ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2004, 11, 129–138

EXPOSURE OF HOP GROWERS TO BIOAEROSOLS

Anna Góra[1], Czesława Skórska[1], Jolanta Sitkowska[1], Zofia Prażmo[1], Ewa Krysińska-Traczyk[1], Barbara Urbanowicz[2], Jacek Dutkiewicz[1]

[1]Department of Occupational Biohazards, Institute of Agricultural Medicine, Lublin, Poland
[2]Laboratory of Electron Microscopy, Institute of Pediatrics, Collegium Medicum, Jagiellonian University, Kraków, Poland

Góra A, Skórska C, Sitkowska J, Prażmo Z, Krysińska-Traczyk E, Urbanowicz B, Dutkiewicz J: Exposure of hop growers to bioaerosols. *Ann Agric Environ Med* 2004, **11**, 129–138.

Abstract: Air sampling was performed during picking and sorting of hop (*Humulus lupulus*) cones on 19 hop farms located in eastern Poland. The concentration and composition of airborne microflora and the concentration of airborne dust and endotoxin were determined. Additionally, 7 samples of settled hop dust were collected and examined for the presence of microorganisms and endotoxin. Total concentrations of airborne microorganisms were within a range of 2.08–129.58 × 10^3 cfu/m^3. Gram-positive bacteria formed 22.2–96% of the total count. Among them, prevailed corynebacteria and endospore-forming bacilli. Fungi constituted 3.7–65.4% of the total count. The dominant species were *Penicillium citrinum*, *Alternaria alternata*, and *Cladosporium epiphyllum*. Thermophilic actinomycetes and Gram-negative bacteria were detected in the air of only 10 and 6 farms, respectively. Airborne dust concentrations at the workplace ranged from 0.17–31.67 mg/m^3. The concentrations of airborne endotoxin were in the range of 26–6250 ng/m^3. In the samples of settled dust, the concentrations of total microorganisms ranged from 0.25 × 10^6 to 2.87 × 10^8 cfu/g. Gram-positive and Gram-negative bacteria constituted respectively 3.2–98% and 0-93.5% of the total count. Fungi formed 0–30.3% of the total count. The most common species were *Penicillium* spp. and *Alternaria alternata*. The concentrations of endotoxin were in the range of 312.5–6250 µg/g (median 6250 µg/g). The presence of microorganisms and endotoxin in the samples of settled dust was confirmed by electron microscopy. The hop growers seem to be exposed to lower concentrations of dust, microorganisms and endotoxin compared to other branches of agriculture. This may be partly due to antimicrobial properties of hop plant. Among microbial factors associated with hop dust, bacterial endotoxin and allergenic fungi pose the greatest potential hazard for exposed hop farmers.

Address for correspondence: Anna Góra, MD, Department of Occupational Biohazards, Institute of Agricultural Medicine, Jaczewskiego 2, 20-090 Lublin, Poland. E-mail: annagora@galen.imw.lublin.pl

Key words: hop (*Humulus lupulus*), farmers, occupational exposure, organic dust, bioaerosols, bacteria, fungi, endotoxin, ultrastructure.

INTRODUCTION

Farmers may be exposed to large quantities of bioaerosols. Recently, the level of this exposure has been studied extensively in different branches of agriculture such as: herb or flax processing [6, 11, 13, 20], grain handling [11, 43], poultry farming [16, 18, 35], cattle-breeding [36] or pig-raising [28]. However, little is known about the exposure of hop farmers to dust and endotoxin in their work environment. To our knowledge, only one study on hop-growers exposure to bioaerosol was carried out by Aleksandrov and Gyeorgyev in 70s [1].

Received: 2 February 2004
Accepted: 10 April 2004
Presented at the 1st International Scientific-Training Congress *Organic Dust Induced Pulmonary Diseases*, 10-12 Oct. 2003, Kazimierz Dolny, Poland

Hop (*Humulus lupulus*) is a perennial herbaceous liana of the family Cannabinaceae [31, 32]. The female inflorescences of the plant (cones) are commonly used in industry as a component of drugs and cosmetics, and as a flavouring and preservative agent in breweries [4, 41]. Hop is cultivated extensively in Germany, the Czech Republic, UK and USA. Poland is the third biggest hop-producer in Europe.

The aim of this study was to determine the levels of microorganisms, dust and endotoxin in the work environment of hop growers, and to examine the species composition of the airborne and settled dust microflora.

MATERIALS AND METHODS

The study was conducted during the hop picking season (August/September 2000) in 19 randomly selected hop farms located in the Lublin region of eastern Poland. The hop picking is carried in several stages. First, the hop shoots are cut at the height of 1.2 m and transported to the picking machines inside farm buildings. Each shoot is hooked to a conveyor belt and transported through the system of picking and cleaning drums of a machine. Next, the separated hop cones are cleaned (parts of the leaves or peduncles are removed) and sorted manually. The cones are then transported to a drying house where they are dried for 5-8 hours at a temperature of 55–60ºC and finally packed in sacks. All farm buildings were ventilated through the system of doors and windows.

The air sampling was performed during picking and sorting of hop cones (farms 1-6, 15-16, 18-19), and while drying and packing cones (farms 7-14, 17). The samples were taken by use of an AP-2A personal sampler (TWOMET, Zgierz, Poland), at the flow rate of 2 l/min. The glass fiber filters, with 1 µm pore size and 37 mm diameter, were used. At each site, two samples were collected, one for determination of the concentration and species composition of microorganisms, and the other for determination of endotoxin. The concentration of dust in the air was determined gravimetrically from the difference between weight of the filter measured before and after sampling. The concentration of airborne dust estimated for each farm was a mean of 2 single determinations.

Microbiological examination of the air. The concentration and species composition of microorganisms in collected air samples were determined by dilution plating. The filters were extracted in 5 ml of sterile saline (0.85% NaCl) with 0.05% Tween 80, and after shaking, serial 10-fold dilutions were made. The 0.1 ml aliquots of each dilution were spread on duplicate sets of the 4 following media: blood agar for estimation of Gram-positive bacteria, eosin methylene blue (EMB) agar (Difco, MI, USA) for estimation of Gram-negative bacteria, half-strength trypic soya agar (BioCorp, Poland) for estimation of thermophilic actinomycetes, and malt agar (Difco, MI, USA) for estimation of fungi. The blood agar plates and EMB agar plates were subsequently incubated for 1 day at 37ºC, then 3 days at room temperature (22ºC) and finally 3 days at 4ºC. The malt agar plates were subsequently incubated for 4 days at 30ºC and next 4 days at room temperature. The prolonged incubation at lower temperatures aimed to isolate as wide a spectrum of bacteria and fungi as possible. The tryptic soya agar plates were incubated for 5 days at 55ºC. The grown colonies were counted and differentiated, and the data reported as cfu per 1 cu m of air (cfu/m^3). The total concentration of the microorganisms in the air was obtained by the addition of the concentrations of Gram-positive and Gram-negative bacteria, thermophilic actinomycetes and fungi.

Bacterial isolates were identified by microscopic and biochemical methods as recommended by Bergey's Manual [19, 42, 44]. Additionally, the selected isolates were identified with microtests: API Systems 20E and NE (bioMérieux, Marcy l'Etoile, France), and BIOLOG System (Biolog, Inc., Hayward, CA, USA). Fungi were identified by microscopic methods, according to Barron [2] and Litvinov [27].

The concentration of bacterial endotoxin in the airborne dust was determined by the *Limulus* amebocyte lysate (LAL) gel clot test (Associates of Cape Code, Inc., Falmouth, MA, USA) [25]. The filters were extracted for 1 hr in 10 ml of pyrogen-free water at room temperature.

Table 1. Concentrations of microorganisms, dust, and endotoxin in the air during harvesting and processing of hop (*Humulus lupulus*).

	No. of positive samples	Arithmetic mean (AM)	Standard deviation (SD)	Median	Range
Microorganisms					
Gram-positive bacteria (cfu × $10^3/m^3$)	19	11.73	27.47	5.0	1.25–124.58
Gram-negative bacteria (cfu × $10^3/m^3$)	6	1.58	5.42	0.0	0–23.75
Thermophilic actinomycetes (cfu × $10^3/m^3$)	11	0.9	1.73	0.42	0–7.5
Fungi (cfu × $10^3/m^3$)	19	3.31	2.92	2.08	0.42–9.58
Total count (cfu × $10^3/m^3$)	19	17.52	28.32	9.16	2.08–129.58
Dust and endotoxin					
Total dust (mg/m^3)	19	7.05	8.09	3.33	0.17–25.83
Total endotoxin (ng/m^3)	19	712.5	1636.78	52.2	26.1–6250

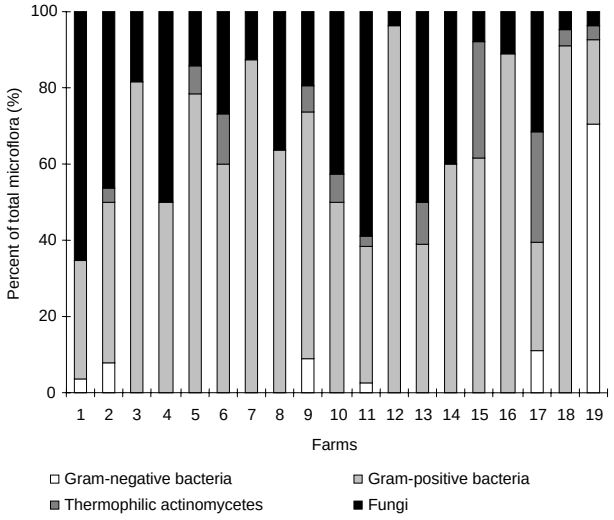

Figure 1. Composition of airborne microflora on hop farms (total count).

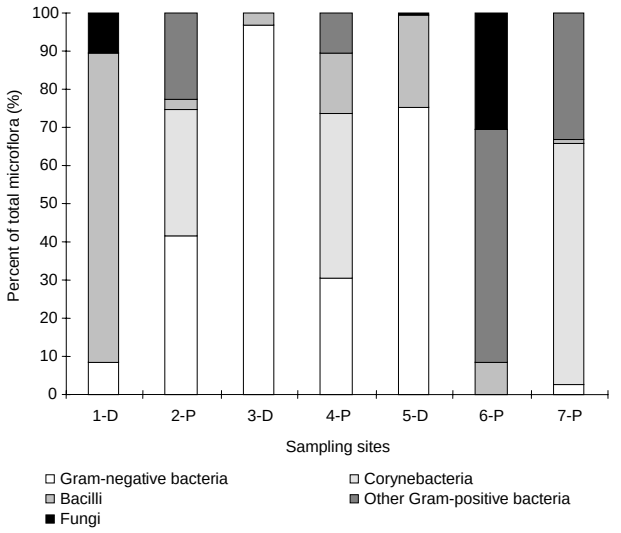

Figure 2. Composition of the microflora of settled hop dust (total count). Samples 2-P, 4-P, 6-P, 7-P were collected at picking machines while samples 1-D, 3-D, 5-D were collected in drying houses.

After extraction, serial dilutions were prepared. The 0.1 ml dilutions were mixed equally with *Limulus* reagent, pyrogen-free water (negative control) and commercial lipopolysaccharide of *Escherichia coli* O113:H10 (positive control). The tests were incubated for 1 hr in a water bath at 37ºC. The formation of a stable clot was regarded as a positive result. The estimated concentration of endotoxin in the airborne dust (ng/mg) was multiplied by estimated concentration of dust in the air (mg/m^3). The final result was reported as nanograms of the equivalents of the *E. coli* O113:H10 endotoxin per 1 m^3 of air. To convert to Endotoxin Units (EU), the value in nanograms was multiplied by 10.

Microbiological examination of settled dust. Additionally, on 4 farms 7 samples of settled hop dust were collected in sterile Erlenmeyer flasks for the determination of microorganisms and endotoxin. The dust samples were taken from 7 sites: at picking machines (sampling sites 2, 4, 6, 7), and in drying houses (sampling sites 1, 3, 5). Microbiological examination of the collected dust samples was performed with dilution plating method [33]. One gram of each sample was suspended in 100 ml of the sterile saline (0.85% NaCl) with 0.1% (v/v) Tween 80, and after vigorous shaking, serial 10-fold dilutions in saline were made up to 10^{-8}. The 0.1 ml aliquots of each dilution were spread on duplicate sets of the following media: blood agar for Gram-positive bacteria, eosin methylene blue (EMB) agar (Difco, MI, USA) for Gram-negative bacteria, half-strength tryptic soya agar (BioCorp, Poland) for thermophilic actinomycetes, and malt agar (Difco, MI, USA) for fungi. The incubation conditions and identification methods were the same as described above for air samples.

The concentration of bacterial endotoxin in the samples of settled dust was determined by *Limulus* amebocyte lysate (LAL) gel clot test (Associates of Cape Code, Inc., Falmouth, MA, USA). Ten milligrams of each dust sample were extracted for 1 hr in 10 ml of sterile pyrogen-free water. The test was performed as described above for air samples.

Table 2. List of microbial species and genera identified in the air samples collected on hop farms.

Gram-negative bacteria: ***Enterobacter*** **spp.+** (17, 19), ***Pantoea agglomerans****+ (synonyms: ***Erwinia herbicola***, ***Enterobacter agglomerans***) (2, 17), *Flavimonas* spp. (17).

Bacilli: *Bacillus* spp. (1-19).

Corynebacteria: ***Arthrobacter globiformis**** (9, 12), *Aureobacterium testaceum* (1), *Aureobacterium saperdae* (9), ***Brevibacterium linens**** (6), *Corynebacterium aquaticum* (1), *Corynebacterium* spp. (3, 10, 12, 16, 17), *Gordona terrae* (9), *Microbacterium lacticum* (10).

Other mesophilic bacteria: *Micrococcus* spp. (1, 2, 4, 5, 7, 8, 10, 12-14, 17), *Staphylococcus haemolyticus* (5, 11, 18), *Staphylococcus intermedius* (3, 12), *Staphylococcus* spp. (1, 2, 4, 6-8, 10, 11, 13, 17), *Streptococcus* spp. (8, 15), ***Streptomyces albus**** (3, 10, 12, 13), *Streptomyces* spp. (17).

Thermophilic actinomycetes: *Actinomadura pusilla* (17), ***Thermoactinomyces thalpophilus**** (3, 9, 11, 15), ***Thermoactinomyces vulgaris**** (2, 5, 6, 9, 10, 13, 17-19).

Fungi: ***Alternaria alternata****+ (1-4, 6, 8-10, 12, 13, 17), *Alternaria brassicae* (8, 13), *Alternaria humicola* (7, 8, 13, 14), ***Aspergillus candidus****+ (6), ***Aspergillus ustus****+ (1, 2), ***Cladosporium epiphyllum**** (1-5, 9-12, 14, 17), ***Cladosporium elegantum**** (3, 4, 11, 12), ***Mucor mucedo**** (17), ***Mucor racemosus**** (12), ***Penicillium citrinum****+ (1-4, 6, 9, 11, 12, 16, 18, 19), ***Penicillium*** **spp**.*+ (2, 4, 5, 10-13, 17), ***Trichoderma viride**** (10).

Numbers of farms on which strains were isolated are given in parentheses. Names of the species reported as having allergenic and/or immunotoxic properties (see text) are in bold and marked as follows: * allergenic species; + immunotoxic species.

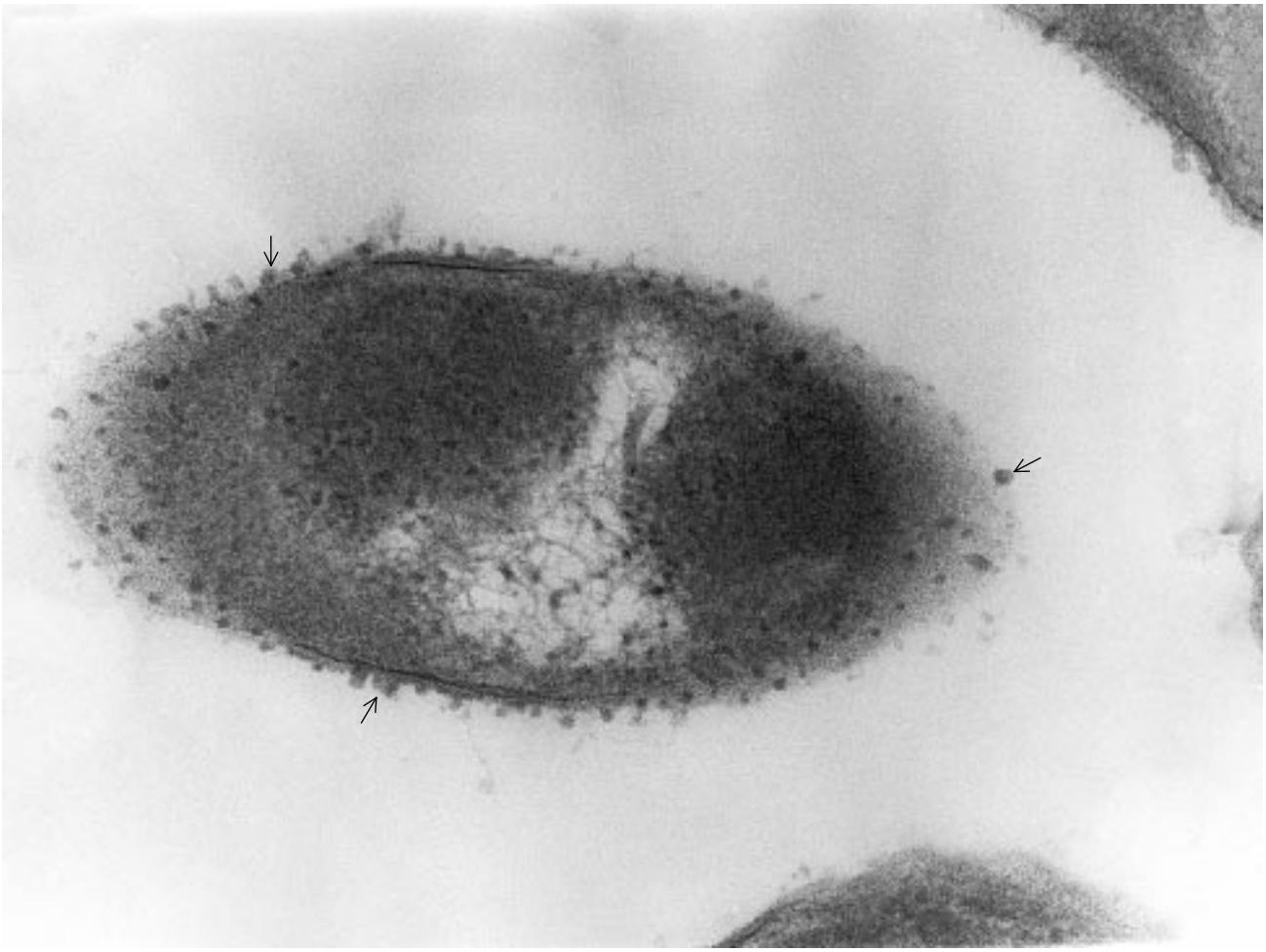

Figure 3. Thin-sectioned sample of settled hop dust, sampling site 4. Note a structure corresponding to the cell of Gram-negative bacterium bearing numerous endotoxin-containing membrane vesicles (marked with arrows), budding from outer membrane. EM, × 96,600.

Electron microscopy. To confirm the presence of bacteria and endotoxin-containing membrane vesicles (ECMV) in samples of settled hop dust, the samples were examined by electron microscopy. The examination was carried out in the Laboratory of Electron Microscopy, Institute of Pediatrics, Collegium Medicum, Jagiellonian University, Kraków, Poland, as described earlier [8, 33, 34]. Briefly, small portions of dust samples were pre-fixed in 2% glutaraldehyde in phosphate buffer at pH 7.3 and post-fixed in 1% buffered osmium tetroxide. After dehydration in graded series of ethanol, the samples were embedded in Low Viscosity (by dr Spurr), thin sectioned (silver colour) and stained with 2% uranyl acetate and lead citrate. The micrographs were taken with a Philips EM 300 electron microscope operating at 80 KV.

Statistical analysis. The data were analysed for distribution with the Kolmogorov-Smirnov test. Not-normal distributed data were analysed with Mann-Whitney test. All statistical analyses were conducted using STATISTICA for Windows v. 5.1 package (Statsoft©, Inc., Tulsa, OK, USA). $P < 0.05$ was regarded as a level of significance.

RESULTS

The concentrations of microorganisms in the air during harvesting and processing of hop (*Humulus lu*pulus) are presented in Table 1. The total concentrations of airborne microorganisms were within the range of 2.08–129.58 × 10^3 cfu/m^3 (median 9.16 × 10^3 cfu/m^3). The level of 100 × 10^3 (10^5) cfu/m^3 was exceeded on only 1 farm. The mesophilic Gram-positive bacteria and fungi were found in the air of all 19 examined farms, and their median concentrations were respectively 5.0 × 10^3 cfu/m^3 and 2.08 × 10^3 cfu/m^3. The Gram-negative bacteria and thermophilic actinomycetes were less numerous. Qualitative examination of the air samples revealed that Gram-positive bacteria were dominant in the air of most of the farms, forming 22.2–96% of the total count (Fig. 1). Among them, most numerous were corynebacteria (mainly *Corynebacterium* spp., *Aureobacterium testaceum*, *Aureobacterium saperdae*) which constituted on the average 57.5% of all Gram-positive isolates (range 0-91.3%), bacilli (*Bacillus* spp.) - 22.5% (range 4.0–94.4%) and cocci (mainly *Staphylococcus* spp.) - 16.1% (range 0–81.8%). Fungi constituted 3.7–65.4% of the total count,

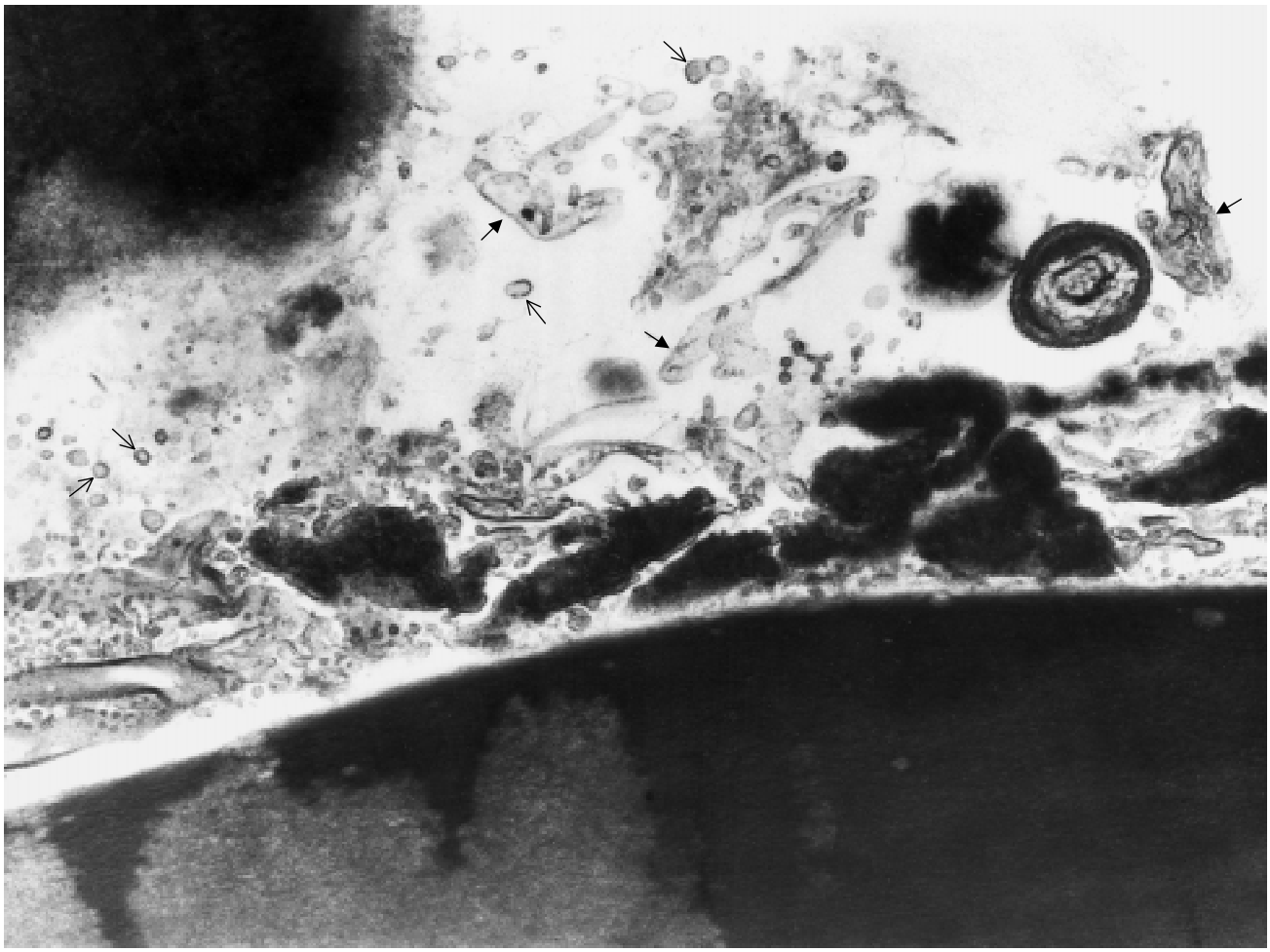

Figure 4. Thin-sectioned sample of settled hop dust, sampling site 4. Note light structures corresponding to dead Gram-negative bacteria (marked with solid arrows) and numerous endotoxin-containing membrane vesicles (marked with open arrows). EM, × 36,800.

and in the air of 5 farms formed the dominant component of microflora (Fig. 1). The prevailing species were *Penicillium citrinum*, *Alternaria alternata*, and *Cladosporium epiphyllum*. *Penicillium* spp. constituted 32.7% of all fungal isolates, *Alternaria* spp. - 23.3%, and *Cladosporium* spp. - 20.7%. Thermophilic actinomycetes and Gram-negative bacteria were detected in the air of only 11 and 6 farms, respectively, where they formed 2.6–30.8% and 2.6–70.4% of the total count. *Pantoea agglomerans* constituted 50% of all isolated Gram-negative bacteria, *Enterobacter* spp. - 30%, and *Flavimonas* spp. - 20%. *Thermoactinomyces* species dominated among thermophilic actinomycetes: *Th. vulgaris* formed 76.9% of all isolates and *Th. thalpophilus* - 17.9%.

Table 3. Concentrations of microorganisms and endotoxin in the samples of settled dust collected during picking and drying of hop.

Sampling site	Gram-positive bacteria cfu × 10^6/g	Gram-negative bacteria cfu × 10^6/g	Thermophilic actinomycetes cfu × 10^4/g	Fungi cfu × 10^6/g	Total microorganisms cfu × 10^6/g	Endotoxin µg/g
1 - Drying house	0.2	0.02	0.1	0.025	0.25	625
2 - Picking machine	37.5	26.5	0	0.02	64.02	3125
3 - Drying house	0.01	0.29	0.05	0	0.31	312.5
4 - Picking machine	200.0	86.75	0	0	286.75	6250
5 - Drying house	1.9	6.0	0.05	0.053	7.95	6250
6 - Picking machine	23.9	0	0.05	10.4	34.30	6250
7 - Picking machine	164.0	4.10	0.6	0.018	168.12	6250
Median (range)	23.9 (0.01-200.0)	4.10 (0-86.75)	0.05 (0-0.6)	0.02 (0-10.4)	34.30 (0.25-286.75)	6250 (312.5-6250)

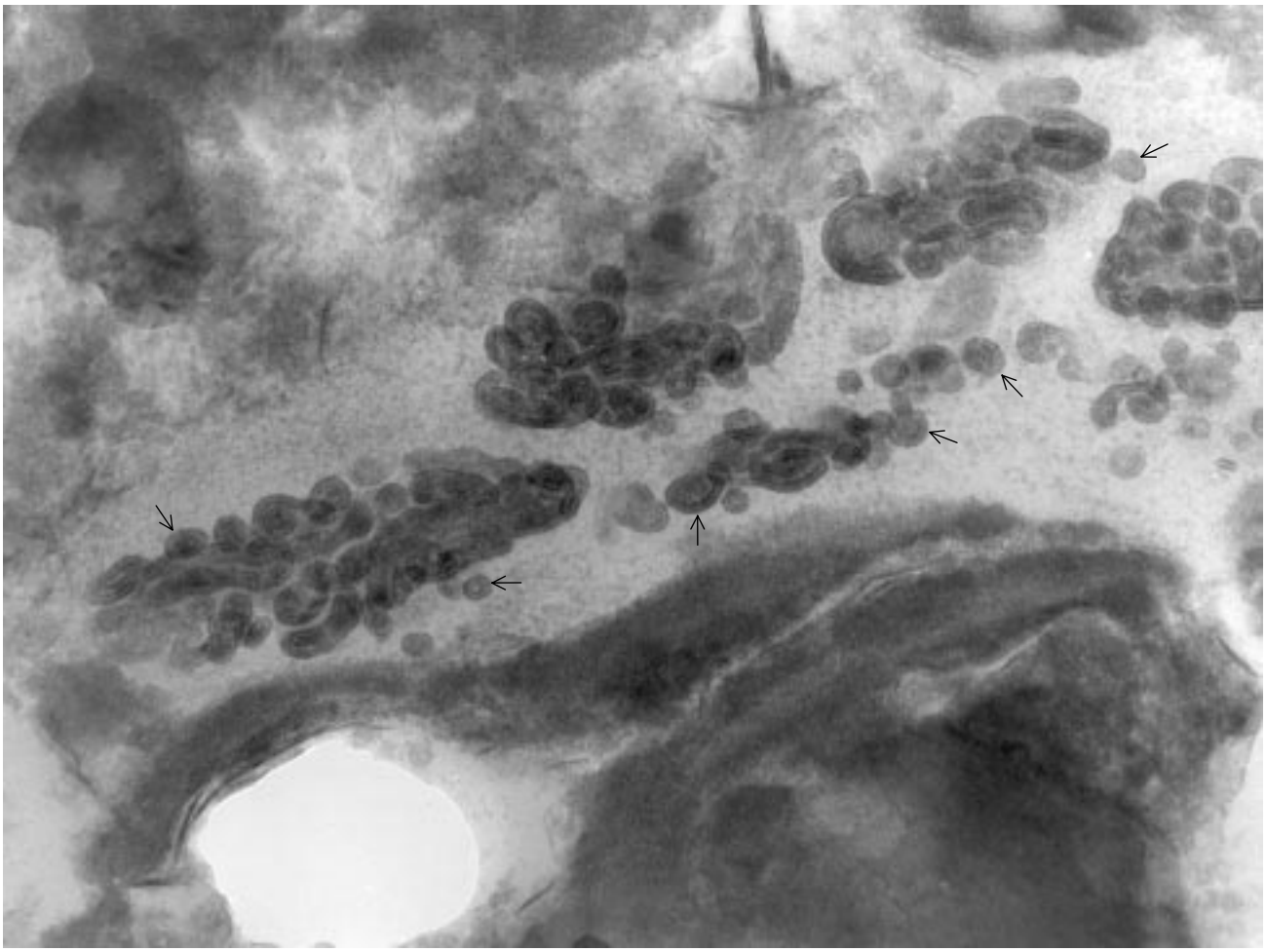

Figure 5. Thin-sectioned sample of settled hop dust, sampling site 1. Note aggregations of the structures corresponding to endotoxin-containing membrane vesicles (marked with arrows), surrounded by remnants of plant tissues. EM, × 62,100.

In the air samples taken on examined farms, 22 species or genera of bacteria and 12 species or genera of fungi were identified (Tab. 2); of these, 7 and 10 species or genera respectively were reported as having allergenic and/or immunotoxic properties [7, 21, 22, 33].

Airborne dust concentrations at the workplace ranged from 0.17–25.83 mg/m^3 (median 3.33 mg/m^3) and on 9 farms exceeded Polish OEL of 4 mg/m^3 (Tab. 1). The concentrations of airborne endotoxin were in the range of 26–6250 ng/m^3 (median 52.2 ng/m^3) and on 7 farms exceeded the level of 200 ng/m^3. No statistically significant differences were found between the concentrations of airborne microorganisms, dust and endotoxin noted at sorting machines and inside drying houses.

The concentrations of total microorganisms in the samples of settled dust ranged from 0.25×10^6 to 2.87×10^8 cfu/g (median 3.43×10^7 cfu/g). The dominant organisms were Gram-positive bacteria which constituted 3.2–98% (mean 76.1%) of the total count (Tab. 3, Fig. 2). Among them, prevailed corynebacteria (*Corynebacterium* spp., *Aureobacterium flavescens*, *Rhodococcus rhodochrous*) and bacilli (*Bacillus* spp.). The *Staphylococcus* species (*S. equorum*, *S. sciuri*), *Kocuria kristinae* and *Streptomyces* spp. were also identified.

Gram-negative bacteria constituted 0–93.5% (mean 22.0%) of the total count and dominated in the samples collected in drying houses (Fig. 2). Among Gram-negative bacteria, species of *Alcaligenes* (*A. faecalis*, *A. denitrificans*) and *Pseudomonas* (*P. fluorescens*, *P. pickettii*) prevailed. *Pantoea agglomerans* was found in only 2 dust samples. Moreover, the following species of Gram-negative bacteria were identified in the samples of settled hop dust: *Cedecea lapagei*, *Escherichia coli*, *Klebsiella* spp., *Leclercia adecarboxylata*, *Ochrobactrum anthropi*, *Oligella urethralis*, *Serpens flexibilis*, *Serratia fonticola*, *Sphingobacterium spiritovorum*, and *Stenotrophomonas maltophilia*.

Fungi formed 0–30.3% (mean 1.9%) of the total count (Tab. 3, Fig. 2). The most common species were *Penicillium* spp. and *Alternaria alternata*. Other isolates included: *Aspergillus terreus*, *Fusarium* spp., *Monilia* spp., *Mucor mucedo*, *Trichoderma viride*, and yeast. Among thermophilic actinomycetes, 2 species were identified: *Thermoactinomyces vulgaris* and *Thermoactinomyces thalpophilus*.

The concentrations of endotoxin were in the range of 312.5–6250 µg/g (median 6250 µg/g) (Tab. 3).

In the examination by electron microscopy, structures corresponding to bacteria were found in 4 samples of

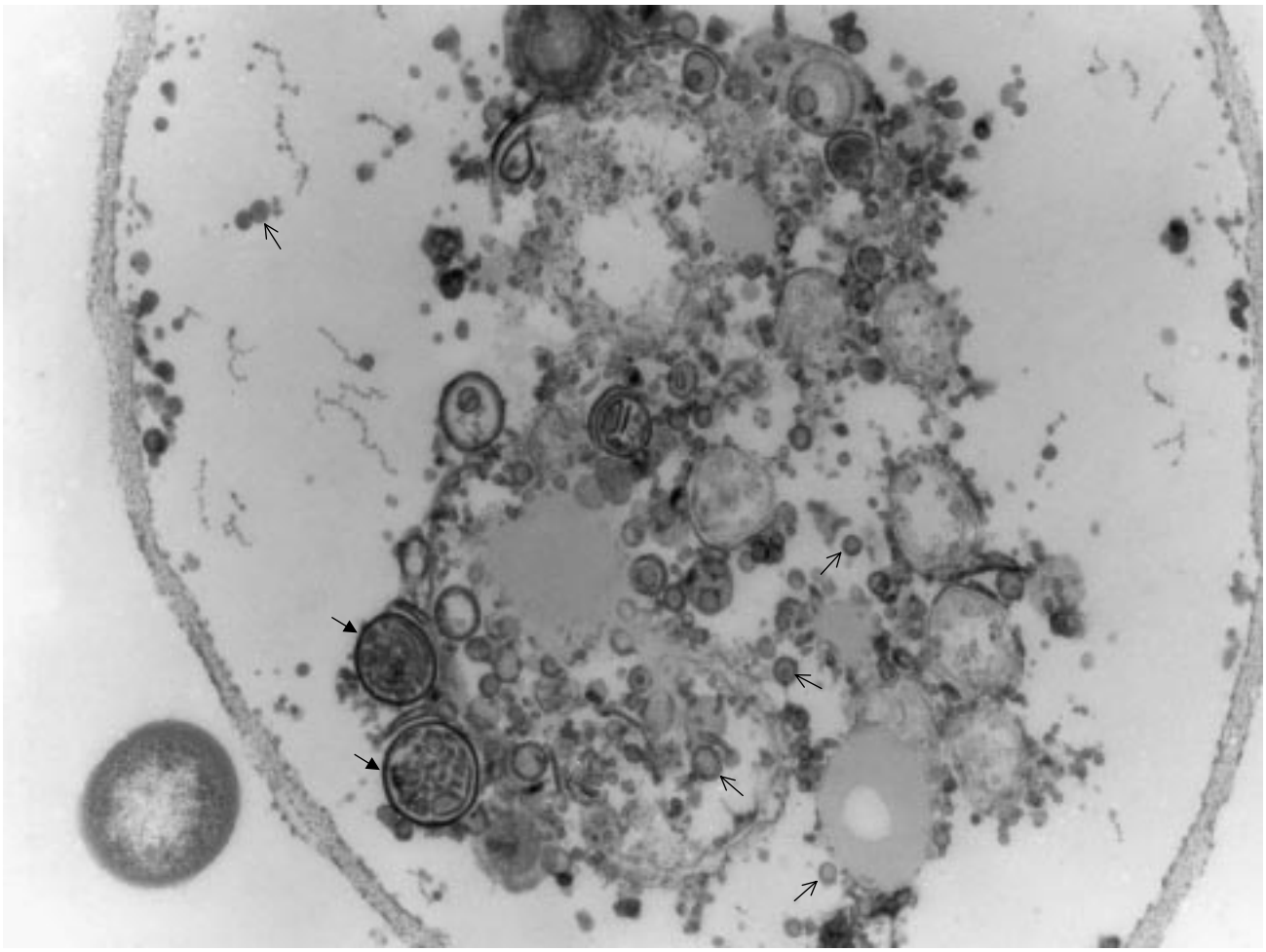

Figure 6. Thin-sectioned sample of settled hop dust, sampling site 2. Note structures corresponding to endotoxin-containing membrane vesicles distributed freely (marked with open arrows) and unidentified round, thick-walled structures containing small vesicles (marked with solid arrows). EM, × 46,000.

settled dust out of 7 examined. Both the structures corresponding to Gram-negative and Gram-positive bacteria were found. Gram-negative bacteria were lighter and released endotoxin-containing membrane vesicles (ECMVs) (Fig. 3) which formed large aggregations between remnants of plant tissues (Fig. 4-6). Structures corresponding to Gram-positive bacteria were covered with dark microfibrils (Fig. 7-8). Some of Gram-positive bacteria released unidentified rod-shaped structures ca. 80 nm long, covered also with fibrils (Fig. 8).

DISCUSSION

The results of this study indicate that hop growers are exposed to relatively low levels of bioaerosols compared to other agricultural workers. The concentrations of total airborne microorganisms found in the present study (median 9.16×10^3 cfu/m^3, range $2.08–129.58 \times 10^3$ cfu/m^3) were 1-4 orders of magnitude lower compared to those found in herb, flax or grain processing facilities, swine confinement buildings or cattle barns [6, 10, 11, 13, 15, 16, 20, 28, 43]. Nevertheless, on 9 out of 19 examined farms the concentration of total airborne microorganisms exceeded the Occupational Exposure Limit (OEL) value of 10^4 cfu/m^3 proposed by Malmros *et al.* [29], and on 1 farm the values of 5×10^4 cfu/m^3 proposed by Erman *et al.* [16] and 10^5 cfu/m^3 proposed by Dutkiewicz and Jabłoński [7]. Gram-negative bacteria were found only in the air of 6 out of 19 farms, but on 3 of them their concentration exceeded the OEL value of 10^3 cfu/m^3 proposed by Clark [3] and Malmros *et al.* [29] and on 1 the OEL value of 2×10^3 cfu/m^3 proposed by Dutkiewicz and Jabłoński [7]. The concentrations of fungi and termophilic actinomycetes nowhere exceeded the OEL values of 5×10^4 cfu/m^3 and 2×10^4 cfu/m^3 respectively, proposed by Dutkiewicz and Jabłoński [7].

Similarly low concentrations of bioaerosols in a hop processing plant were reported by the Russian researchers Aleksandrov and Gyeorgyev [1] in the late 70s. The authors suggested that this may be due to antimicrobial properties of hop. This explanation is in agreement with more recent studies conducted by Langezaal *et al.* [24] who demonstrated that hop extract reveals strong antimicrobial properties against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and fungi (*Trichophyton mentagrophytes*), but not against Gram-negative bacteria (*Escherichia coli*) and yeast (*Candida albicans*).

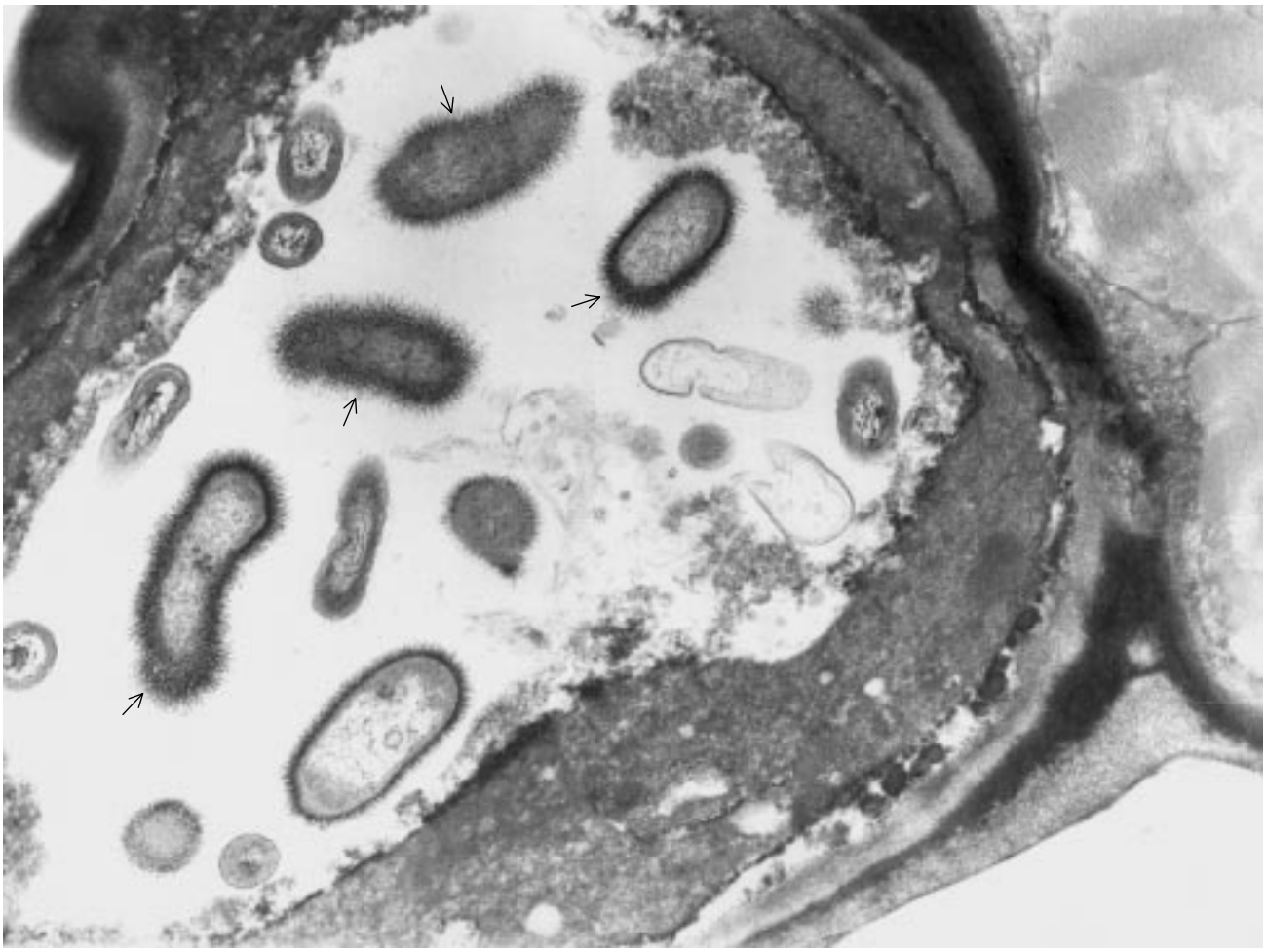

Figure 7. Thin-sectioned sample of settled hop dust, sampling site 2. Note structures corresponding to Gram-positive bacteria (corynebacteria) covered with dark microfibrils (marked with arrows), located inside a plant vesicle. EM, × 28,750.

In this study, the species composition of airborne microflora was characterised by the dominance of Gram-positive bacteria. The most common were corynebacteria, in particular *Corynebacterium* and *Aureobacterium* species. Corynebacteria are commonly associated with organic dusts and were isolated in large quantities from the air of animal farms [9, 28], herb processing plants [13], sawmills [12], potato processing plants [14] and during handling of grain and flax [11]. To date, little is known about the health effects of the environmental exposure to corynebacteria. Hagiwara *et al.* [17] reported a case of hypersensitivity pneumonitis caused by a domestic humidifier and suggested that *Aureobacterium liquefaciens* could be one of the causative agents. Milanowski *et al.* [33] described 8 cases of allergic alveolitis caused by *Arthrobacter globiformis* in agricultural workers exposed to grain dust. According to these authors, *Arthrobacter globiformis*, *Pantoea agglomerans* and *Alcaligenes faecalis* are the commonest offending agents causing allergic alveolitis among agricultural workers in eastern Poland. In our study, *Pantoea agglomerans* constituted 50% of the isolated Gram-negative bacteria, but occurred in relatively small quantities and therefore may cause lesser health hazard than in other work environments [7, 11, 33, 43].

The concentration of fungi noted in the air of examined hop processing farms was relatively low. The most common were strains of *Alternaria* and *Cladosporium*. This finding is in agreement with the results of other aerobiological studies conducted during handling grain, herbs and other plant products [11, 13, 20, 22, 43]. *Alternaria* and *Cladosporium* are classified among so-called "field fungi" which colonise plants during their growth and become a main component of organic dust in plant processing farms. *Alternaria*, *Cladosporium* and other fungal species detected in the air of hop farms (*Penicillium* spp., *Mucor* spp., *Aspergillus* spp.) pose potent allergenic and/or immunotoxic properties and may evoke allergic rhinitis, allergic alveolitis or decrease lung function in asthmatics [7, 21, 22, 26, 30].

The concentrations of dust and bacterial endotoxin observed in our study were much lower compared to those found in other agricultural environments [6, 10, 11, 13, 28, 35]. Nevertheless, the concentration of airborne dust exceeded the Polish OEL value of 4 mg/m^3 [37] on 9 out of 19 examined hop farms by 1.1–6.5 times.

The concentration of airborne endotoxin on hop farms ranged from 26.1–6250 ng/m^3. To date, there is no standard OEL for endotoxin, the results obtained in the

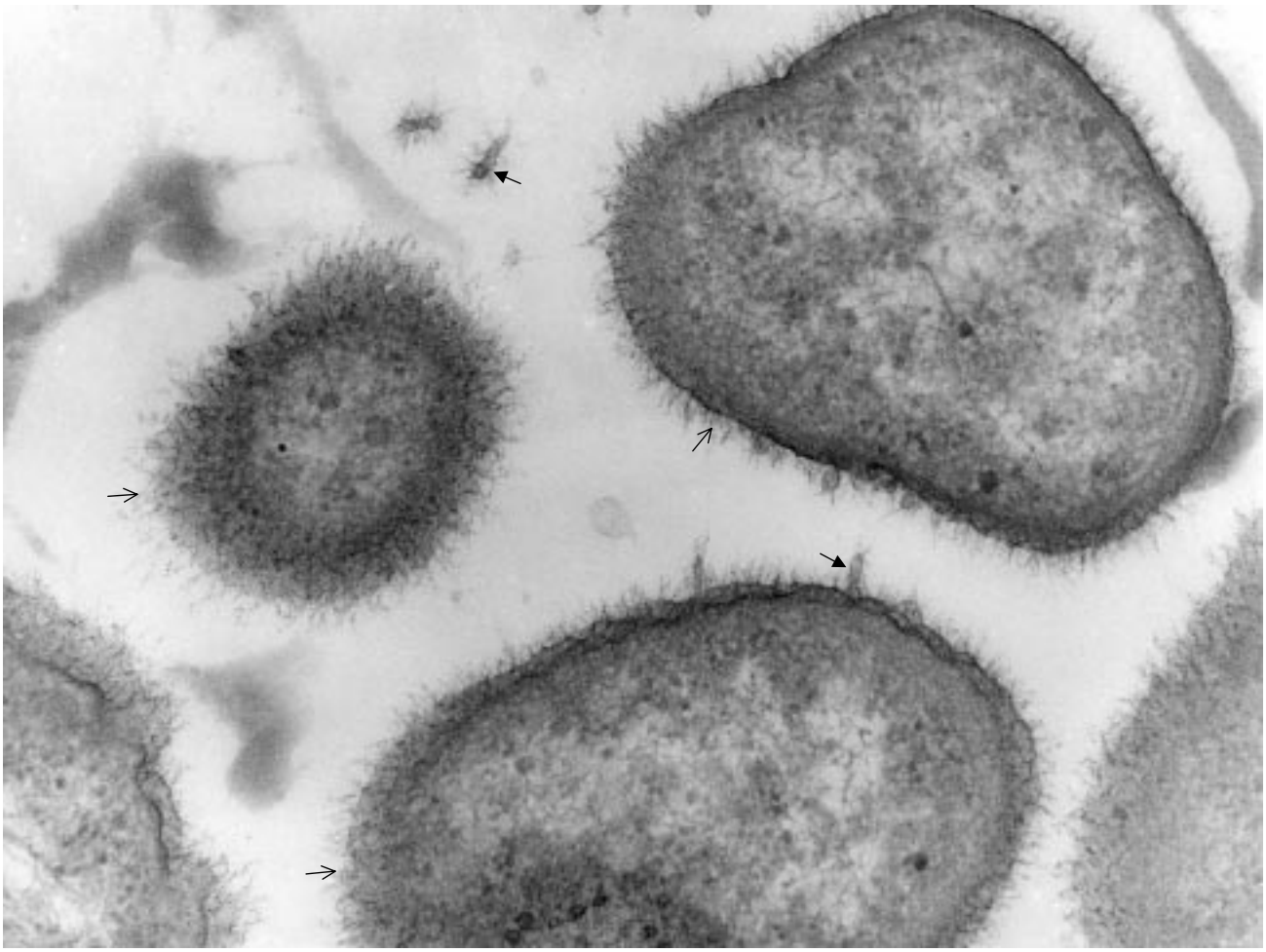

Figure 8. Thin-sectioned sample of settled hop dust, sampling site 4. Note structures corresponding to Gram-positive bacteria (corynebacteria) covered with microfibrils (marked with open arrows). Noteworthy are small, rod-shaped structures released by bacteria (marked with solid arrows) which are also covered with fibrils. EM, × 62,100.

present work may therefore be compared only to the proposals raised by several other authors. The concentrations of airborne endotoxin recorded on hop farms exceeded on all 19 farms the OEL values of 5 ng/m^3 and 25 ng/m^3 proposed respectively by DECOS [5] and Laitinen *et al.* [23], and on 7 farms, the level of 100 ng/m^3 proposed as an OEL by Clark [3], Rylander [38, 40] and Malmros *et al.* [29]. On 7 farms the concentrations of airborne endotoxin exceeded the value of 200 ng/m^3 supposed to cause a decrease of lung function during workshift [39], and on 2 farms the values of 1000–2000 ng/m^3 which are supposed to evoke ODTS symptoms [39].

The samples of settled hop dust contained relatively large concentrations of microorganisms of the order 10^5–10^8 cfu/g and very large concentrations of endotoxin of the order 10^2–10^5 µg/g (10^5–10^8 ng/g), approximating the highest values of endotoxin concentration reported hitherto for settled organic dusts [6, 7, 13]. The concentrations of microorganisms in the samples of settled dust collected from picking machines were higher compared to those derived from drying houses. This may by due to the climatic conditions in drying houses. High temperature (55–60ºC) and low humidity may damage the bacterial cells and therefore impair their growth on culture media. By contrast, thermostable endotoxin persists in damaged cells and may pose a potential risk to exposed workers.

The presence of bacteria and endotoxin in the samples of settled hop dust was confirmed by electron microscopy. The observed structures corresponded exactly to Gram-negative bacteria and endotoxin-containing membrane vesicles (ECMVs) which had been identified in organic dusts in the course of earlier studies [8, 34]. ECMVs are produced by the fragmentation of the outer membrane of Gram-negative bacteria in the form of characteristic spherical structures with a triple-tracked membrane, measuring on the average 30–50 nm [8]. Large quantities of ECMVs were found to be deposited in hop dust in the form of aggregations between remnants of plant tissues.

Besides Gram-negative bacteria and ECMVs, cell structures covered with dark microfibrils, corresponding morphologically to Gram-positive bacteria (specifically corynebacteria) [33] were observed in the samples of settled hop dust. It is noteworthy that some of Gram-positive bacteria released peculiar rod-shaped structures, covered also with fibrils.

CONCLUSIONS

1. The work-related exposure of hop farmers to bioaerosols is much lower compared to farmers cultivating grain and other crops.

2. Among microbial factors associated with hop dust, bacterial endotoxin and allergenic fungi pose a greatest potential hazard for exposed hop farmers.

Acknowledgements

The skillful assistance of Ms. Wiesława Lisowska, Ms. Halina Wójtowicz and Eng. Grażyna Cholewa at performing the study is gratefully acknowledged. The study was supported by the Polish State Committee for Scientific Research (Grant No. 4PO5D 03719).

REFERENCES

1. Aleksandrov LA, Gyeorgyev VG: Voprosy gigieny truda khmelevodov pri ruchnoi uborke i sushke khmela. *Gig Truda Prof Zabol* 1977, **12**, 46-47.

2. Barron GL: *The Genera of Hyphomycetes from Soil*. Williams & Wilkins, Baltimore 1968.

3. Clark CS: Report on prevention and control. **In:** Rylander R, Peterson Y, Donham KJ (Eds): Health Effects of Organic Dusts in the Farm Environment. Proceedings of an International Workshop held in Skokloster, Sweden, April 23-25, 1985. *Am J Ind Med* 1986, **10**, 267-273.

4. Dubiel Ł: Stosowanie chmielu w polskim przemyśle piwowarskim. Część I. *Przem Ferment Owoc Warzyw* 1992, **6**, 41-43.

5. Dutch Expert Committee on Occupational Standards (DECOS): *Endotoxins, Health-Based Recommended Occupational Exposure Limit*. Gezondheidsraad, The Netherlands 1998.

6. Dutkiewicz J: Microbial hazards in plants processing grain and herbs. **In:** Rylander R, Peterson Y, Donham KJ (Eds): Health Effects of Organic Dusts in the Farm Environment. Proceedings of an International Workshop held in Skokloster, Sweden, April 23-25, 1985. *Am J Ind Med* 1986, **10**, 300-302.

7. Dutkiewicz J, Jabłoński L: Biologiczne Szkodliwości Zawodowe. PZWL, Warszawa 1989.

8. Dutkiewicz J, Tucker J, Burrell R, Olenchock SA, Schwegler-Berry D, Keller III GE, Ochalska B, Kaczmarski F, Skórska C: Ultrastructure of endotoxin produced by Gram-negative bacteria associated with organic dust. *System Appl Microbiol* 1992, **15**, 474-485.

9. Dutkiewicz J, Pomorski ZJH, Sitkowska J, Krysińska-Traczyk E, Skórska C, Prażmo Z, Cholewa G, Wójtowicz H: Airborne microorganisms and endotoxin in animal houses. *Grana* 1994, **33**, 185-190.

10. Dutkiewicz J: Bacteria and fungi in organic dust as potential health hazard. **In:** Midtgård U, Poulsen OM (Eds): Waste Collection and Recycling - Bioaerosol Exposure and Health Problems. Proceedings of an International Meeting held in Køge, Denmark, 13-14 September 1996. *Ann Agric Environ Med* 1997, **4**, 11-16.

11. Dutkiewicz J, Krysińska-Traczyk E, Skórska C, Sitkowska J, Prażmo Z, Urbanowicz B: Exposure of agricultural workers to airborne microorganisms and endotoxin during handling of various vegetable products. *Aerobiologia* 2000, **16**, 193-198.

12. Dutkiewicz J, Krysińska-Traczyk E, Prażmo Z, Skórska C, Sitkowska J: Exposure to airborne microorganisms in Polish sawmills. *Ann Agric Environ Med* 2001, **8**, 71-80.

13. Dutkiewicz J, Krysińska-Traczyk E, Skórska C, Sitkowska J, Prażmo Z, Golec M: Exposure to airborne microorganisms and endotoxin in herb processing plants. *Ann Agric Environ* Med 2001, **8**, 201-211.

14. Dutkiewicz J, Krysińska-Traczyk E, Skórska C, Cholewa G, Sitkowska J: Exposure to airborne microorganisms and endotoxin in a potato processing plant. *Ann Agric Environ Med* 2002, **9**, 225-235.

15. Eduard W: Exposure to non-infectious microorganisms and endotoxins in agriculture. *Ann Agric Environ Med* 1997, **4**, 179-186.

16. Erman MI, Eglite ME, Olefir AI, Kalinina LN: Aerogennaya mikroflora zhivotnovodcheskikh proizvodstvennykh pomeshchennyi, kriterii eyo vrednogo deistva i gigyenicheskaya reglamentatsia. *Gig Truda Prof Zabol* 1998, **4**, 19-22.

17. Hagiwara S, Ishii Y, Sugiyama Y, Kitamura S: Hypersensitivity pneumonitis caused by a home humidifier. *Nihon Kyobu Shikkan Gakkai Zassahi* 1995, **33**, 1024-1029.

18. Hagmar L, Schütz A, Hallberg T, Sjöholm A: Health effects of exposure to endotoxin and organic dust in poultry slaughter-house workers. *Int Arch Occup Environ Health* 1990, **62**, 159-164.

19. Krieg NR, Holt JG (Eds): *Bergey's Manual of Systematic Bacteriology*. Vol. 1. Williams & Wilkins, Baltimore 1984.

20. Krysińska-Traczyk E, Dutkiewicz J, Skórska C, Prażmo Z, Cisak E, Sitkowska J, Cholewa G: Narażenie zawodowe rolników indywidualnych na bioaerozole występujące w pyłach z ziela tymianku. *Med Ogólna* 1999, **5**, 186-192.

21. Lacey J, Crook B: Fungal and actinomycete spores as pollutants of the workplace and occupational allergens. *Ann Occup Hyg* 1988, **32**, 515-533.

22. Lacey J, Dutkiewicz J: Bioaerosols and occupational lung disease. *J Aerosol Sci* 1994, **25**, 1371-1404.

23. Laitinen S, Kangas J, Husman K, Susitaival P: Evaluation of exposure to airborne bacterial endotoxins and peptidoglycans in selected work environments. *Ann Agric Environ Med* 2001, **8**, 213-219.

24. Langezaal CR, Chandra A, Scheffer JJ: Antimicrobial screening of essential oils and extracts of some *Humulus lupulus* L. cultivars. *Pharm Weekbl Sci* 1992, **14**, 353-356.

25. Levin J, Bang FB: The role of endotoxin in the extracellular coagulation of *limulus* blood. *Bull Johns Hopkins Hosp* 1964, **115**, 265-274.

26. Licorish K, Novey H, Kozak P, Fairshelter R, Wilson A: Role of *Alternaria* and *Penicillium* spores in pathogenesis of asthma. *J Allergy Clin Immunol* 1985, **76**, 819-825.

27. Litvinov MA: *Opredelitel' Mikroskopicheskikh Pochvennykh Gribov*. Izd. Nauka, Leningrad 1967.

28. Mackiewicz B: Study on exposure of pig farm workers to bioaerosols, immunologic reactivity and health effects. *Ann Agric Environ Med* 1998, **5**, 169-175.

29. Malmros P, Sigsgaard T, Bach B: Occupational health problems due to garbage sorting. *Waste Manag Res* 1992, **10**, 227-234.

30. Marchisio VF, Sulotto F, Botta GC, Chiesa A, Airaudi D, Anastasi A: Aerobiological analysis in a salami factory: a possible case of extrinsic allergic alveolitis by *Penicillium camembertii*. *Med Mycol* 1999, **37**, 285-289.

31. Migdal J: Rejonizacja upraw chmielu w Polsce. *Przem Ferment Owoc Warzyw* 2001, **8**, 41-43.

32. Migdal J (Ed): *Chmielarskie ABC*. Instytut Uprawy Nawożenia i Gleboznawstwa, Puławy 1995.

33. Milanowski J, Dutkiewicz J, Potoczna H, Kuś L, Urbanowicz B: Allergic alveolitis among agricultural workers in eastern Poland: A study of twenty cases. *Ann Agric Environ Med* 1998, **5**, 31-43.

34. Prażmo Z, Krysińska-Traczyk E, Skórska C, Sitkowska J, Cholewa G, Urbanowicz B, Dutkiewicz J: Birch wetwood as a source of potential bacterial hazard for woodworkers. *Ann Agric Environ Med* 1996, **3**, 67-70.

35. Radon K, Danuser B, Iversen M, Monso E, Weber C, Hartung J, Donham KJ, Palmgren U, Nowak D: Air contaminants in different European farming environments. *Ann Agric Environ Med* 2002, **9**, 41-48.

36. Radon K, Opravil U, Hartung J, Szadkowski D, Nowak D: Work-related respiratory disorders and farming characteristics among cattle farmers in Northern Germany. *Am J Ind Med* 1999, **36**, 444-449.

37. Rozporządzenie Ministra Pracy i Polityki Socjalnej z dnia 17 czerwca 1998 r. w sprawie najwyższych dopuszczalnych stężeń i natężeń czynników szkodliwych dla zdrowia w środowisku pracy. Dz. U. 1998, nr 79, poz. 513. Warszawa 1998.

38. Rylander R: The role of endotoxin for reactions after exposure to cotton dust. *Am J Ind Med* 1987, **12**, 687-697.

39. Rylander R: Organic dusts - from knowledge to prevention. *Scand J Work Environ Health* 1994, **20**, 116-122.

40. Rylander R: Evaluation of the risks of endotoxin exposure. **In:** Rylander R (Ed): Endotoxin in Environment: a Criteria Document. *Int J Occup Environ Health* 1997, **3**, s32-s36.

41. Schulz J, Überhuber E: *Leki z Bożej Apteki*. Znaki Czasu, Warszawa 1985 (in Polish).

42. Sneath PHA, Mair N, Sharpe ME, Holt JG (Eds): *Bergey's Manual of Systematic Bacteriology*. Vol. 2. Williams & Wilkins, Baltimore 1986.

43. Swan JRM, Crook B: Airborne microorganisms associated with grain handling. *Ann Agric Environ Med* 1998, **5**, 7-15.

44. Williams ST, Sharpe ME, Holt JG (Eds): *Bergey's Manual of Systematic Bacteriology*. Vol. 4. Williams & Wilkins, Baltimore 1989.