

EXPOSURE TO AIRBORNE MICROORGANISMS, DUST AND ENDOTOXIN DURING FLAX SCUTCHING ON FARMS

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Abstract: Microbiological air sampling was performed on 5 flax farms located in eastern Poland. Air samples for determination of the concentrations of microorganisms, dust and endotoxin were collected in barns during machine scutching of flax stems by the farmers. The concentrations of mesophilic bacteria ranged from $203.5\text{--}698.8 \times 10^3$ cfu/m³, of Gram-negative bacteria from $27.2\text{--}123.4 \times 10^3$ cfu/m³, of thermophilic actinomycetes from $0.5\text{--}2.6 \times 10^3$ cfu/m³, and of fungi from $23.4\text{--}99.8 \times 10^3$ cfu/m³. The concentrations of total airborne microorganisms (bacteria + fungi) were within a range of $245.0\text{--}741.0 \times 10^3$ cfu/m³. The values of the respirable fraction of total airborne microflora on the examined farms were between 45.5–98.3%. Corynebacteria (irregular Gram-positive rods, mostly *Corynebacterium* spp.) were dominant at all sampling sites, forming 46.8–67.8% of the total airborne microflora. Among Gram-negative bacteria, the most numerous species was *Pantoea agglomerans* (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*), known to have strong endotoxic and allergenic properties. Among fungi, the allergenic species *Alternaria alternata* prevailed. Altogether, 25 species or genera of bacteria and 10 species or genera of fungi were identified in the farm air during flax scutching; of these, 11 and 6 species or genera respectively were reported as having allergenic and/or immunotoxic properties. The concentrations of airborne dust ranged within 43.7–648.1 mg/m³ (median 93.6 mg/m³), exceeding on all farms the Polish OEL value of 4 mg/m³. The concentrations of airborne endotoxin ranged within 16.9–172.1 µg/m³ (median 30.0 µg/m³), exceeding at all sampling sites the suggested OEL value of 0.2 µg/m³. In conclusion, flax farmers performing machine scutching of flax could be exposed to large concentrations of airborne microorganisms, dust and endotoxin, posing a risk of work-related respiratory disease.

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INTRODUCTION

Processing of vegetable matter may be associated with exposure to large quantities of organic dust and bioaerosols causing allergic and/or immunotoxic reactions and

respiratory disease in the workers [8, 9, 17, 39, 40, 45, 59, 60, 70]. The risk concerns also the farmers cultivating flax (*Linum usitatissimum*) and flax processing workers of the textile industry. Common occurrence of byssinosis, asthma, chronic pneumonia, and bronchitis was found

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among the workers of the flax industry and attributed to the effects of bacteria and fungi present on flax stems [1, 2, 4, 5, 25, 27, 72, 80, 81]. The concentrations of bacteria and fungi found by various authors in the air of flax processing factories ranged from 1.7×10^3 – 6.0×10^5 cfu/m³ and from 1.3×10^2 – 7.4×10^4 cfu/m³, respectively [5, 27, 31, 58, 72, 80]. The concentrations of dust in this environment were within a range of 3.2–50.5 mg/m³ [2, 5, 30, 72, 80]. The concentration of bacterial endotoxin on the premises of the flax industry was determined as between 0.5–2.5 µg/m³ [66, 67].

Compared to flax industry workers, much less is known about the exposure of farmers growing flax to dust and bioaerosols and health effects of the exposure in farmers' population. Flax farmers either provide harvested flax directly to textile factories or separate flax fibres by themselves by the machine scutching (threshing) flax before delivery of fibres to factory. Malenky [48, 49] found that out of 593 female workers of a cooperative farm in the former Soviet Union, exposed during flax scutching to extremely large dust concentrations (1000–3458 mg/m³), 148 (25%) had respiratory disorders and 43 (7.3%) had radiological changes in the lungs. Workers with the job duration of 20–35 years showed decrease of vital capacity of the lungs (VC) by 25%. The author has confirmed a high degree of respiratory risk by an experiment, in which he found inflammatory changes in the lungs of white mice exposed for 3 months to flax dust in the vicinity of scutching machines. Gemke *et al.* [28] described a case of lung granuloma resembling allergic alveolitis in a female flax scutcher with 20-year job duration. Noweir *et al.* [55] reported the 22.9% frequency of byssinosis among the Egyptian workers processing flax manually in small workshops or homes and exposed to large quantities of dust (8.5–67.4 mg/m³).

So far, there are no reliable data on microbial pollution of the air during scutching of flax on farms. The results of Malenky [49] obtained by an outdated sedimentation method are inconclusive, as the number of bacteria could not be determined because of confluent growth on agar plates and the reported number of fungi 2.2 – 2.6×10^1 cfu/m³ is definitely underestimated.

The aim of the present work was to determine the levels of microorganisms, dust and endotoxin in the farm air during flax scutching, and to examine the species composition of airborne microflora.

MATERIALS AND METHODS

Examined farms. Air sampling was performed on 5 farms owned by flax cultivating farmers, located in Lublin province (eastern Poland) on the territory of 2 villages, at the distance of circa 35 km in a southeasterly direction from the city of Lublin. Samples were collected in winter 1997 (29 January–7 March) during machine scutching (threshing) of flax by farmers in the barns.

Flax plants were combine harvested in July 1996 and left for about 6 weeks in the field for natural, dew retting.

Then, after drying and machine deseeding, flax stems were transported to the barn and stored for several months until scutching. Scutching was performed inside a barn with a big machine in which the flax fiber is separated from boon by a system of rollers in a 2-stage process. Each time, 7–10 farm workers were occupied at machine scutching of flax, of whom 3–4 persons split bundles of flax stems and loaded them into the inlet of machine, 2 watched the stems during scutching and the other 2–4 persons bound up separated flax fibers at the outlet of the machine.

Air samples were taken at the height of 145 cm, as close as possible (circa 1.0 m) to the workers loading flax stems where the dust release into air was always very high.

Microbiological examination of the air. Air samples were taken on the farms with a custom-designed particle-sizing slit sampler [13] which enabled 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 2 parallelly exposed agar plates: one, "a", sampled directly for all organisms and used for the estimation of the total concentration of cfu per m³; and the other, "b", sampled through a pre-selector (consisting of a system of glass tubes and regulated deposition discs 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-off point for the respirable fraction was 3.0 µm, approximating the recommendations of the American Conference of Governmental Industrial Hygienists [78]. The used sampler enabled the determination of concentrations of microorganisms in the air in the range of 10^0 – 10^8 cfu/m³.

On each farm, a series of 5 double samples was taken on each of the following agar media: blood agar for total mesophilic Gram-negative and Gram-positive bacteria, eosin methylene blue (EMB) agar for Gram-negative bacteria, half-strength tryptic soya agar for thermophilic actinomycetes, and malt agar for fungi. The blood agar plates were subsequently incubated for 1 day at 37°C, then 3 days at 22°C, and finally 3 days at 4°C. The malt agar plates were subsequently incubated for 4 days at 30°C and 4 days at 22°C [15]. The prolonged incubation at lower temperatures aimed at isolating as wide a spectrum of bacteria and fungi as possible. The EMB agar plates were incubated in the same way as the blood agar plates and the tryptic soya agar plates were incubated for 5 days at 55°C. The grown colonies were counted and differentiated and the data reported as cfu per 1 cubic metre of air (cfu/m³). The total concentration of viable microorganisms in the air was obtained by the addition of the concentrations of total mesophilic bacteria (grown on blood agar medium), thermophilic actinomycetes and

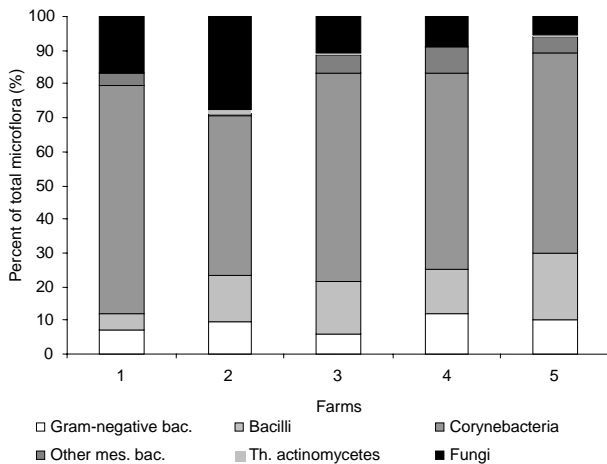


Figure 1. Composition of airborne microflora on farms during scutching of flax: total count, including mesophilic bacteria, thermophilic actinomycetes and fungi.

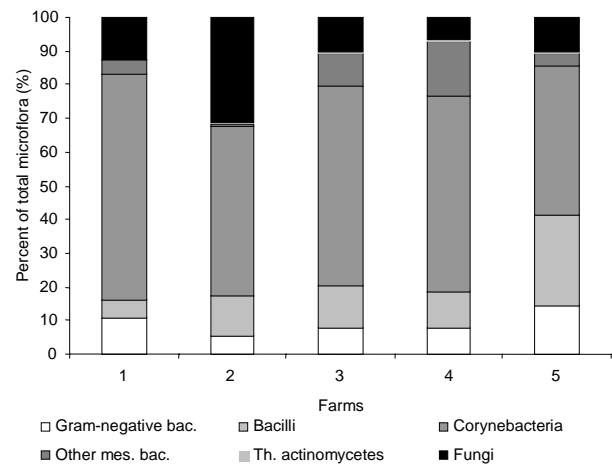


Figure 2. Composition of airborne microflora on farms during scutching of flax: respirable fraction, including mesophilic bacteria, thermophilic actinomycetes and fungi.

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 [35, 75, 79] and Cowan & Steel [7]. Additionally, the Gram-negative strains isolated on EMB agar were identified with microtests, using API Systems 20E and NE (bioMérieux, Marcy l'Etoile, France). Fungi were classified with microscopic methods, according to Barron [3], Larone [42], Litvinov [44], Ramirez [61], and Raper & Fennell [62].

For determination of the dust and endotoxin concentrations, the air samples were collected on the polyvinyl chloride filters by use of an AS-50 one-stage sampler (TWOMET, Zgierz, Poland). Two samples were taken on each farm. The concentration of dust in the air was estimated gravimetrically. The concentration of bacterial endotoxin in the airborne dust was determined by the *Limulus* amoebocyte lysate gel tube test (LAL) [43]. The filters were extracted for 1 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 (Associations of Cape Code, Palmouth, MA, USA). The test was incubated for 1 hour in a water bath at 37°C, using pyrogen-free water as a negative control and the standard lipopolysaccharide (endotoxin) of *Escherichia coli* 0113:H10 (Difco) 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/m³) and the results were reported as micrograms of the equivalents of the *E. coli* 0113:H10 endotoxin per 1 m³ of air. To convert to Endotoxin Units (EU), the value in nanograms was multiplied by 10.

The study was performed during the years 1997–2004. Preliminary results of this work have been reported elsewhere [20, 36].

Table 1. Microorganisms in the farm air during scutching of flax: concentrations and respirable fractions (Rf).

| Sampling site | Total mesophilic bacteria (Blood agar) | | Gram-negative bacteria (EMB agar) | | Thermophilic actinomycetes (Tryptic soya agar) | | Fungi (Malt agar) | | Total microorganisms* | |
|---------------|--|--------|--|--------|--|--------|--|--------|--|--------|
| | Concentration (mean ± S.D., cfu/m ³ × 10 ³) | Rf (%) | Concentration (mean ± S.D., cfu/m ³ × 10 ³) | Rf (%) | Concentration (mean ± S.D., cfu/m ³ × 10 ³) | Rf (%) | Concentration (mean ± S.D., cfu/m ³ × 10 ³) | Rf (%) | Concentration (mean ± S.D., cfu/m ³ × 10 ³) | Rf (%) |
| Farm 1 | 203.5 ± 51.9 | 100 | 37.2 ± 9.9 | 66.8 | 0.5 ± 0.3 | 75 | 41.0 ± 6.6 | 74.0 | 245.0 ± 46.3 | 98.3 |
| Farm 2 | 255.8 ± 38.6 | 70.4 | 123.4 ± 19.5 | 80.6 | 2.6 ± 2.6 | 61.5 | 99.8 ± 40.3 | 83.2 | 358.2 ± 36.4 | 73.9 |
| Farm 3 | 374.8 ± 174.0 | 57.2 | 59.8 ± 18.4 | 59.5 | 2.0 ± 3.5 | 50.0 | 46.6 ± 13.9 | 51.9 | 423.4 ± 182.9 | 57.3 |
| Farm 4 | 247.4 ± 131.8 | 66.5 | 27.2 ± 11.5 | 65.4 | 0.6 ± 1.3 | 66.7 | 23.4 ± 8.7 | 51.3 | 271.4 ± 138.4 | 65.2 |
| Farm 5 | 698.8 ± 115.7 | 43.2 | 46.2 ± 8.2 | 58.0 | 0.6 ± 0.9 | 100 | 41.6 ± 11.7 | 83.2 | 741.0 ± 105.4 | 45.5 |
| Mean | 356.1 ± 211.7 | 67.5 | 58.8 ± 37.1 | 66.1 | 1.3 ± 2.1 | 70.6 | 50.5 ± 32.3 | 68.7 | 407.8 ± 210.4 | 68.0 |

*Sum of the concentrations of mesophilic bacteria, thermophilic actinomycetes and fungi.

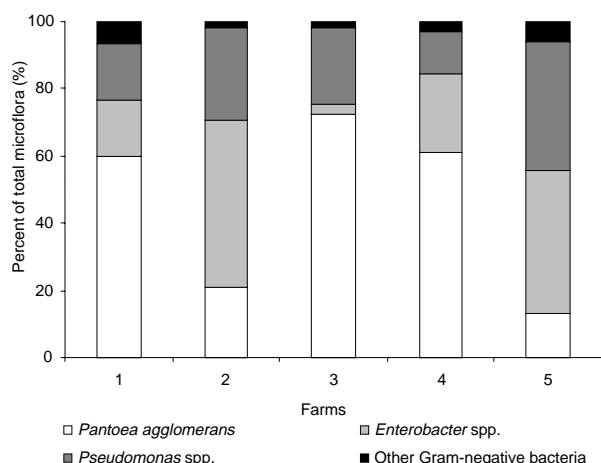


Figure 3. Composition of airborne Gram-negative flora on farms during scutching of flax: total count.

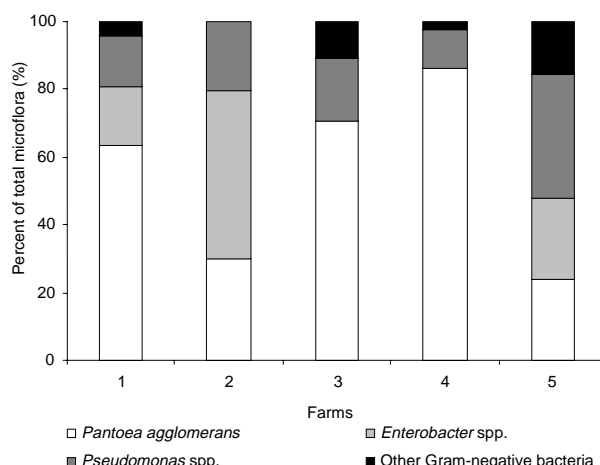


Figure 4. Composition of airborne Gram-negative flora on farms during scutching of flax: respirable fraction.

RESULTS

Concentrations of total viable microorganisms in the air of barns during flax scutching were very large on all farms, ranging from $245.0\text{--}741.0 \times 10^3$ cfu/m³ (Tab. 1).

Mesophilic bacteria were dominant on all farms with the concentrations ranging from $203.5\text{--}698.8 \times 10^3$ cfu/m³ (Tab. 1). They formed 71.5–94.3% of the total airborne microflora (Fig. 1) and 68.0–93.0% of the respirable fraction of airborne microflora (Fig. 2). Among the mesophilic bacteria, there distinctly prevailed corynebacteria (irregular Gram-positive rods, mostly *Corynebacterium* spp.) which accounted for 46.8–67.8% of the total airborne microflora (Fig. 1) and 44.1–67.3% of the respirable fraction (Fig. 2). The endospore-forming bacilli (*Bacillus* spp.) constituted 4.6–19.5% of the total airborne microflora (Fig. 1) and 5.3–26.8% of the respirable fraction (Fig. 2). Gram-negative bacteria recovered on blood agar formed 5.9–11.8% of the total airborne microflora (Fig. 1) and 5.6–14.5% of the respirable fraction (Fig. 2). A group described as “other mesophilic bacteria” which consisted mostly of Gram-positive cocci (*Micrococcus* spp., *Staphylococcus* spp.) formed 1.1–8.0% of the total count (Fig. 1) and 0.5–16.1% of the respirable fraction (Fig. 2).

The concentrations of airborne Gram-negative bacteria recovered on EMB agar during flax scutching were large on all farms, ranging from $27.2\text{--}123.4 \times 10^3$ cfu/m³ (Tab. 1). The most common was the species *Pantoea agglomerans* (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*) which distinctly prevailed on 3 farms, forming 60.0–72.6% of the total count of Gram-negative bacteria (Fig. 3) and 63.3–86.5% of the respirable fraction (Fig. 4). On the remaining 2 farms, the species formed respectively 13.0–20.9% and 23.9–29.8%. The species of the genera *Enterobacter* (*Enterobacter amnigenus*, *Enterobacter sakazakii*, *Enterobacter* spp.) and *Pseudomonas* (*Pseudomonas maltophilia*, *Pseudomonas oryzihabitans*, *Pseudomonas paucimobilis*, *Pseudomonas* spp.) were also numerous in the farm air during flax scutching, forming respectively 2.7–49.8% and 12.5–38.5% of the total count of Gram-negative bacteria (Fig. 3), and 0–49.9% and 11.2–36.5% of the respirable fraction (Fig. 4).

The concentrations of airborne thermophilic actinomycetes were small on all farms, ranging from $0.5\text{--}2.6 \times 10^3$ cfu/m³ (Tab. 1). They constituted only 0.1–0.7% of the total airborne microflora (Fig. 1) and 0.2–0.6% of the respirable fraction (Fig. 2). The distinctly dominant species was *Thermoactinomyces thalophilus* which formed

Table 2. List of microbial species and genera identified in the samples of air taken on farms during scutching of flax.

Gram-negative bacteria: *Enterobacter amnigenus*+ (2), *Enterobacter sakazakii*+ (2), *Enterobacter* spp.+ (1-5), *Pantoea agglomerans**+ (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*) (1-5), *Pseudomonas maltophilia* (5), *Pseudomonas oryzihabitans* (2), *Pseudomonas paucimobilis* (2), *Pseudomonas* spp. (1-5).

Bacilli: *Bacillus cereus* (1-5), *Bacillus megaterium* (1-5), *Bacillus subtilis** (1-5), *Bacillus* spp. (1-5).

Corynebacteria: *Arthrobacter globiformis** (1-5), *Arthrobacter* spp. (1-5), *Brevibacterium linens** (2-4), *Brevibacterium* spp. (1-5), *Corynebacterium* spp. (1-5).

Other mesophilic bacteria: *Micrococcus* spp. (1-5), *Staphylococcus* spp. (1-5), *Streptococcus* spp. (2-5), *Streptomyces albus** (1-3), *Streptomyces* spp. (2-5).

Thermophilic actinomycetes: *Saccharomonospora viridis** (2), *Thermoactinomyces thalophilus** (1-5), *Thermoactinomyces vulgaris** (1, 2).

Fungi: *Alternaria alternata**+ (1-5), *Aspergillus fumigatus**+ (1), *Candida* spp.* (5), *Fusarium* spp.+ (1-5), *Monilia* spp. (5), *Mucor* spp.* (2), *Oospora glauca* (1), *Penicillium* spp.*+ (1, 2), *Rhodotorula rubra* (1), *Rhodotorula* spp. (5).

Numbers of farms on which strains were isolated are given in parentheses. 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. *Bacillus cereus* and *Aspergillus fumigatus* may cause infectious disease in man.

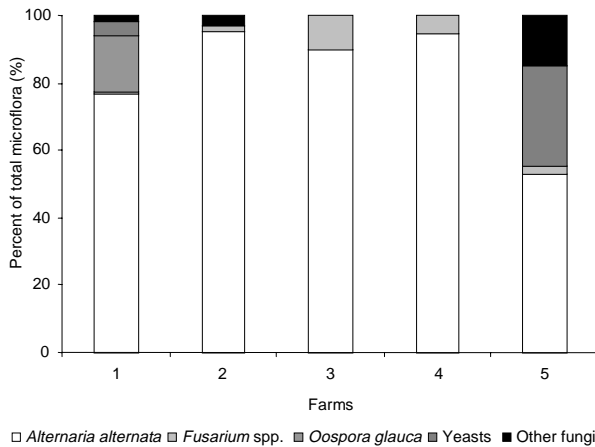


Figure 5. Composition of airborne fungal flora on farms during scutching of flax: total count.

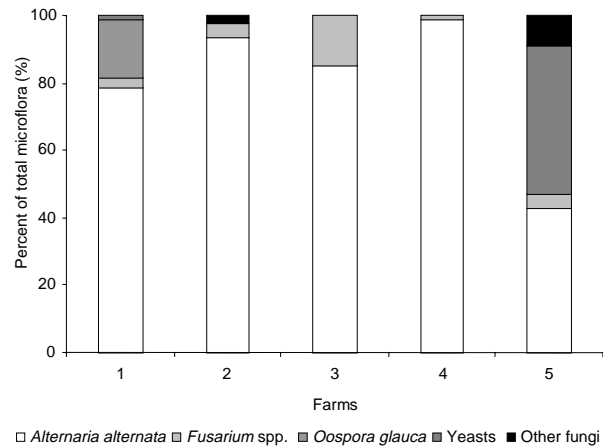


Figure 6. Composition of airborne fungal flora on farms during scutching of flax: respirable fraction.

75–100% of the total count of thermophilic actinomycetes and 87.5–100% of the respirable fraction.

Concentrations of airborne fungi during flax scutching ranged from $23.4\text{--}99.8 \times 10^3$ cfu/m³ (Tab. 1). Fungi constituted 5.6–27.8% of the total airborne microflora (Fig. 1) and 6.8–31.4% of the respirable fraction (Fig. 2). The distinctly prevailing species was *Alternaria alternata* which constituted 52.7–95.4% of the total count of fungi (Fig. 5) and 42.8–98.6% of the respirable fraction (Fig. 6). *Fusarium* strains occurred in the air of all farms but in much smaller proportions: 0.6–9.9% of the total count (Fig. 5) and 1.4–14.9% of the respirable fraction (Fig. 6).

The values of the respirable fraction of airborne microflora isolated during flax scutching were large and for total microorganisms were within the range 45.5–98.3% (Tab. 1). For total mesophilic bacteria, they ranged from 43.2–100%, for Gram-negative bacteria from 58.0–80.6%, for thermophilic actinomycetes from 50.0–100%, and for fungi from 51.3–83.2% (Tab. 1).

In the air samples taken on the examined farms, 25 species or genera of bacteria and 10 species or genera of fungi were identified; of these, 11 and 6 species or genera respectively were reported as having allergenic and/or immunotoxic properties [17, 24, 34, 39, 40, 53] (Tab. 2). These figures are certainly an underestimation, as a part of bacterial and fungal strains could be identified only to generic level.

Table 3. Concentrations of dust and bacterial endotoxin in the farm air during scutching of flax.

| Sampling site | Concentration of dust (mg/m ³) | Concentration of endotoxin (µg/m ³) |
|---------------|--|---|
| Farm 1 | 110.6 | 80.4 |
| Farm 2 | 648.1 | 16.9 |
| Farm 3 | 93.6 | 25.7 |
| Farm 4 | 43.7 | 30.0 |
| Farm 5 | 46.8 | 172.1 |
| Median | 93.6 | 30.0 |

The concentrations of airborne dust and endotoxin recorded on the examined farms during flax scutching were very large and ranged from 43.7–648.1 mg/m³, and from 16.9–172.1 µg/m³, respectively (Tab. 3).

DISCUSSION

The present study has demonstrated that farm workers performing machine scutching of flax inside the barns could be exposed to large concentrations of airborne microorganisms, dust and endotoxin posing an occupational hazard. The concentrations of total airborne microorganisms were of the order 10^5 cfu/m³, resembling those reported for working environments with the highest bioaerosol pollution, such as: grain stores, seed stores, animal feed factories, malt houses, herb processing plants, pig farms, poultry farms, and waste composting facilities [8, 15, 18, 19, 20, 22, 26, 39, 40, 41, 46, 59, 74, 76].

On average, the numbers of microorganisms found in farm air during flax scutching were greater by one order of magnitude compared to those reported from the premises of the flax industry [5, 27, 31, 58, 72, 80]. They are closest to the results obtained by Cinkotai *et al.* [5] in small scutching mills in Normandy using old machinery, which resembled in some respects the work environment examined in this study.

As, so far, there are no internationally recognised 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. As regards total viable airborne microorganisms, the OEL values proposed by Malmros *et al.* (10×10^3 cfu/m³) [50], and by Dutkiewicz and Jabłoński (50×10^3 cfu/m³ at the value of respirable fraction equal to or above 50%, 100×10^3 cfu/m³ at the value of respirable fraction below 50%) [17, 19] were exceeded on all farms examined. Similarly, on all farms there were exceeded: the OEL value for airborne Gram-negative bacteria proposed by Clark [6] and Malmros *et al.* [1×10^3 cfu/m³) and by Dutkiewicz and Jabłoński [17, 19] and

Górny and Dutkiewicz [33] (10×10^3 cfu/m³ at the value of respirable fraction equal to or above 50%, and 20×10^3 cfu/m³ at the value of respirable fraction below 50%). The OEL value proposed by Dutkiewicz and Jabłoński [17, 19] and Górny and Dutkiewicz [33] for airborne fungi (25×10^3 cfu/m³ at the value of respirable fraction equal to or above 50%, and 50×10^3 cfu/m³ at the value of respirable fraction below 50%), was exceeded on 4 out of 5 examined farms, while nowhere was exceeded the OEL value proposed by these authors for airborne thermophilic actinomycetes (10×10^3 cfu/m³ at the value of respirable fraction equal to or above 50%, and 20×10^3 cfu/m³ at the value of respirable fraction below 50%).

Values of respirable fraction, determined for airborne microorganisms in the course of the present study, were usually large, in most cases above 50%. This result is in accordance with those reported by Malenky [48, 49] and Gościcki *et al.* [30] who determined microscopically that on average above 50% of the airborne flax dust particles were below 5 µm.

The viable airborne microflora found on examined farms during flax scutching was distinctly dominated by corynebacteria that constituted on average nearly 60% of the total count. These bacteria are commonly associated with organic dusts [53] and isolated in large quantities from the air of animal farms [15, 18], sawmills [21], herb processing plants [22], potato processing plant [23], and during handling of grain and hop [20, 32].

So far, little is known about the potentially pathogenic properties of corynebacteria associated with organic dusts. Cases of allergic alveolitis caused by *Arthrobacter globiformis* and *Brevibacterium linens* have been reported [53], and the involvement of peptidoglycan produced by these bacteria in causing organic dust toxic syndrome (ODTS) cannot be excluded. Because of the common occurrence of corynebacteria in organic dusts, future studies on the potential role of these organisms in causing work-related respiratory disorders among agricultural workers are highly desirable.

The epiphytic species *Pantoea agglomerans* (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*), prevailing among Gram-negative bacteria isolated from the farm air during flax scutching, was proved to possess strong endotoxic and allergenic properties [14, 16, 38, 51, 52, 65, 71]. It was identified as a cause of allergic alveolitis [38, 53] and other respiratory disorders [10, 11] in agricultural workers exposed to grain dust, and as a cause of allergic pneumopathies in cattle [57]. The results obtained by Mackiewicz *et al.* [47] and Golec *et al.* [29] suggest the important role of this bacterium as an occupational allergen in herb dust. Śpiwak *et al.* [77] found a correlation between cellular reactivity to *Pantoea agglomerans* and the occurrence of work-related dermatitis in farming students.

The present results indicating the important role of *Pantoea agglomerans* as an occupational hazard for flax farmers corroborate those of our earlier study [73] in which a significantly greater ($p < 0.01$) immunologic

response of flax farmers to antigen of this bacterium was found compared to a reference group not exposed to organic dust, both in the precipitin test and the test for inhibition of leukocyte migration in the presence of specific antigen.

The concentration of the airborne Gram-negative bacteria found in this study was notably greater compared to that reported by Cinkotai *et al.* [5]. The reported by Malenky [68] and Gościcki *et al.* [31] of the high prevalence of endospore-forming bacteria (*Bacillus* spp.) in airborne flax dust was not confirmed by the present study.

Among fungi recovered from the farm air during flax scutching, there distinctly dominated the species *Alternaria alternata* which constituted, on average, over 80% of total isolates. This is a known allergenic species that could be a cause of allergic rhinoconjunctivitis and asthma [39]. *Fusarium* species, which occurred in the air of all farms, are producers of trichothecene mycotoxins (deoxynivalenol, nivalenol, moniliformin, T-2, HT-2). These mycotoxins, often referred to as fusariotoxins, occur commonly in grain and grain dust [37, 54] and are considered as a potential cause of mycotoxicoses in exposed agricultural workers [39, 40].

The presence of potentially hazardous mycotoxins in flax dust is indicated also by the experimental study of Pieckova and Jesenska [56]. These authors found that over 50% of the metabolite samples of filamentous fungi isolated from flax revealed ciliostatic activity on tracheal cultures of 1-day-old chicks *in vitro*.

The concentrations of dust and bacterial endotoxin in the farm air during flax scutching were extremely large, and on all farms exceeded the existing and proposed OEL values. The concentrations of dust were of the order 10^1 – 10^2 mg/m³, exceeding the Polish OEL value of 4 mg/m³ [64] by 11–162 times. The dust levels found in the present work were on average by 1–2 orders of magnitude greater compared to those reported from the premises of the flax industry in various countries [2, 30, 72, 80] and small Egyptian workshops [55], and by 1–2 orders of magnitude lower compared to those reported by Malenky [48, 49] from farms during flax scutching in the former Soviet Union.

The concentrations of airborne endotoxin recorded during flax scutching were of the order 10^1 – 10^2 µg/m³ and exceeded on all the examined farms the OEL values proposed by various authors [6, 12, 17, 33, 41, 50, 68] and the values supposed to cause decrease of lung function over work shift and ODTS symptoms [69]. On average, the concentration of endotoxin in the air exceeded over 100 times the OEL values proposed by Clark [6] (0.1 µg/m³), Rylander [68] (0.1–0.2 µg/m³), Malmros *et al.* [50] (0.1 µg/m³), and by Górny and Dutkiewicz [33] (0.2 µg/m³), and over 1,000 times the OEL values proposed by Laitinen *et al.* [41] (0.025 µg/m³), and by the Dutch Expert Committee on Occupational Standards (DECOS) [12] (0.005 µg/m³). The endotoxin levels found in the present work were, on

average, by 1–2 orders of magnitude greater compared to those reported from the premises of flax industry [66, 67].

The potential adverse effect of exposure to the inhalation of large amounts of endotoxin during flax scutching has been demonstrated by our earlier study [73] in which we found that as many as 62.7% of interviewed farmers reported the occurrence of work-related, general and respiratory symptoms during flax scutching, largely resembling those considered as characteristic for ODS [63].

CONCLUSION

Flax farmers could be exposed during machine scutching of flax to large concentrations of airborne microorganisms, dust and endotoxin posing a risk of work-related respiratory disease. The risk is increased by the presence of microbial species possessing allergenic and/or immunotoxic properties.

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REFERENCES

- Baskakova AE: Bronchialna astma i preastma u rabochikh Inokombinatov (Bronchial asthma and preasthma in flax plant workers). *Vrach Delo* 1979, **(8)**, 108-109 (in Russian).
- Baskakova AE, Kapitonova ME: K voprosu o allergennikh svoistvakh proizvodstvennoi Inianoi pyli (On allergenic properties of industrial flax dust). *Gig Tr Prof Zabol* 1979, **(5)**, 24-28 (in Russian).
- Barron GL: *The Genera of Hyphomycetes from Soil*. Williams & Wilkins, Baltimore 1968.
- Bouhuys A, Van Duyn J, Van Lennep HJ: Byssinosis in flax workers. *Arch Environ Health* 1961, **3**, 499-509.
- Cinkotai FF, Emo P, Gibbs ACC, Caillard JF, Jouany JM: Low prevalence of byssinotic symptoms in 12 flax scutching mills in Normandy, France. *Br J Ind Med* 1988, **45**, 325-328.
- 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.
- Cowan ST, Steel KJ: *Manual for the Identification of Medical Bacteria*. University Press, Cambridge 1965.
- Crook B, Olenchock SA: Industrial workplaces. In: Cox CS, Wathes CM (Eds): *Bioaerosols Handbook*, 531-545. CRC Press, Boca Raton 1995.
- Danuser B, Monn C: Endotoxins in the workplace and in the environment. *Schweiz Med Wochenschr* 1999, **129**, 475-483 (in German).
- Durda M, Dutkiewicz J, Skublewski A, Borkowska L, Dutkiewicz E, Ziemecka-Rakowska E: Wczesne wyniki swoistego odczulania alergenem *Erwinia herbicola* chorych na astmę oskrzelową (Early results of specific desensitization with *Erwinia herbicola* allergen in patients with bronchial asthma). *Pneum Pol* 1980, **48**, 707-713 (in Polish).
- Durda M, Skublewski A, Borkowska L, Dutkiewicz J, Jakubowski R, Ziemecka-Rakowska E, Mołocznik A, Tymiński J, Bylina J, Dutkiewicz E, Krysińska-Traczyk E, Smerdel-Skórska C, Bartoszczyk H: Przewlekłe choroby układu oddechowego u mężczyzn narażonych na działanie pyłów organicznych (Chronic respiratory diseases in men exposed to organic dusts). *Med Komunikacyjna* 1986, **22(5)**, 125-132 (in Polish).
- Dutch Expert Committee on Occupational Standards (DECOS): *Endotoxins, Health-based Recommended Occupational Exposure Limit*. Gezondheidsraad, The Netherlands 1998.
- Dutkiewicz J, Kwapiszewski C: Nowy aparat do badania mikrobiologicznego zanieczyszczenia powietrza (New sampler for microbiological examination of the air). *Ochrona Powietrza* 1975, **9(2)**, 37-42 (in Polish).
- Dutkiewicz J: Studies on endotoxins of *Erwinia herbicola* and their biological activity. *Zbl Bakt Hyg I Abt Orig A* 1976, **236**, 487-508.
- Dutkiewicz J: Exposure to dust-borne bacteria in agriculture. I. Environmental studies. *Arch Environ Health* 1978, **33**, 250-259.
- Dutkiewicz J: Exposure to dust-borne bacteria in agriculture. II. Immunological survey. *Arch Environ Health* 1978, **33**, 260-270.
- Dutkiewicz J, Jabłoński L: *Biologiczne Szkodliwości Zawodowe (Occupational Biohazards)*. PZWL, Warsaw 1989 (in Polish).
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- Dutkiewicz J, Śpiewak R, Jabłoński L: *Klasyfikacja Szkodliwych Czynników Biologicznych Występujących w Środowisku Pracy oraz Narażonych na Nie Grup Zawodowych (Classification of Occupational Biohazards and the Exposed Professional Groups)*. 3rd Ed. Ad Punctum, Lublin 2002 (in Polish).
- Elwood PC, Pemberton J, Merrett JD, Carey GCR, McAulay IR: Byssinosis and other respiratory symptoms in flax workers in Northern Ireland. *Br J Ind Med* 1965, **22**, 27-37.
- Eduard W: Exposure to non-infectious microorganisms and endotoxins in agriculture. *Ann Agric Environ Med* 1997, **4**, 179-186.
- Fetisova AA, Titova SM, Aleksandrova OG: Allergennye svoistva pyli khlopko- i Inopriadilnikh fabrik (Allergenic properties of the cotton and flax spinning mill dust). *Gig Tr Prof Zabol* 1970, **14(5)**, 19-22 (in Russian).
- Gemke GR, Kaupasa MM, Lekakh ER, Iofel GB: O oddalennikh iskhodakh porazhenia logkikh u trepalshchikov Ina (Long-term consequences of lung injuries in flax scutchers). *Gig Tr Prof Zabol* 1991, **(1)**, 5-8 (in Russian).
- Golec M, Skórska C, Mackiewicz B, Dutkiewicz J: Immunologic reactivity to work-related airborne allergens in people occupationally exposed to dust from herbs. *Ann Agric Environ Med* 2004, **11**, 121-127.
- Gościcki J, Więcek E, Matecki W, Bielichowska G: Ocena środowiska pracy w zakładach Iniarskich. IV. Stężenie pyłu na stanowiskach pracy w przędzalniach i tkalniach Inu (Evaluation of work environment in flax industry. IV. Dust concentration in the air at workposts in flax spinning and flax weaving mills). *Med Pracy* 1980, **31**, 21-26 (in Polish).
- Gościcki J, Włodarczyk L, Bielichowska G: Ocena środowiska pracy w zakładach Iniarskich. V. Mikroflora powietrza na stanowiskach pracy w przędzalniach i tkalniach Inu (Evaluation of work environment in flax industry. V. Microflora of the air at workposts in flax spinning and flax weaving rooms). *Med Pracy* 1980, **31**, 91-97 (in Polish).
- 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.
- Górny RL, Dutkiewicz J: Bacterial and fungal aerosols in indoor environment in Central and Eastern European countries. *Ann Agric Environ Med* 2002, **9**, 17-23.

34. Johnson CE, Bernstein L, Gallagher JS, Bonventre PF, Brooks SM: Familial hypersensitivity pneumonitis induced by *Bacillus subtilis*. *Am Rev Respir Dis* 1980, **122**, 339-348.
35. Krieg NR, Holt JG (Eds): *Bergey's Manual of Systematic Bacteriology. Vol. 1*. Williams & Wilkins, Baltimore 1984.
36. Krysińska-Traczyk E, Skórska C, Prażmo Z, Dutkiewicz J, Sitkowska J: Drobnoustroje i endotoksyny w powietrzu zanieczyszczonym pyłami omlotowymi z lnu jako potencjalne czynniki narażenia zawodowego rolników indywidualnych (Microorganisms and endotoxins in the air polluted with flax dust as potential occupational hazards for farmers). V Polish Symposium on Health Hazards in Work Environment, Łódź, 6-8 November 1997, Abstracts, 76 (in Polish).
37. Krysińska-Traczyk E, Kiećana I, Perkowski J, Dutkiewicz J: Levels of fungi and mycotoxins in samples of grain and grain dust collected on farms in Eastern Poland. *Ann Agric Environ Med* 2001, **8**, 269-274.
38. Kuś L: Alergiczne zapalenie pęcherzyków płucnych w wyniku ekspozycji na antygeny występujące w pyłach zbożowych w świetle własnych badań klinicznych i doświadczalnych (Allergic alveolitis in effect of the exposure to antigens present in grain dust: An experimental and clinical study). *Med Wiejska* 1980, **15**, 73-80 (in Polish).
39. Lacey J, Crook B: Review: Fungal and actinomycete spores as pollutants of the workplace and occupational allergens. *Ann Occup Hyg* 1988, **32**, 515-533.
40. Lacey J, Dutkiewicz J: Bioaerosols and occupational lung disease. *J Aerosol Sci* 1994, **25**, 1371-1404.
41. 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.
42. Larone DH: *Medically Important Fungi: A Guide to Identification*. American Society for Microbiology, Washington, D.C. 1993.
43. Levin J, Bang FB: The role of endotoxin in the extracellular coagulation of *Limulus* blood. *Bull Johns Hopkins Hosp* 1964, **115**, 265-274.
44. Litvinov MA: *Opredelitel' Mikroskopicheskikh Pochvennykh Gribov (Guide for Determination of the Microscopic Soil Fungi)*. Izd. Nauka, Leningrad 1967 (in Russian).
45. Lundholm M, Palmgren U, Malmberg P: Exposure to endotoxin in the farm environment. 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**, 314-315.
46. Mackiewicz B: Study on exposure of pig farm workers to bioaerosols, immunologic reactivity and health effects. *Ann Agric Environ Med* 1998, **5**, 169-175.
47. Mackiewicz B, Skórska C, Dutkiewicz J, Michnar M, Milanowski J, Prażmo Z, Krysińska-Traczyk E, Cisak E: Allergic alveolitis due to herb dust exposure. *Ann Agric Environ Med* 1999, **6**, 167-170.
48. Malenky VP: Sanitarno-gigienicheskaya ocenka pylevovo faktora punktov pervichnoy pererabotki lna i evo wlyanye na organy dyhanya (Sanitary hygienic evaluation of the dust factor at stations of initial flax processing and its effect on the respiratory organs). *Vrach Delo* 1968, (2), 103-106 (in Russian).
49. Malenky VP: K gigenicheskoi kharakteristike uslovyi truda i sostoyania zdorovia kolkhoznikov pererabativayushchikh len (On the hygienic characteristics of the working conditions and status of health of cooperative farm workers engaged in processing flax). *Gig Tr Prof Zabol* 1969, **13**(2), 47-49 (in Russian).
50. Malmros P, Sigsgaard T, Bach B: Occupational health problems due to garbage sorting. *Waste Manag Res* 1992, **10**, 227-234.
51. Milanowski J: Effects of *Pantoea agglomerans* on the respiratory system. Part I. Studies *in vitro*. *Ann Agric Environ Med* 1994, **1**, 44-51.
52. Milanowski J: Effects of *Pantoea agglomerans* on the respiratory system. Part II. Studies *in vivo*. *Ann Agric Environ Med* 1994, **1**, 52-56.
53. 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.
54. Nordby KC, Halstensen AS, Elen O, Clasen PE, Langseth W, Kristensen P, Eduard W: Trichothecene mycotoxins and their determinants in settled dust related to grain production. *Ann Agric Environ Med* 2004, **11**, 75-83.
55. Noweir MH, El-Sadik YM, El-Dakhkhny AA, Osman HA: Dust exposure in manual flax processing in Egypt. *Br J Ind Med* 1975, **32**, 147-154.
56. Pieckova E, Jesenska Z: Filamentous microfungi in raw flax and cotton for textile industry and their ciliostatic activity on tracheal organ cultures *in vitro*. *Mycopathologia* 1996, **134**, 91-96.
57. Pomorski ZJH, Dutkiewicz J, Taszkun I, Woźniak M, Sitkowski W, Skórska C, Cholewa G: Badania nad alergicznymi uwarunkowaniami pneumopatii bydła, wynikłych z wdychania zawartych w pyłach organicznych pneumoalergenów (Studies on allergic conditioning of cattle pneumopathies resulting from inhaling pneumoallergens comprised in organic dusts). *Ann UMCS Sect DD* 1993, **48**, 183-193 (in Polish).
58. Przyłęcka J, Włodarczyk L, Gościński J: Ocena środowiska pracy w roszarniach lnu i konopi. III. Mikrobiologiczne zanieczyszczenia powietrza na stanowiskach pracy w roszarniach lnu i konopi (Evaluation of work environment in the flax and hemp factories. III. Air microflora in the flax and hemp factories). *Med Pracy* 1975, **26**, 77-83 (in Polish).
59. Radon K, Danuser B, Iversen M, Monso E, Weber C, Hartung J, Donham K, Palmgren U, Nowak D: Air contaminants in different European farming environments. *Ann Agric Environ Med* 2002, **9**, 41-48.
60. Radon K, Monso E, Weber C, Danuser B, Iversen M, Opravil U, Donham K, Hartung J, Pedersen S, Garz S, Blainey D, Rabe U, Nowak D: Prevalence and risk factors for airway diseases in farmers - summary of results of the European Farmers' Project. *Ann Agric Environ Med* 2002, **9**, 207-213.
61. Ramirez C: *Manual and Atlas of the Penicillia*. Elsevier, Amsterdam 1982.
62. Raper KB, Fennell DI: *The Genus Aspergillus*. Williams & Wilkins, Baltimore 1965.
63. Rask-Andersen A: Organic dust toxic syndrome among farmers. *Br J Ind Med* 1989, **46**, 233-238.
64. 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.
65. Rylander R, Lundholm M: Bacterial contamination of cotton and effects on the lung. *Br J Ind Med* 1978, **35**, 204-207.
66. Rylander R, Morey P: Airborne endotoxin in industries processing vegetable fibers. *Am Ind Hyg Assoc J* 1982, **43**, 811-812.
67. Rylander R, Vesterlund J: Airborne endotoxins in various occupational environments. In: Watson SW, Levin J, Novitsky TJ (Eds): *Endotoxins and Their Detection With the Limulus Amebocyte Lysate Test*, 399-409. Alan R. Liss, Inc., New York 1982.
68. Rylander R: The role of endotoxin for reactions after exposure to cotton dust. *Am J Ind Med* 1987, **12**, 687-697.
69. Rylander R: Organic dusts - from knowledge to prevention. *Scand J Work Environ Health* 1994, **20**, 116-122.
70. Rylander R, Jacobs RR (Eds): *Organic Dusts. Exposure, Effects and Prevention*. Lewis Publishers, Boca Raton, FL 1994.
71. Salkinoja-Salonen MS, Helander I, Rylander R: Toxic bacterial dusts associated with plants. In: Rhodes-Roberts M, Skinner FA (Eds): *Bacteria and Plants*, 219-233. Soc. Appl. Bact. Symp. Ser. No. 10. Academic Press, London 1982.
72. Shchepochkin AM, Garasko EV, Arzhakova TI: O vliyanií uvlazhneniya volokna na zapylennost i bakterialnyuy zagryaznennost vozdukhá v lnopriadilnom proizvodstve (Effect of fiber humidification on the dust content and bacterial pollution of the air in a flax-spinning mill). *Gig Sanit* 1976, (4), 106-108 (in Russian).
73. Skórska C, Mackiewicz B, Dutkiewicz J: Effects of exposure to flax dust in Polish farmers: work-related symptoms and immunologic response to microbial antigens associated with dust. *Ann Agric Environ Med* 2000, **7**, 111-118.
74. Smid T, Heederik D, Mensink G, Houba R, Boleij JSM: Exposure to dust, endotoxins and fungi in the animal feed industry. *Am Ind Hyg Assoc J* 1992, **53**, 362-368.
75. Sneath PHA, Mair N, Sharpe ME, Holt JG (Eds): *Bergey's Manual of Systematic Bacteriology. Vol. 2*. Williams & Wilkins, Baltimore 1986.
76. Swan JRM, Crook B: Airborne microorganisms associated with grain handling. *Ann Agric Environ Med* 1998, **5**, 7-15.

77. Śpiewak R, Skórska C, Góra A, Horoch A, Dutkiewicz J: Young farmers with cellular reactivity to airborne microbes suffer more frequently from work-related skin symptoms and allergic dermatitis. *Ann Agric Environ Med* 2001, **8**, 255-259.

78. *Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices, 1993-1994*. American Conference of Governmental Industrial Hygienists, Cincinnati 1993.

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

80. Zaritskaya LP: Zagraznenie proizvodstvennoi sredy i professionalnye zaboлевania logkikh u rabochikh Inoobrabativayushchevo kombinata (Environmental pollution in relation to occupational lung diseases among workers of a flax mill). *Gig Tr Prof Zabol* 1979, **(5)**, 20-23 (in Russian).

81. Zaritskaya LP: Immunologicheskie issledovaniya pri bissinoze ot vozdeistvia smieshannoi rastitelnoi pyli (dzhuta, lna, kenafa) (Immunological studies on byssinosis from exposure to a mixed plant dust (jute, flax and kenaf)). *Gig Tr Prof Zabol* 1992, **(7)**, 15-17 (in Russian).