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OCCUPATIONAL EXPOSURE TO ORGANIC DUST, MICROORGANISMS, ENDOTOXIN AND PEPTIDOGLYCAN AMONG PLANTS PROCESSING WORKERS IN POLAND

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Abstract: The objective of present work was to determine and compare the components of bioaerosol in several sectors of plant processing industries. The study was conducted in 10 facilities engaged in herb and grain processing, flax threshing, grain storing, baking, and cereals production. The air samples were taken on glass fibre filters with an AS-50 sampler. We determined the concentrations of airborne microorganisms, dust, endotoxin and peptidoglycan. Total concentrations of viable airborne microorganisms ranged from 0.18–861.4 × 10^3 cfu/m^3. The highest levels of microbial contamination of the air were observed at flax farms, in grain elevators and in a herb processing plant. Gram-positive bacteria and fungi were detected at all sampling sites and their median concentrations were respectively 18.1 × 10^3 cfu/m^3 and 0.66 × 10^3 cfu/m^3. The concentration of Gram-negative bacteria ranged from 0.0–168.0 × 10^3 cfu/m^3. The concentration of thermophilic actinomycetes ranged from 0.0–1.45 × 10^3 cfu/m^3. Qualitatively, Gram-positive bacteria constituted 23–93% of the total microbial count. The most common species were: *Staphylococcus* spp., *Curtobacterium pusillum*, *Rhodococcus fascians*, *Aureobacterium testaceum*, *Sanguibacter keddieii*, *Microbacterium* spp., and *Bacillus* spp. Gram-negative bacteria formed 0–48% of the total count. The species *Pantoea agglomerans* dominated in all examined air samples. Fungi constituted 2.5–76.9% of the total microbial count. Among them, *Penicillium* spp., *Mucor* spp., *Alternaria* spp., *Aspergillus niger*, and *Aspergillus* spp. were found. The dust concentration ranged from 0.18–86.9 mg/m^3. The concentration of endotoxin was large and ranged from 0.0041–1562.6 µg/m^3. Muramic acid, the chemical marker of peptidoglycan, was detected in 9 out of 13 (69.2%) collected samples. The concentration of peptidoglycan ranged from 1.93–416 ng/m^3. A highly significant correlation was found between the individual components of bioaerosol determined in this study. The concentration of endotoxin was correlated with the concentration of Gram-negative bacteria, total microorganisms, and peptidoglycan ($R>0.9$, $p<0.001$). The concentration of peptidoglycan was correlated with the concentration of Gram-positive bacteria, Gram-negative bacteria, and total microorganisms ($R>0.9$, $p<0.001$).

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INTRODUCTION

Agriculture and related industries belong to the most hazardous occupations [1, 17, 28]. Workers involved in cultivating, harvesting, storing or processing of agricultural products may be exposed, via the respiratory route, to a wide range of airborne biological agents such as microorganisms, endotoxin, peptidoglycan, glucans, lipoteichoic

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acid, and allergens. This complex and heterogeneous mixture of particles is collectively regarded as bioaerosols [1, 14, 15, 17, 20, 26, 51, 59]. The concentration of bioaerosols at a work place may vary between different sectors of production, but in most branches of agricultural industry it often exceeds existing recommended limits [1, 10, 11, 12, 20, 26, 51]. Inhalation of high levels of bioaerosols may lead to lung function decline and the rise of respiratory diseases such as asthma, chronic obstructive pulmonary diseases (COPD) or allergic alveolitis [9, 28, 46]. Endotoxin (LPS), the major constituent of the outer membrane of Gram-negative bacteria, has been recognised as important but not the only causative agent of both short-term and long-term effects on human health [55].

There is also a growing evidence of modifying inflammatory effects of other components of bioaerosols such as peptidoglycan (PGN). Chemically, peptidoglycan is a polymer formed by (1,4) linked N-acetylglucosamine and N-acetylmuramic acid. It is a main cell wall component of almost all bacteria, especially Gram-positive bacteria. Although the biological potency of PGN is much lower compared to endotoxin, inhalation of this substance may result in the increased influx of polymorphonuclear cells into the alveolar compartment, and the local production of proinflammatory cytokines and chemokines, such as TNF-α, which may have pirogenic properties [30, 31, 56]. The epidemiologic study by Zhiping *et al.* [61] confirmed that inhalation of PGN from swine-dust may cause a fever. On the other hand, *in vitro* study demonstrated that PGN can inhibit human mast cells development from pluripotent progenitor cells CD34+, and decreases the number of mature mast cells in culture by stimulation of apoptotic processes [23]. Therefore, the environmental exposure to PGN could be protective from asthmatic symptoms, as shown in epidemiologic studies [57, 60]. Moreover, the animal studies carried recently by Murphey *et al.* [40, 41] showed that exposure to PGN may induce resistance to Gram-positive and Gram-negative bacteria challenge, induce cross-tolerance to LPS, and non-specifically enhance innate immune functions. This suggests that airborne PGN may modify inflammatory reaction to other components of bioaerosol.

During the last two decades, occupational exposure to endotoxin has been intensively studied [13, 29, 42, 46, 48, 53]; however, there is only scant evidence of exposure to peptidoglycan at a work place [22, 24, 29]. The objective of our study was to determine and compare the concentration of airborne dust, microorganisms, endotoxin and peptidoglycan in several sectors of plant processing industries. This is the first study assessing exposure to peptidoglycan in Poland.

MATERIAL AND METHODS

Examined facilities. The study was conducted in 10 facilities located in eastern Poland, engaged in the processing of agricultural plants' products. The following plants were included in our study: herb processing plant "P", grain mills ("D", "M", and "W"), flax farms ("S" and "K"), grain elevators ("T" and "L"), bakery "L", plant producing cereals from barley "L". The air samples were taken on filters by the use of an AS-50 sampler (TWOMET, Zgierz, Poland), at the flow rate of 50 l/min. Glass fibre filters, with 1 μm pore size and 37 mm diameter were used. At each site, 3 samples were collected: 1 for the determination of microorganisms' concentration and composition, the second for the determination of dust and endotoxin concentration, and the third for determination of peptidoglycan concentration. The concentration of dust in the air was estimated gravimetrically from the difference between weight of the filter measured before and after sampling. The measurements were performed on 2 consecutive days, during daily performance, on the sites showing exposure typical for examined branches. The air samples were collected during: • cleaning, crumbling and granulating the peppermint leaves at the herb plant "P"; • threshing flax in the barns (farm "S" and "K"); • sacking wheat brans (mill "W"); • pouring cleaned wheat grain (mill "D"); • production of semolina, half-product for the production of noodles (mill "M"); • transport of uncleaned wheat grain (elevators "T" and "L"); • sieving the rye flour (bakery "L"); • production of pearl barley (cereals producing plant).

Determination of the concentration of microorganisms in 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 mesophilic bacteria, eosin methylene blue agar (EMB agar, Merck, Darmstadt, Germany) for estimation of Gram-negative mesophilic bacteria, half-strength tryptic soya agar (Trypticase Soy Agar, Difco, Baltimore, MI, USA) for estimation of thermophilic actinomycetes, and malt agar (Malt Agar, Difco, Baltimore, MI, USA) for estimation of fungi. The incubation conditions have been described earlier [6, 15]. Blood agar plates and EMB agar plates were incubated for 1 day at 37°C, then 3 days at room temperature (22°C), and finally 3 days at 4°C. The prolonged incubation at lower temperatures aimed to isolate as wide a spectrum of bacteria as possible. The tryptic soya agar plates were incubated for 5 days at 55°C. The malt agar plates were incubated for 4 days at 30°C, and for next 4 days at 22°C. After incubation, the grown colonies were counted and differentiated and the results reported as cfu per 1 cubic metre of air (cfu/m^3). The total concentration of microorganisms in the air was estimated by the addition of the concentrations of Gram-positive bacteria, Gram-negative bacteria, thermophilic actinomycetes and fungi. Bacterial isolates were identified with microscopic and biochemical methods as recommended by Bergey's

Manual [25, 52, 59]. 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). The species composition of isolated fungi was identified by microscopic methods according to Barron [2] and Litvinov [33].

Determination of the concentration of endotoxin in the air. The air samples for the detection of endotoxin were stored at -20°C until further processing. Concentration of bacterial endotoxin was determined by the Limulus amebocyte lysate (LAL) gel clot test [32]. 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 (Associates of Cape Code, Falmouth, 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 the airborne dust (ng/mg) was multiplied per estimated concentration of dust in the air (mg/m^3) and the results reported as micrograms of the equivalents of the *E. coli* 0113:H10 endotoxin per 1 m^3 of air. To convert to Endotoxin Units (EU), the value in micrograms was multiplied by 10,000.

Determination of the concentration of peptidoglycan in the air. The concentration of muramic acid (MuAc, the chemical marker of peptidoglycan) in collected air samples was estimated by gas chromatography – tandem mass spectrometry (GC-MS/MS) technique. The analyses were carried out at the Department of Laboratory Medicine, Division of Medical Microbiology, Lund University, Sweden. The air samples were frozen at -20°C, until the time of analyses.

The test was performed as described previously [49, 54]. Briefly, the dust samples were heated overnight at 85°C in 2 M methanolic HCl. This procedure resulted in the release of chemical markers from larger bacterial structures. Next, the internal standard (13 C-labelled MuAc in a methanolysate of 13 C-labelled algal cells) was added to each sample, and the mixture was extracted with 1 ml of heptane to remove hydrophobic compounds. The lower phase (containing more hydrophilic compounds) was evaporated to dryness under nitrogen at 50°C and further dried under vacuum in a desiccator. The samples were acetylated by heating in a mixture of acetic anhydride and pyridine at 60°C for an hour. Then the reaction mixtures were evaporated and dissolved in dichloromethane, and subsequently washed with 0.05 M HCl and water. The samples were evaporated to dryness, dissolved in chloroform and analysed.

The prepared samples were analysed using Saturn 2000 ion trap GC-MS instrument (Varian, Palo Alto, CA, USA) equipped with a fused-silica capillary column (CP-Sil 8 CB, low bleed, 0.25 µm film thickness, 30 m, 0.25 mm i.d.) (Chompack, Middelburg, The Netherlands). Volume of 2 µl was injected in the splitless mode with a helium head column pressure of 69 kPa using the Combi Pal auto sampler. The temperature of the column was programmed from 90–280°C, the temperature of the injector was 280°C, of the ion trap 220°C, and of the transfer line 290°C. All analyses were made in electron impact mode [49]. The results are shown as the amount of peptidoglycan per the cubic meter of air (ng/m^3). The calculations were made based on the fact that muramic acid constitutes about 15% of peptidoglican.

Statistical analysis. The data were analysed by Kolmogorov-Smirnov test for distribution. Not-normal distributed data were analysed with Mann-Whitney and Spearman's tests. The analyses were conducted using Statistica for Windows v. 5.0 package (Statsoft©, Inc., Tulsa, Oklahoma, USA).

RESULTS

The microbial pollution of the air of examined plants and farms is presented in Table 1. Concentrations of the total viable airborne microorganisms ranged from 0.18–861.4 × 10^3 cfu/m^3 (median 25.6 × 10^3 cfu/m^3). The highest microbial contamination of the air was observed on flax farms (median 630.9 × 10^3 cfu/m^3), in elevators (median 49.7 × 10^3 cfu/m^3) and in herb processing plant (median 26.0 × 10^3 cfu/m^3). Gram-positive mesophilic bacteria and fungi were detected at all sampling sites, and their median concentrations were respectively 18.1 × 10^3 cfu/m^3 (range 0.045–724.0 × 10^3 cfu/m^3) and 0.66 × 10^3 cfu/m^3 (range 0.03–80.6 × 10^3 cfu/m^3).

Gram-positive bacteria constituted 23–93% of the total microbial count (Fig. 1). The most common species found

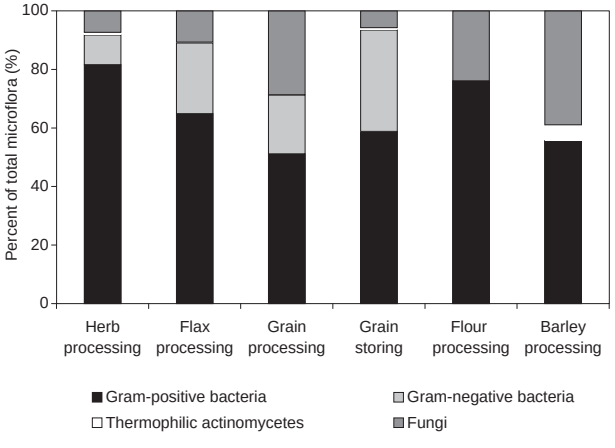

Figure 1. Composition of airborne microflora in different sectors of plant processing industry.

Table 1. Concentrations of microorganisms in the air (cfu $\times 10^3/m^3$) during processing of different plant products.

Type of production, facilities	Activity	Mesophilic bacteria		Thermophilic actinomycetes	Fungi	Total microorganisms
		Gram-positive	Gram-negative			
Herb processing						
Plant "P"	Production of granulates from the peppermint leaves	52.5	19.0	0.21	5.1	76.8
	Cleaning and crumbling of the peppermint leaves	9.0	0.1	0.1	0.46	9.7
	Cleaning of the peppermint leaves (1)	19.4	1.9	0.38	3.9	25.6
	Cleaning of the peppermint leaves (2)	23.6	2.0	0.1	0.66	26.4
Median		21.5	1.95	0.16	2.28	26.0
Flax processing						
Farm "S"	Flax threshing	724.0	56.4	0.44	80.6	861.4
Farm "K"	Flax threshing	183.0	168.0	1.45	48.0	400.4
Median		453.5	112.2	0.95	64.3	630.9
Grain processing						
Mill "W"	Sacking wheat brans	0.975	0.79	0.0	0.45	1.8
Mill "D"	Pouring cleaned wheat grain	0.345	0.075	0.0	0.03	0.45
Mill "M"	Production of semolina	0.045	0.0	0.0	0.15	0.19
Median		0.345	0.075	0	0.15	0.45
Grain storing						
Elevator "T"	Transport of uncleaned wheat grain	35.1	35.5	0.3	3.0	73.7
Elevator "L"	Transport of uncleaned wheat grain	18.1	5.4	0.345	1.96	25.8
Median		26.6	20.45	0.32	2.48	49.7
Flour processing						
Bakery "L"	Sieving the rye flour	0.56	0.0	0.0	0.18	0.74
Barley processing						
Plant "L"	Production of pearl barley	0.1	0.0	0.01	0.07	0.18
Total (median, range)		**18.1 (0.045–724.0)**	**1.9 (0.0–168.0)**	**0.1 (0.0–1.45)**	**0.66 (0.03–80.6)**	**25.6 (0.18–861.4)**

in the examined air samples were: *Staphylococcus* spp. *Curtobacterium pusillum*, *Rhodococcus fascians*, *Aureobacterium testaceum*, *Sanguibacter keddieii*, *Microbacterium* spp., *Bacillus* spp. Some mesophilic actinomycetes such as *Streptomyces albus* and *Streptomyces* spp. were also found.

Gram-negative bacteria were detected in 77% of collected air samples, and formed 0–48% of the total count. Their concentration ranged from 0–168.0 × 10^3 cfu/m^3 (median 1.9 × 10^3 cfu/m^3). The highest concentrations were detected on flax farms (median 112.2 × 10^3 cfu/m^3) and in elevators (median 20.45 × 10^3 cfu/m^3). No Gram-negative bacteria were found in the air during flour and barley processing. The species *Pantoea agglomerans* dominated in all air samples where the growth of Gram-negative bacteria was observed. In addition, the species of *Enterobacter amnigenus*, *Pseudomonas* spp., *Sphingomonas paucimobilis*, *Flavimonas oryzihabitans* and *Rahnella aquatilis* were also found. The concentration of thermophilic actinomycetes ranged from 0–1.45 × 10^3 cfu/m^3 (median 0.1 × 10^3 cfu/m^3). The species *Thermomonospora fusca*, *Thermoactinomyces thalpophilus*, and *Thermoactinomyces vulgaris* prevailed among thermophilic actinomycetes.

Fungi constituted 2.5–76.9% of the total microbial count. They dominated only at one sampling side (in mill "M" at semolina production). Among fungi, we identified *Penicillium* spp., *Mucor* spp., *Alternaria* spp., *Aspergillus niger*, *Aspergillus penicillioides*, *Aspergillus* spp., *Ulocladium* spp., *Fusarium* spp., *Rhizopus* spp., and *Scopulariopsis* spp.

The concentrations of airborne dust, endotoxin and muramic acid, the chemical marker of peptidoglycan, are presented in Table 2. In general, the airborne dust concentration at all examined workplaces ranged from 0.18–86.9 mg/m^3 (median 6.27 mg/m^3), being the highest during flax threshing (median 53.2 mg/m^3), flour processing (median 15.82 mg/m^3) and grain storing (median 6.52 mg/m^3). The

Table 2. Concentrations of dust, bacterial endotoxin and peptidoglycan in the air during processing of different plant products.

Type of production, facilities	Activity	Dust [mg/m^3]	Endotoxin [μg/m^3]	Muramic acid (Peptidoglycan) [ng/m^3]
Herb processing				
Plant "P"	Production of granulates from the peppermint leaves	68.33	62.5	N.d.
	Cleaning and crumbling of the peppermint leaves	2.22	41.7	10.3(68.7)
	Cleaning of the peppermint leaves (1)	4.22	41.7	10.9 (72.6)
	Cleaning of the peppermint leaves (2)	4.53	42.7	10.8 (72.0)
Median		4.37	42.2	10.8 (72.0)
Flax processing				
Farm "S"	Flax threshing	19.52	200.3	62.4 (416.0)
Farm "K"	Flax threshing	86.9	1562.6	N.d.
Median		53.2	881.4	62.4 (416.0)
Grain processing				
Mill "W"	Sacking wheat brans	20.57	3.2	N.d.
Mill "D"	Pouring cleaned wheat grain	0.47	0.00625	2.4 (15.0)
Mill "M"	Production of semolina	0.37	0.29	0.47 (3.13)
Median		0.47	0.29	1.43 (9.53)
Grain storing				
Elevator "T"	Transport of uncleaned wheat grain	6.27	156.3	38.4 (256.0)
Elevator "L"	Transport of uncleaned wheat grain	6.77	312.5	20.1 (134.0)
Median		6.52	234.0	29.5 (196.7)
Flour processing				
Bakery "L"	Sieving the rye flour	15.82	8.34	N.d.
Barley processing				
Plant "L"	Production of pearl barley	0.18	0.0041	0.29 (1.93)
Total (median, range)		**6.27 (0.18-86.9)**	**41.7 (0.0041-1562.6)**	**10.8(0.29-62.4) 72.0 (1.93-416.0)**

N.d. – not determined

lowest exposure to dust (0.18 mg/m^3) was recorded during barley processing. However, it must be noticed that only one plant was examined in this sector, so it may be not representative for the whole barley processing branch. Overall, almost 70% of the measurements exceeded Polish OEL of 4 mg/m^3 [43].

The median concentration of endotoxin in the air of the examined plants was large and amounted to 41.7 μg/m^3. The values varied within wide limits (0.0041–1562.6 μg/m^3) between different branches. The highest concentrations of airborne endotoxin were detected in the flax sector (median 881.4 μg/m^3), and in the grain storing sector (234.0 μg/m^3). The proposed exposure limit of 0.2 μg/m^3 [21] was exceeded in all branches except barley processing.

Muramic acid, the chemical marker of peptidoglycan, was detected in 9 out of 13 (70%) collected samples. In 4 collected air samples the concentration of dust was so high that amount of muramic acid could not be estimated, even after 3-fold dilution of the samples in chloroform. In general, the concentration of muramic acid ranged from 0.29–62.4 ng/m^3 (median 10.8 ng/m^3) while the concentration of recalculated peptidoglycan ranged from 1.93–416.0 ng/m^3 (median 72.0 ng/m^3) (Tab. 2). The highest amounts of peptidoglycan were recorded during flax processing, grain storing and herb processing.

A highly significant correlation was found by Spearman's test between the individual components of bioaerosol determined in this study. The concentration of endotoxin was correlated with the concentration of Gram-negative bacteria, total microorganisms, and peptidoglycan ($R>0.9$, $p<0.001$). The concentration of peptidoglycan was correlated with the concentration of Gram-positive bacteria, Gram-negative bacteria, and total microorganisms ($R>0.9$, $p<0.001$).

DISCUSSION

The present study demonstrates that workers employed in a broad spectrum of plant processing agricultural industry facilities in Poland may be exposed to large concentrations of microorganisms, dust, endotoxin and peptidoglycan. There was a considerable variability in exposure between examined sectors of agriculture. Overall, the highest

levels of microbial air contamination were found during primary processing of raw plant material such as flax, herbs and grain. The recorded concentrations of airborne microorganisms in these sectors were of the order of 10^4–10^5 cfu/m^3, comparable to microbial pollution observed in such hazardous sectors as animal production [12, 34] or potato processing [18]. Flax threshing especially seems to generate the greatest hazards to the health of exposed workers as during this occupation the highest concentrations of all types of microorganisms were detected. This data is in line with a previous study conducted in this sector of agriculture by Krysińska-Traczyk *et al.* [26]. It must be noticed, however, that both in the quoted study and in our work the samples were taken on small private farms, where the old types of threshing machines were used.

The concentrations of airborne microorganisms found in the present study in the herb processing sector were one order of magnitude lower compared to microbial pollution detected in a previous study carried out in herb processing plants 20–25 years ago [15], and 2 orders of magnitude lower compared to exposure found on herb farms engaged in processing peppermint [51]. Similarly, the concentrations of airborne microorganisms found in the present study in the big grain storing and processing plants were 1–2 orders of magnitude lower compared to those recorded 30–35 years ago in the similar facilities located in the same area of Poland [6, 8]. The above-mentioned data suggest the significant improvement of working conditions and marked progress in the reduction of airborne biohazards in the big Polish agricultural industry facilities after the change of the economic system in the last 20 years.

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 value proposed by Dutkiewicz and Jabłoński (100×10^3 cfu/m^3) [11] was exceeded only at 2 sampling sites associated with flax threshing while that proposed by Malmros *et al.* (10×10^3 cfu/m^3) [36] was exceeded at 7 out of 13 sampling sites, associated with flax threshing, herb processing and grain storage. The OEL value for airborne Gram-negative bacteria proposed by Dutkiewicz and Jabłoński [11] and Górny and Dutkiewicz [21] (20×10^3 cfu/m^3) was exceeded at 3 sampling sites associated with flax threshing and grain storage while that proposed by Clark [3] and Malmros *et al.* [36] (1×10^3 cfu/m^3) was exceeded at 7 sampling sites, associated with flax threshing, herb processing and grain storage. The OEL value proposed by Dutkiewicz and Jabłoński [11] and Górny and Dutkiewicz [21] for airborne fungi (50×10^3 cfu/m^3) was exceeded only on 2 farms processing flax, while nowhere was the OEL value proposed by these authors for airborne thermophilic actinomycetes (20×10^3 cfu/m^3) exceeded.

Gram-positive bacteria dominated at all but 2 sampling sites, while Gram-negative bacteria and fungi dominated at 1 sampling site each. Gram-positive bacteria consisted mostly of corynebacteria and cocci which are so far poorly known as occupational pathogens. Among Gram-negative bacteria, the dominant species was the epiphytic bacterium *Pantoea agglomerans* (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*) possessing strong endotoxic and allergenic properties [5, 27, 37, 38, 44, 47]. It has been documented that *Pantoea agglomerans* evokes a strong immunologic response in agricultural workers processing grain and herbs [7, 16, 19] and could be a cause of allergic alveolitis in these workers [9, 27, 35, 39]. The results of the present work confirm the potential role of this bacterium as an occupational allergen in plant dust. Among fungi isolated in the examined facilities, the representatives of the genera *Aspergillus*, *Penicillium*, and *Fusarium* may pose a hazard for exposed workers as a potential source of allergens and mycotoxins (ochratoxin, zearalenon, fumonisins, nivalenol and others) [1, 11, 28].

The median concentrations of airborne dust and endotoxin were large, amounting to 6.27 mg/m^3 and 41.7 μg/m^3, respectively. Both endotoxin and dust showed a large variability between different branches of agriculture. For dust, the Polish occupational exposure limit of 4 mg/m^3 [43] was exceeded at 9 out of 13 sampling sites (almost 70%). The greatest concentrations of airborne dust were observed during production of herb granulates (68.3 mg/m^3), flax processing (19.5–86.9 mg/m^3), sieving rye flour in a bakery (15.8 mg/m^3), and grain storage in elevators (6.3–6.8 mg/m^3). The concentrations of airborne dust and endotoxin recorded in the present study were similar to or lower compared to earlier investigations conducted on Polish flax farms [26], in herb processing plants [15], on herb farms [50, 51], and during grain processing [10, 13], but much higher then those observed in the corresponding sectors in the Netherlands [53].

As, to date, there is no legal occupational exposure limit (OEL) for endotoxin, the results obtained in the present work may be compared to some proposals based on health effects. Altogether, the concentrations of airborne endotoxin exceeded at 11 out of 13 sampling sites (84.6%) the OEL values proposed by Clark [3] (0.1 μg/m^3), Rylander [45] (0.1–0.2 μg/m^3), Malmros *et al.* [36] (0.1 μg/m^3), Górny and Dutkiewicz [21] (0.2 μg/m^3), and Laitinen *et al.* [29] (0.025 μg/m^3), and at 12 sites (92.3%) proposed by the Dutch Expert Committee on Occupational Standards (DECOS) [4] (0.005 μg/m^3). At 10 out of 13 examined sites (76.9%), airborne endotoxin occurred in very large quantities of the order 10^0–10^3 μg/m^3, exceeding values supposed to cause decrease of lung function over work shift and ODTS symptoms [46]. These data show that the work in most of plant processing facilities in Poland may be associated with a serious health hazard to the employees.

The present study, providing the first data on exposure to airborne muramic acid in Polish agriculture, showed rather low concentrations, ranging from 0.29–62.4, which correspond to peptidoglycan concentration of 1.93–416 ng/m^3.

The highest levels were measured during flax threshing and grain storing. Compared to PGN concentrations recorded in the air of agricultural facilities in other countries, the present results are similar to those reported by Krahmer *et al.* [24], slightly lower compared to those reported by Laitinen *et al.* [29], and distinctly lower compared to those obtained by Jolie *et al.* [22] and Wang *et al.* [58].

To summarize, the results of this work suggest that although viable bacteria may be largely inactivated during plant processing in modern facilities, the thermo resistant high molecular components of the cell wall, such as bacterial endotoxin and peptidoglycan, persist in the work environment and may pose a significant hazard to the workers.

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