOR RESEARCH AND PREVENTION

Research needs. Microflora of organic dusts and its effects are very diverse and an extensive research should be done to solve the most important problems. Among them, two seem to be particularly significant:

a) Identification of all major fungal immunotoxicants and the modes of their pathogenic effects on human respiratory system;

b) Study of the pathogenic significance of corynebacteria and other Gram-positive bacteria which in most cases constitute the dominant fraction of the microflora of organic dusts.

Establishing of Occupational Exposure Limit. To the best of my knowledge, so far the Occupational Exposure Limit (OEL) or Threshold Limit Values (TLV) for air microflora and its constituents have not been elaborated and introduced as law regulations anywhere in the world. The basic difficulties lie upon the marked diversity of microflora and the large differences in methods of microbiological air study and sampling instruments. Nevertheless, the necessity of such values is unquestionable and this meeting is a good opportunity for formulation of the introductory project. In my opinion, the first step for introduction of the microbiological OEL would be publication of the relevant values, based on unified methodics, which could be edited by an expert group as a separate brochure and published by any of the international health agendas. In this way would be obtained the internationally recognised reference values which could function for 3–5 years as a basis for comparisons of the epidemiological exposure/effect studies in different countries and should be subject of further developments. Then, the introduction of the OEL values to law regulations in particular countries would be much easier.

The Scandinavian cfu-oriented projects for occupational OEL suggest 5 – 10×10^3 cfu/m³ for total microorganisms, 1×10^3 cfu/m³ for Gram-negative bacteria and 1 – 2×10^2 ng/m³ for endotoxin [19]. These are reliable values but in some environments (such as animal farms) their execution could be difficult at the present state of technology. The project prepared in Poland suggests 1×10^5 cfu/m³ for total microorganisms, 5×10^4 cfu/m³ for fungi and 2×10^4 cfu/m³ for Gram-negative bacteria and thermophilic actinomycetes, with a reduction of the values by half if the respirable fraction equals to or exceeds 50% of the total count [6]. This project is based on the fact that at the continuous exposure to microbial concentrations above 10^5 cfu/m³, the work-related respiratory disorders in the workers are very common [6, 10].

Prevention. The most effective measure for the prevention of occupational diseases due to exogenous microorganisms is the reduction of dust at workplace by effective ventilation and exhaust systems [6, 17]. Of great importance also is the proper storing of the plant raw materials (grain, hay) at low temperature and humidity that prevents growth of microorganisms and overheating. Some physical methods as ionization of air, cleaning of production rooms with aerosols (fogging) or sterilization of the products by gamma-irradiation, seem as yet to be more promising than using chemicals for the prevention of the growth of bacteria and fungi or for inactivation of endotoxin [17].

Good preventive measures are also: the use of positive-pressure helmets during work with decomposed vegetable materials, providing of machines with ventilated cabins and the remote control of the production processes. Very important is also the health education and periodical medical examination of the workers exposed to organic dusts.

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