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EXPOSURE TO AIRBORNE MICROORGANISMS IN POLISH SAWMILLS

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Abstract: Microbiological air sampling was performed in four sawmills located in eastern Poland, of which two were processing coniferous wood (pine, fir) and other two deciduous wood (oak, birch). Total concentration of microorganisms (bacteria and fungi) in the air of sawmills processing coniferous wood was on average $20.2 \pm 5.6 \times 10^3$ cfu/m^3 (mean ± S.E.) and significantly ($p < 0.05$) higher compared to those processing deciduous wood where the mean concentration of airborne microorganisms was $9.8 \pm 3.0 \times 10^3$ cfu/m^3. The greatest concentrations of microorganisms in the sawmills processing coniferous wood were noted at debarking and at first-cut frame sawing of pine logs ($42.1 \pm 7.6 \times 10^3$ cfu/m^3 and $39.8 \pm 7.0 \times 10^3$ cfu/m^3, respectively). Microflora released into air during debarking consisted mostly of allergenic fungi (mainly *Aspergillus fumigatus*) and corynebacteria, whereas airborne microflora recovered during first-cut frame sawing constituted mostly of endotoxin-producing Gram-negative bacteria of the genus *Rahnella*, developing in the sapwood of pine. In the sawmills processing deciduous wood, the largest concentration of microorganisms ($30.6 \pm 3.4 \times 10^3$ cfu/m^3) was found at sorting of the oak parquet boards and was due to the secondary infection of the boards with moulds *Penicillium citrinum* during prolonged storing in the open air. Values of the respirable fraction of airborne microflora in the examined sawmills varied within fairly wide limits and were between 22.5–86.6%. Altogether, 34 species or genera of bacteria and 21 species or genera of fungi were identified in the air of sawmills, of which respectively 13 and 9 species or genera were reported as having allergenic and/or immunotoxic properties. The concentrations of airborne bacterial endotoxin which were determined on two sampling sites in the sawmills processing pine and fir, were 0.24 µg/m^3 and 4.00 µg/m^3 respectively, distinctly exceeding the suggested safe level. In conclusion, the workers of Polish sawmills may be exposed on some working stands to airborne microorganisms posing respiratory hazard, of which the greatest risk is represented by allergenic fungi developing on bark of logs or stored wood products and endotoxin-producing Gram-negative bacteria of the genus *Rahnella*, developing in sapwood of coniferous logs.

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Key words: sawmills, woodworkers, occupational exposure, wood dust, bioaerosols, bacteria, fungi, endotoxin, *Rahnella*.

INTRODUCTION

Sawmill workers may be exposed at work to the inhalation of various allergenic and immunotoxic agents, comprising wood derivatives (e.g. terpenes, resin acids) and microorganisms associated with timber [8, 9, 11, 30, 33, 64]. They cause decrease in lung function, bronchial hyperresponsiveness and respiratory disorders, such as: organic dust toxic syndrome (ODTS), allergic alveolitis, asthma, non-asthmatic chronic airflow obstruction, chronic bronchitis, mucous membrane irritation syndrome (MMI) and rhinitis [5, 12, 30, 31, 34, 50, 51, 58, 64, 69]. Microorganisms and their products known as potential causative agents of these disorders (Gram-negative

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bacteria, endotoxin, mould fungi, (1→3)-β-D-glucans) has been detected in the air of sawmills in the course of studies performed on different continents: Africa [1], Australia [2, 50, 51], Europe [27, 28, 37, 40, 65] and North America [10, 11, 13].

The concentration and composition of airborne microflora in sawmills may vary to a great degree depending on the kind of timber being processed and the technology of production [11, 50, 51]. The pollution of air with microorganisms results from the primary or secondary infection of timber [19, 27, 58]. The primary infection develops in timber logs stored in forests and in lumber yards, initially with bacteria (described as “pioneer organisms”) and then with fungi which may eventually cause wood decay [32, 39, 46, 59]. A study of six kinds of stored wood performed in the USA has shown that bark usually contains large amounts of Gram-positive bacteria and moulds while inner wood (sapwood and heartwood) contains great quantities of Gram-negative bacteria and yeasts [21, 63]. Secondary infection of wood proceeds on chopped wood (chips, planks) which are stored in sawmills and other wood processing facilities in conditions favouring microbial growth. It is characterized by abundant growth of moulds and often causes respiratory illnesses in workers [27, 28, 37, 40, 58].

The aim of the present work was to study the concentration and species composition of the microflora of air in Polish sawmills processing coniferous and deciduous wood.

MATERIALS AND METHODS

Examined sawmills. Air sampling was performed in four sawmills located in eastern Poland, of which two (“A” and “B”) were processing coniferous wood and other two (“C” and “D”) were processing deciduous wood. The plants included (bracketed figures indicate numbers of sampling sites in each facility): sawmill “A” processing wood of Scots pine (*Pinus sylvestris*) (6), sawmill “B” processing wood of silver fir (*Abies alba*) (1), sawmill “C” processing wood of English oak (*Quercus robur*) (3), and sawmill “D” processing wood of English oak (*Quercus robur*) and white warty birch (*Betula verrucosa*) (5). The sampling sites in subsequent sawmills were marked as: A1-A6, B1, C1-C3 and D1-D5. In the sawmills “A”, “B”, “C” and “D” were employed 43, 18, 33 and 57 workers, respectively.

In sawmill “A” the air samples were taken, in the sequence of production cycle, at following sites: manual sawing of long pine logs into shorter blocks (A1); machine debarking of logs (A2); first-cut frame sawing, removing sapwood (A3); cellar under sawing machines, housing conveyor belt removing sawdust (A4); second-cut frame sawing, slicing heartwood core into planks (A5); plank trimming (A6). In sawmill “B”, the samples were taken at only one site, at first-cut frame sawing of fir logs (B1). Except for site “A1”, all samples in sawmills “A” and “B” were taken indoors.

In sawmill “C”, the samples were taken at the following sites: frame sawing of oak logs (C1); multi-stage machine, sawing planks into raw parquet boards (C2); sorting of raw parquet boards stacked in piles, earlier stored in the open air for 6-9 months (C3). In sawmill “D”, the samples were taken at the following sites: cutting of oak logs for veneer (D1); feeding oak veneer to a dryer (D2); trimming of dried oak veneer (D3); machine finishing oak parquet boards (D4); multi-stage machine, manufacturing floor mosaic boards from birch wood (D5). All samples in sawmills “C” and “D” were taken indoors.

Microbiological examination of the air. Air samples were taken in sawmills with a custom-designed particle-sizing slit sampler [15] enabling estimations of both total and respirable fractions of the microbial aerosol (Polish Patent 87612 assigned on 6 June 1977). Each air sample was a duplicate, taken at a flow rate of 20 l/min. It consisted of two parallelly exposed agar plates: one "a" sampled directly for all organisms and used for the estimation of the total concentration of colony forming units (cfu) per m^3; and another “b” sampled through a pre-selector (consisting of a system of glass tubes and regulated deposition disks covered with a sticky substance) for the respirable fraction. The value of respirable fraction was expressed as a percent (%) of the total count, calculated by division of the number(s) of cfu on plate(s) “b” through the number(s) of cfu on plate(s) “a” and multiplication by 100. The median cut point for the respirable fraction was 3.0 μm, approximating the recommendations of the American Conference of Governmental Industrial Hygienists [66]. The used sampler enables the determinations of concentrations of microorganisms in the air in the range of 10^0-10^8 cfu/m^3.

On each sampling site, series of five double samples were taken on each of the following agar media: blood agar for total mesophilic Gram-negative and Gram-positive bacteria, whey agar for lactobacilli, half-strength tryptic soya agar for thermophilic actinomycetes and malt agar for fungi. The blood agar plates were subsequently incubated for one day at 37°C, then three days at 22°C and finally three days at 4°C. The malt agar plates were subsequently incubated for four days at 30°C and four days at 22°C [16]. The prolonged incubation at lower temperatures aimed to isolate as wide a spectrum of bacteria and fungi as possible. The whey agar plates were incubated as the blood agar plates and the tryptic soya agar plates were incubated for five days at 55°C. The grown colonies were counted and differentiated and the data were reported as cfu per one cubic meter of the air (cfu/m^3). The total concentration of microorganisms in the air was obtained by the addition of the concentrations of mesophilic bacteria, thermophilic actinomycetes and fungi. The percent composition of the total microflora of the air was then determined.

Bacterial isolates were identified with microscopic and biochemical methods, as recommended by Bergey's Manual [41, 62, 68] and Cowan & Steel [7]. 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 classified with microscopic methods, according to Barron [4], Larone [44], Litvinov [47], Ramirez [56], and Raper & Fennell [57].

On two sampling sites (A3, B1) the concentration of dust and endotoxin in the air was determined. The air samples were collected on the polyvinyl chloride filters by the use of an AS-50 one-stage sampler (TWOMET, Zgierz, Poland). Two samples were taken at each sampling site. The concentration of dust in the air was estimated gravimetrically. The concentration of bacterial endotoxin in the airborne dust was determined by the *Limulus* amebocyte lysate gel tube test (LAL) [45]. The filters were extracted for one hour in 10 ml of pyrogen-free water at room temperature, heated to 100°C in a Koch apparatus for 15 min (for better dissolving of endotoxin and inactivation of interfering substances), and after cooling, serial dilutions were prepared. The 0.1 ml dilutions were mixed equally with the "Pyrotell" *Limulus* reagent (Associates of Cape Code, Inc., Woods Hole, Mass., USA). The test was incubated for one hour in a water bath at 37°C, using pyrogen-free water as a negative control and the commercial lipopolysaccharide (endotoxin) of *Escherichia coli* 0111:B4 (Difco Laboratories, Detroit, USA) as positive control. The formation of a stable clot was regarded as a positive result. The estimated concentration of endotoxin in dust (ng/mg) was multiplied per estimated concentration of dust in the air (mg/m^3) and the results were reported as micrograms of the equivalents of the *E. coli* 0111:B4 endotoxin per 1 m^3 of air. To convert to Endotoxin Units (EU), multiply the value in nanograms by 1.2 [53].

The study was performed mostly during the years 1981-1986 and continued during 1995-2000. Preliminary results of this work have been reported elsewhere [17, 19, 23].

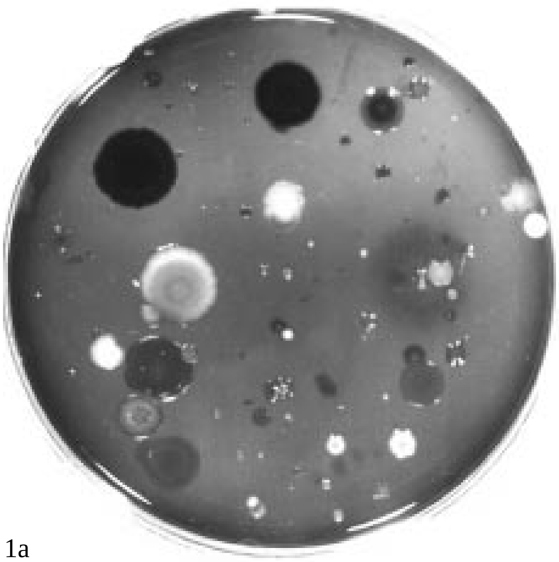
RESULTS

The concentrations of microorganisms in the air of sawmills processing coniferous wood are presented in Table 1 and composition of microflora depicted in Figure 1. In the course of production cycle in the pine processing sawmill, noteworthy changes both in the concentration and composition of airborne microflora could be observed. At the initial stage, the transverse cutting of long logs in the open air, the concentration of microorganisms in the air was very low. At debarking, the airborne microflora increased about six times up to the level of 4.2 × 10^4 cfu/m^3 and was clearly dominated by two groups of microorganisms: corynebacteria (mostly *Corynebacterium* spp.) forming nearly 50% of the total count, and fungi

Table 1. Microorganisms in the air of sawmills processing coniferous wood: concentrations and respirable fractions (Rf).

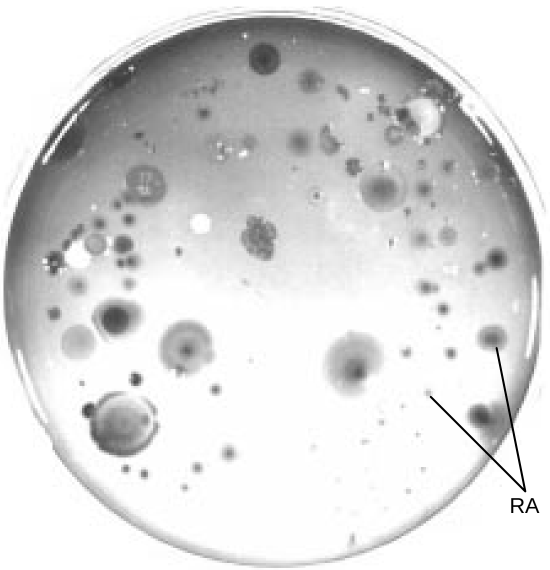
Plant, sampling site	Mesophilic bacteria		Thermophilic actinomycetes		Fungi		Total microorganisms[a]	
	Concentration (mean ± S.E., cfu/m^3 × 10^3)	Rf (%)	Concentration (mean ± S.E., cfu/m^3 × 10^3)	Rf (%)	Concentration (mean ± S.E., cfu/m^3 × 10^3)	Rf (%)	Concentration (mean ± S.E., cfu/m^3 × 10^3)	Rf (%)
Sawmill "A" processing pine								
A1. Transverse cutting of logs	5.2 ± .0.8	50.9	0.4 ± 0.2	0	1.6 ± 0.8	75.0	7.2 ± 1.1	53.5
A2. Debarking room	26.4 ± 3.7	34.1	0.3 ± 0.3	0	15.4 ± 11.8	11.7	42.1 ± 7.6	25.7
A3. First-cut frame sawing	36.2 ± 7.3	19.7	0.3 ± 0.3	100	3.3 ± 0.7	45.5	39.8 ± 7.0	22.5
A4. Cellar under sawing machines	15.6 ± 2.7	23.8	0.2 ± 0.2	100	3.5 ± 0.6	20.7	19.3 ± 2.7	23.8
A5. Second-cut frame sawing	8.4 ± 1.0	48.9	0.3 ± 0	0	2.0 ± 0.6	45.5	10.7 ± 1.0	46.9
A6. Trimming of planks	6.2 ± 2.3	70.2	0.1 ± 0.1	0	1.3 ± 0.6	100	7.6 ± 2.2	86.6
Sawmill "B" processing fir								
B1. First-cut frame sawing	12.7 ± 0.6	37.3	0		2.0 ± 1.3	54.5	14.8 ± 1.5	39.2
Mean	15.8 ± 4.4	40.7	0.2 ± 0.1	33.3	4.2 ± 1.9	50.4	20.2 ± 5.6	42.6

[a]Samples for lactobacilli taken on whey agar were negative in all cases except for small amount detected at frame sawing fir (mean ± S.E = 0.1 ± 0.1 cfu/m^3 × 10^3, Rf = 0).

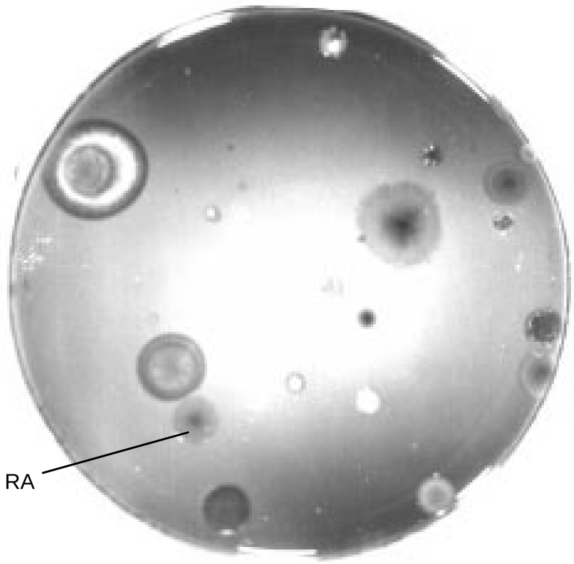


1a

1b



2a



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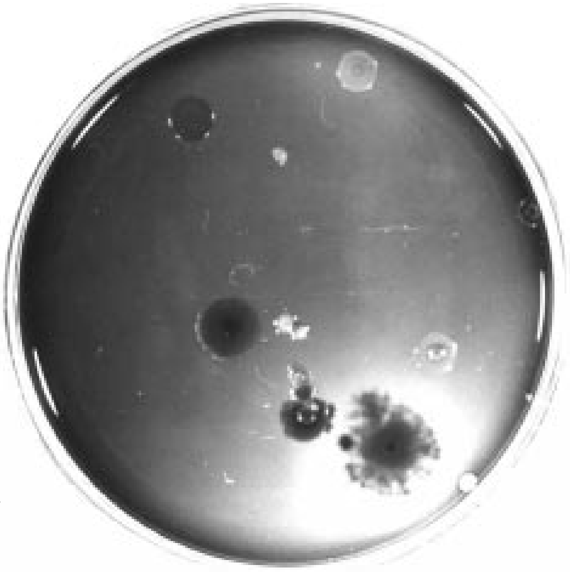
3a

3b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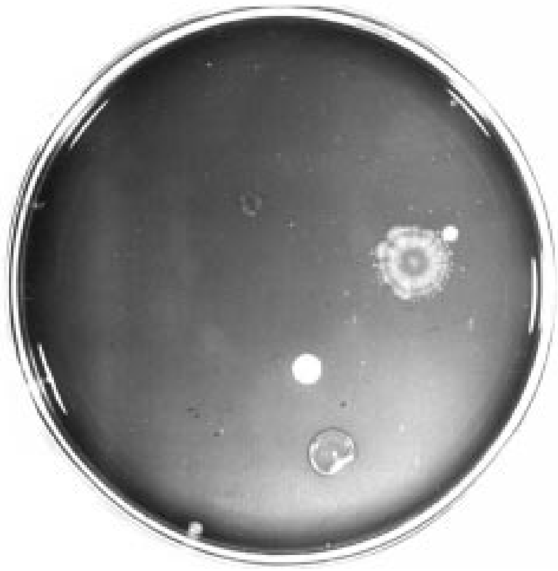
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Figure 1. Photographs of air samples for mesophilic bacteria taken in sawmill "A" processing pine at following sites: 1a-1b debarking room; 2a-2b first-cut frame sawing; 3a-3b second cut frame sawing; 4a-4b trimming of planks. The samples were taken using particle-sizing sampler on blood agar plates, each in volume of 3.33 l. Photographs 1a, 2a, 3a, 4a show total bacterial flora of the air, while photographs 1b, 2b, 3b, 4b show the respirable fraction. It may be seen that the concentration of bacteria in the air was high at initial stages of wood processing (debarking, first-cut sawing) and decreased in the further stages. Corynebacteria, growing in the form of colonies of uniform consistency of variable size and colour, dominated during debarking, whereas during first-cut frame sawing Gram-negative bacteria of the genus *Rahnella* prevailed, growing in the form of characteristic glistening, transparent colonies with dark centre (indicated by letters "RA").

(mostly *Aspergillus fumigatus*) forming 30% of the total count (Tab. 1, Figs 1–2). A high level of microbial pollution (approximating 4.0×10^4 cfu/m^3) persisted at the next production stage, the first-cut frame sawing; however, the composition of airborne microflora was quite different compared to the former stage. The dominant organisms were Gram-negative bacteria, released into air with the pine sapwood sawdust. They formed 55% of the total count and the dominant genus among them was *Rahnella* (*Rahnella aquatilis, Rahnella* spp.) which alone formed nearly half (47.9%) the total count (Tab. 1, Figs 1–2, 4). At the next production stages, the second-cut frame sawing

Table 2. Microorganisms in the air of sawmills processing deciduous wood: concentrations and respirable fractions (Rf).

Plant, sampling site	Mesophilic bacteria		Thermophilic actinomycetes		Fungi		Total microorganisms[a]	
	Concentration (mean ± S.E., cfu/m^3 × 10^3)	Rf (%)	Concentration (mean ± S.E., cfu/m^3 × 10^3)	Rf (%)	Concentration (mean ± S.E., cfu/m^3 × 10^3)	Rf (%)	Concentration (mean ± S.E., cfu/m^3 × 10^3)	Rf (%)
Sawmill "C" processing oak								
C1. Frame sawing	5.1 ± 0.6	41.4	0.6 ± 0.3	0	0.8 ± 0.3	0	6.5 ± 0.6	32.6
C2. Making of parquet boards	8.3 ± 1.0	41.2	0.3 ± 0	0	1.3 ± 0.1	18.2	9.9 ± 1.0	36.9
C3. Sorting of parquet boards	13.2 ± 2.5	36.2	0.1 ± 0.1	0	17.3 ± 5.9	50.5	30.6 ± 3.4	44.2
Sawmill "D" processing oak and birch								
D1. Cutting of oak logs for veneer	3.3 ± 0.2	74.5	0.1 ± 0.1	0	2.0 ± 0.9	24.2	5.4 ± 0.4	54.7
D2. Drying of oak veneer	3.7 ± 0.2	78.9	0		0.8 ± 0.2	57.1	4.5 ± 0.3	74.8
D3. Trimming of oak veneer	4.3 ± 0.4	60.8	0.1 ± 0.1	0	0.6 ± 0.1	40.0	5.5 ± 0.4	55.2
D4. Making of oak parquet boards	2.7 ± 0.3	80.0	0.1 ± 0.1	100	4.0 ± 1.7	47.0	6.8 ± 1.1	60.9
D5. Making of floor mosaic boards from birch wood	4.0 ± 0.6	57.6	0.3 ± 0.2	0	4.6 ± 0.8	48.7	8.9 ± 0.9	52.8
Mean	5.6 ± 1.2	58.8	0.2 ± 0.1	14.3	3.9 ± 2.0	35.7	9.8 ± 3.0	51.5

[a]Samples for lactobacilli taken on whey agar were negative in all cases except for small amount detected at trimming of oak veneer (mean ± S.E = 0.5 ± 0.2 cfu/m^3 × 10^3, Rf = 33.3%).

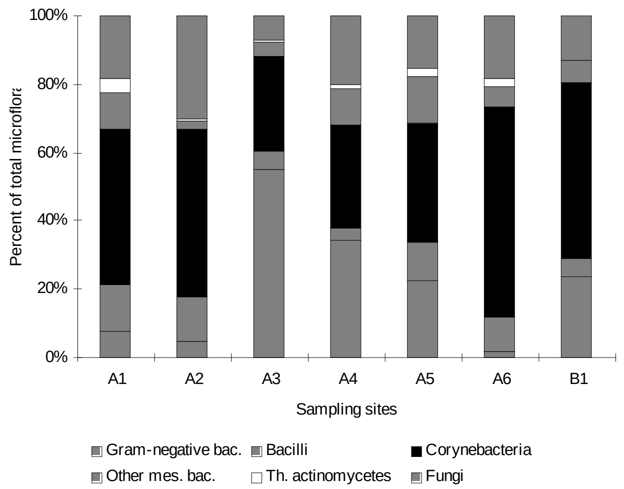

Figure 2. Composition of airborne microflora in sawmills processing coniferous wood (total count, including mesophilic bacteria, thermophilic actinomycetes and fungi).

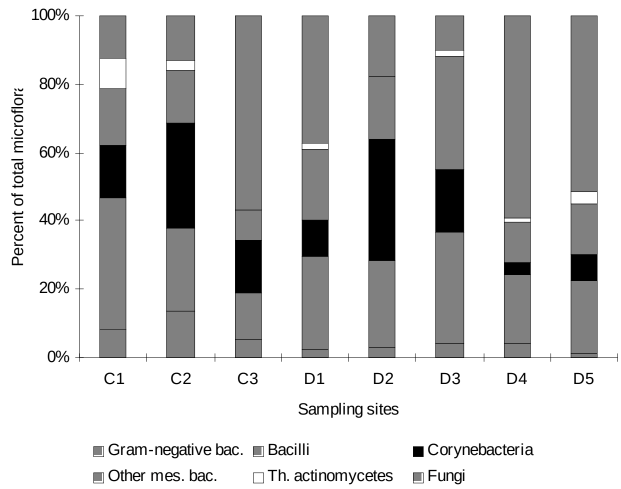

Figure 3. Composition of airborne microflora in sawmills processing deciduous wood (total count, including mesophilic bacteria, thermophilic actinomycetes and fungi).

and trimming of planks, a sharp decrease in the concentration both of total microorganisms and Gram-negative bacteria was noted (Tab. 1, Figs 1-2). The mean concentration of microorganisms in the sawmill processing fir was lower compared to that processing pine (Tab. 1). Corynebacteria prevailed in the air of the fir processing sawmill, forming about 50% of the total count, and Gram-negative bacteria constituted 25% of the total. Generally, the most common organisms in the air of the sawmills processing coniferous wood were corynebacteria. Thermophilic actinomycetes occurred in very small amounts and lactobacilli were detected in trace quantity only on one sampling site.

The concentrations of microorganisms in the air of the sawmills processing deciduous wood were distinctly lower compared to those processing coniferous wood (Tab. 2) and the difference proved to be statistically significant (t-test, $p < 0.05$). Out of eight sampling sites in two oak processing sawmills, the highest concentration of microorganisms was found at sorting of parquet boards (3.2×10^4 cfu/m^3) (Tab. 2). This was due to the secondary infection of the boards, stored earlier in the open air, by the

Table 3. List of microbial species and genera identified in the samples of air from sawmills.

Gram-negative bacteria: ***Acinetobacter calcoaceticus**+** (B, C, D2, D3), ***Alcaligenes faecalis**+** (A2, A5)**,** ***Enterobacter* spp.**+ (A3-A5, B, C1, C3, D1), ***Pantoea agglomerans**+ (synonyms: *Erwinia herbicola, Enterobacter agglomerans*)** (A1, A3, A5), *Pseudomonas* spp. (C3), ***Rahnella aquatilis*+** (A3-A5, B), ***Rahnella* spp.**+ (A2-A5, B).

Bacilli: *Bacillus cereus* (A, C), *Bacillus megaterium* (A, C, D), ***Bacillus subtilis*** * (A, B, C, D), *Bacillus licheniformis* (C), *Bacillus* spp. (A, B, C, D).

Corynebacteria: ***Arthrobacter globiformis*** * (A, B, C2, C3), *Arthrobacter* spp. (A, B, C, D), ***Brevibacterium linens*** * (A, B, D2, D3), *Brevibacterium helvolum* (A, C3), *Corynebacterium pseudodiphteriticum* (A5, C1, C3, D3), *Corynebacterium xerosis* (C1), *Corynebacterium* spp. (A, B, C, D), *Microbacterium lacticum* (A, B, C3).

Other mesophilic bacteria: *Lactobacillus* spp. (B, D3), *Micrococcus luteus* (A), *Micrococcus* spp. (A, B, C, D1-D4), *Nocardia* spp. (A1), *Rhodococcus* spp. (C2), *Staphylococcus epidermidis* (A, D), *Staphylococcus* spp. (A, B, C, D), *Streptococcus lactis* (C3), *Streptococcus* spp. (A5, C, D1-D3), ***Streptomyces albus*** * (A1, A2, A4), *Streptomyces* spp. (A, B, C, D).

Thermophilic actinomycetes: ***Saccharomonospora viridis*** * (A1, A3, A4), ***Saccharopolyspora rectivirgula*** * **(synonyms: *Faenia rectivirgula, Micropolyspora faeni*)** (A3), ***Thermoactinomyces vulgaris**** (A, C, D3-D5).

Fungi: ***Alternaria alternata**+** (A1-A4, A6, C), ***Aspergillus candidus*** *+ (A1, A3-A6, C3), ***Aspergillus fumigatus**+** (A, B, C1, D3, D6)**,** *Aspergillus repens* (A1, A3-A5), *Botryotrichum* spp. (A1), *Botrytis cinerea* (D5), ***Candida* spp.*** (A, B), *Cephalosporium glutineum* (C3, D1-D5), *Geotrichum candidum* (A3-A5), *Monosporium olivaceum* (A1-A2), *Monosporium* spp. (A1), ***Mucor* spp**.* (A1, A4, B, C1, C2, D1, D3-D5), *Paecilomyces* spp. (A2, D4), ***Penicillium citrinum*** *+ (C3), ***Penicillium* spp.*+** (A, C, D1-D3, D5)**,** *Rhinocladiopsis* spp. (A3), ***Rhizopus nigricans*** * + (A5), *Rhodotorula* spp. (C1, C2), *Trichoderma album* (B), ***Trichoderma viride*** * (A1, A2, A6, B, C3, D5), *Trichothecium roseum* (A5, A6).

Sites of isolation are given in parentheses. Quoting only the letter attributed to particular sawmill (“A”, “B”, “C” or “D”, without numbers) means that the species was isolated from all sampling sites within sawmill. The 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.

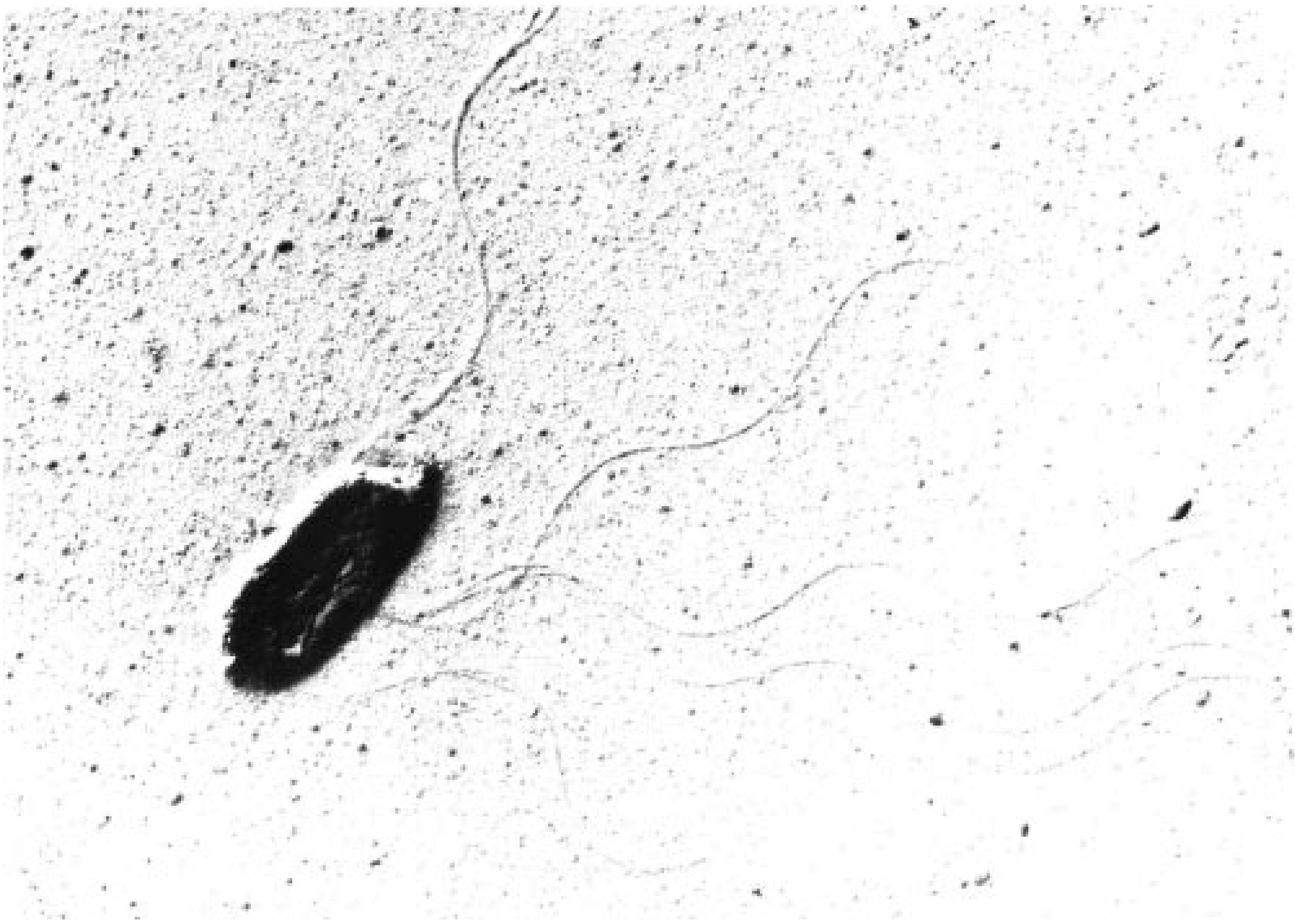

Figure 3. Peritrichous bacterium determined as Rahnella sp. isolated from the air of sawmill “A” processing pine at first-cut frame sawing (strain TR-2). EM preparation shadowed with silica oxide, × 17,000. Photograph by Dr Barbara Urbanowicz, Laboratory of Electron Microscopy, Institute of Pediatrics, Collegium Medicum of Jagiellonian University, Kraków.

fungi of the species *Penicillium citrinum* which constituted 52.3% of the total microflora of the air (Fig. 3). It was the only case of secondary infection of wood products found in the course of the present study. It was confirmed by the dilution plating analysis of the samples of settled dust from the room where parquet boards were sorted (data not shown) which revealed a high contamination of dust with the spores of *Penicillium citrinum* (1.2×10^7 cfu/g, 97.2% of total microflora). At the remaining seven sampling sites in the sawmills processing deciduous wood, the total concentration of microorganisms was low and never exceeded the level of 1.0×10^4 cfu/m^3 (Tab. 2). The commonest microorganisms were fungi (*Cephalosporium glutineum, Botrytis cinerea, Penicillium* spp.), endospore-forming bacilli (*Bacillus* spp.) and corynebacteria (*Arthrobacter* spp., *Corynebacterium* spp.) (Fig. 3). Similar to sawmills processing coniferous wood, thermophilic actinomycetes and lactobacilli occurred in only small quantities (Tab. 2).

Table 4. Concentrations of dust and bacterial endotoxin in the air of two sawmills processing coniferous wood.

Plant, sampling site	Concentration of dust (mean, mg/m^3)	Concentration of endotoxin (mean, µg/m^3)
Sawmill “A” processing pine		
A3. First-cut frame sawing	15.3	0.24
Sawmill “B” processing fir		
B1. First-cut frame sawing	68.9	4.00

The values of the respirable fraction of airborne microflora in all sawmills (both processing coniferous and deciduous wood) varied within fairly wide limits and were between 22.5–86.6%. For reason unknown, these were unusually higher at lower concentrations of microorganisms and *vice versa*.

In the air samples taken in the examined sawmills 34 species or genera of bacteria and 21 species or genera of fungi were identified, of these, 13 and 9 species or genera respectively were reported as having allergenic and/or immunotoxic properties [20, 25, 26, 38, 42, 43, 52] (Tab. 3). These figures are certainly an underestimation, as a part of bacterial and fungal strains could be identified only to the generic level.

Table 4 presents the concentrations of airborne dust and endotoxin which were determined on two sampling sites in the samills processing coniferous wood (Tab. 4). The stated values were high, posing a potential risk of eliciting inflammatory reaction in the lungs of workers exposed to the inhalation of sawdust from pine or fir [14, 25, 60].

DISCUSSION

The woodworkers in the examined Polish sawmills are exposed to the concentrations of airborne microorganisms of the order 10^3–10^4 cfu/m^3, comparable with the degree of exposure found in the sawmills located in various parts of the world [1, 2, 10, 13, 50, 51, 65]. Higher concentrations of microorganisms were reported from Scandinavian sawmills where wood products were secondarily infected by moulds [27, 28, 37, 40], as well as from some agricultural facilities, such as grain storing and processing plants, animal feed factories, animal farms [24, 29]. Because to date there are no internationally recognized Occupational Exposure Limit (OEL) values for bioaerosols, the results obtained in the present work could be compared only to the proposals raised by particular authors. The OEL value of 10×10^3 cfu/m^3 for total airborne micoorganisms proposed by Malmros *et al.* [49] and the OEL values of 1×10^3 cfu/m^3 for airborne Gram-negative bacteria proposed by Clark [6] and Malmros *et al.* [49] were exceeded on five sampling sites out seven examined in the sawmills processing coniferous wood and on one site out eight examined in sawmills processing deciduous wood. Nowhere was the OEL value of 100×10^3 cfu/m^3 proposed for total airborne microorganisms by Dutkiewicz and Jabłoński [20, 24] exceeded and on one site, at first-cut frame sawing of pine logs, was exceeded the OEL value of 20×10^3 cfu/m^3 proposed by these authors for Gram-negative bacteria. These figures indicate that the risk of exposure to microorganisms is usually greater in sawmills processing coniferous wood compared to those processing deciduous wood. Nevertheless, this rule is not always valid, as in the course of other work we have found greater concentration of Gram-negative bacteria in the air of sawmill processing beech wood compared to that processing pine wood [55].

The greatest degree of risk, associated both with the concentration and composition of airborne microflora was found on two working stands in the sawmill processing pine wood and on one working stand in the sawmill processing oak wood. The elevated risk related to processing pine occurred at debarking and first-cut frame sawing. In both cases the risk was due to primary infection of stored pine logs with microorganisms which later, during processing of logs, were released together with sawdust into the air of breathing zone. The people working at debarking were exposed mostly to moulds of the species *Aspergillus fumigatus* and to corynebacteria. *Aspergillus fumigatus* is a known hazardous agent which may cause allergic alveolitis, asthma, and pulmonary aspergillosis [20, 42, 43]. Much less is known about the potentially pathogenic properties of corynebacteria associated with organic dusts. Nevertheless, cases of allergic alveolitis caused by *Arthrobacter globiformis* and *Brevibacterium linens* were reported [52] and the involvement of peptidoglycan produced by these bacteria in causing ODTS cannot be excluded.

Workers engaged in first-cut frame sawing of pine were exposed mostly to Gram-negative bacteria which prevailed in the airborne microflora on this working stand. Dominant isolates, preliminarily determined as *Enterobacter* sp. [17, 19], were finally identified as belonging to the newly described genus *Rahnella*, closely related to *Enterobacter* [35, 41]. The ecological position of this genus, comprising so far only one species *Rahnella aquatilis*, is as yet poorly known. It was isolated from water, soil, breweries and humans [35]. Our observations presented in this paper and another report [55] indicate that wood represents a novel, important ecological niche of *Rahnella*. The typical milieu of these bacteria is sapwood, mostly of pine, where its concentration may reach a level of 10^9 cfu per gram [19]. These bacteria were also isolated by us from the wood of European beech (*Fagus sylvatica*), silver fir (*Abies alba*) and Norwegian spruce (*Picea excelsa*) [55, unpublished data]. Among the *Rahnella* isolates from wood, a part show typical properties of *Rahnella aquatilis*, but the majority of strains reveal some different features and it cannot be excluded that they represent a new taxon.

It was found that the strains of *Rahnella* isolated from wood produce endotoxin possessing strong biological activity [18]. In an inhalation experiment in rabbits, the endotoxin of *Rahnella* induced strong immunologic response with significant elevation of cytokine levels [61]. The presence of these bacteria producing strong endotoxin, may explain, at least in part, the increased bronchial responsiveness in workers sawing Scots pine, described by Malmberg *et al.* [48]. Scots pine is one of the most common industrial woods in Europe and hence the problem of the protection of sawyers against the endotoxin produced by *Rahnella* is an important one. Inhaled endotoxin may cause non specific inflammatory reaction in the lungs and ODTS symptoms [2, 8, 11, 14, 51, 60]. Besides *Rahnella* spp., also other species of endotoxin-producing Gram-negative bacteria (*Pantoea agglomerans*, *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas* spp.) may develop in the sapwood and heartwood of stored timber logs from various species of coniferous and deciduous trees [3, 21, 22, 36, 54, 55]. Bacteria developing in wood tissues release, by the fragmentation of outer membrane, abundant quantities of the endotoxin-containing globular particles, measuring 30–50 nm in diameter [22, 54].

A potential health hazard created by endotoxin-producing bacteria for sawmill workers has been confirmed by finding high concentrations of airborne endotoxin in the sawmills processing coniferous wood. The stated values exceeded 1.5–40 times the OEL values of 0.1–0.2 µg/m^3 proposed by Clark [6], Rylander [60] and Malmros *et al.* [49] and 60-800 the OEL value of 5 ng/m^3 proposed by DECOS [14]. These values were also higher compared to the results obtained by Dennekamp *et al.* [11] and Duchaine *et al.* [13] in Canadian lumber mills, and by Alwis *et al.* [2] and Mandryk *et al.* [50, 51] in Australian sawmills, which may be due both to geographical differences and to more advanced technology and lower dust concentrations in the Canadian and Australian facilities.

The only observed case of increased microbial pollution of the air in oak processing sawmills was due to the secondary infection of the raw parquet boards which were stored on open air for 6–9 months. The boards were colonised by moulds *Penicillium citrinum*, posing a risk of allergy to the workers handling the contaminated boards and inhaling airborne spores [26, 42, 43, 67].

CONCLUSION

The workers of Polish sawmills may be exposed at some working stands to airborne microorganisms posing respiratory hazard, of which the greatest risk represent allergenic fungi developing on bark of logs or stored wood products and endotoxin-producing Gram-negative bacteria of the genus *Rahnella,* developing in sapwood of coniferous logs.

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