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EXPOSURE TO AIRBORNE MICROORGANISMS AND ENDOTOXIN IN A POTATO PROCESSING PLANT

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Abstract: Microbiological air sampling was performed in a big potato processing plant located in eastern Poland. Air samples for determination of concentrations of microorganisms, dust and endotoxin were collected at 6 sites in the division producing potato flakes and meal from dried potato pulp and at 2 sites in the division producing potato syrup from imported starch. The concentrations of total airborne microorganisms were within a range of 28.3–93.1 × 10^3 cfu/m^3. Mesophilic bacteria were dominant at all sampling sites, forming 73.1–98.8% of the total count. Among them, distinctly prevailed corynebacteria (irregular Gram-positive rods) that accounted for 54.3–81.1% of the total airborne microflora. The most common were strains of *Corynebacterium* spp., followed by strains of *Arthrobacter* spp., *Microbacterium* spp., and *Agromyces ramosus*. The latter species, so far not reported from the air of occupational environments, abundantly develops in the parenchyma of potato tubers. Its airborne concentration increased rapidly after peeling of potatoes, and attained maximal values at cutting and blanching (steaming and sulfuration) of potatoes, and at sacking of potato meal. The proportions of Gram-negative bacteria and endospore-forming bacilli were low, respectively 0.6–7.6% and 2.0–8.1% of total count. Fungi constituted 1.2–26.9% of total count. The dominant species was *Aspergillus niger* that formed 99.8% of total airborne fungi. The values of the respirable fraction of airborne microflora varied between 25.3–73.2%. The concentrations of airborne dust were 1.4–26.6 mg/m^3 in the division producing potato flakes and meal and 114.9–200.5 mg/m^3 at pouring of potato and corn starch for syrup. The concentrations of airborne endotoxin were in the range of 0.011–0.089 µg/m^3 during the initial stages of potato processing (unloading, washing, peeling) and drastically increased after blanching to the extraordinarily high levels of 45.9–1893.9 µg/m^3. At pouring of starch for syrup, the concentrations of airborne endotoxin were much lower, within a range of 0.029–0.156 µg/m^3. In conclusion, the workers of potato processing facilities could be exposed to large concentrations of microorganisms, dust and endotoxin posing a risk of work-related respiratory disease.

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Key words: potato industry, occupational exposure, organic dust, bioaerosols, bacteria, fungi, endotoxin, *Agromyces ramosus, Aspergillus niger*.

INTRODUCTION

It has been well-documented that processing of grain and other vegetable matter may be associated with exposure to large quantities of bioaerosols capable of inducing allergic and/or immunotoxic reactions and respiratory disease in workers of the agricultural industry [7, 12, 13, 14, 17, 18, 20, 34, 35, 36, 45, 46, 55, 58, 61, 64]. The risk also concerns the workers of the potato processing industry, as has been evidenced during the recent decade

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by our preliminary report on the essential findings of the present work [15], as well as by a number of Dutch papers [25, 26, 68, 69, 70, 71, 72] including a comprehensive study by Zock [70] and a recent study by Ewers and Tapp [21] in the USA. It was demonstrated that the workers processing potatoes could be exposed to large concentrations of airborne bacteria, endotoxin, and potato antigen [15, 21, 25, 68, 69, 70], produce antibodies against work-related antigens [15, 25, 26, 69, 70], and experience work-related respiratory and general symptoms [15, 21, 25, 69, 70, 71, 72]. Zock [70] and Zock *et al.* [71] found a significant relationship between exposure to airborne endotoxin and cross-shift decrease in the lung function of potato processing workers.

The aim of the present work was to determine the levels of microorganisms, dust and endotoxin in the air of a big potato processing plant and to examine the species composition of airborne microflora.

MATERIALS AND METHODS

Examined facility. Air sampling was performed in a big potato processing plant located in eastern Poland in which a total of 140 workers were employed. The plant consists of 2 main divisions, the first producing potato flakes and meal from dried potato pulp, and the second producing potato syrup from imported starch. The production process in the first division starts outside the factory building with unloading of potato tubers from trucks into a big gutter. The potatoes are transported with water stream to a washing drum inside the building for removing stones and other impurities, poured into a big container, and then transported by conveyor belt to a peeling machine. Peeling is followed by manual trimming of tuber black spots ("specking"). Peeled tubers are transported by conveyor belt to the cutting machine and the small cubes derived (1 × 1 cm) are poured into a big boiler and subjected to a process of "blanching", comprising steaming at 88ºC and sulfuration. After blanching, the cubes of potato pulp are flushed with water and transported to dryers to obtain potato flakes. Some of the dried flakes are ground in milling machines to obtain potato meal, another final product of this division. The production process in the second division producing potato syrup comprises the following stages:

- pouring of imported potato starch and corn starch from sacks into the outlets of containers,
- mixing of the potato and corn starch in the proportion 7 : 3,
- pouring water to the obtained starch mixture and leaving in special vats for fermentation,
- distribution of ready potato syrup into barrels for preservation until dispatch for sale.

Air sampling for microorganisms, dust and endotoxin was conducted throughout whole production process in the division producing potato flakes and meal at 6 sites corresponding to the most important production stages, and at the initial stages of production in the division producing potato syrup from starch (at 2 sites during pouring of potato starch and corn starch to the outlets of containers). Altogether, air samples were taken at following 8 sites: 1) unloading potatoes from trucks; 2) washing potatoes; 3) peeling potatoes; 4) cutting potatoes and blanching potato pulp; 5) milling dried potatoes; 6) sacking potato meal; 7) pouring potato starch for syrup; 8) pouring corn starch for syrup.

Microbiological examination of the air. Air samples were taken in the potato processing plant with a custom-designed particle-sizing slit sampler [11] 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 in 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 cfu per m^3; and the other' "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-off point for the respirable fraction was 3.0 µm, approximating the recommendations of the American Conference of Governmental Industrial Hygienists [65]. The used sampler enabled the determinations of concentrations of microorganisms in the air in the range of 10^0-10^8 cfu/m^3.

At each sampling site, 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 37ºC, then 3 days at 22ºC and finally 3 days at 4ºC. The malt agar plates were subsequently incubated for 4 days at 30ºC and 4 days at 22ºC [12]. The prolonged incubation at lower temperatures aimed to isolate 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 55ºC. The grown colonies were counted and differentiated and the data reported as cfu per 1 cubic meter of air (cfu/m^3). The total concentration of microorganisms in the air was obtained by the addition of the concentrations of total mesophilic bacteria (grown on blood agar medium), 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 [32, 62, 67] and Cowan & Steel [6]. Additionally, the selected isolates were identified with microtests: API Systems 20E and NE (bioMèrieux, Marcy l'Etoile,

Table 1. Microorganisms in the air of a potato processing plant: concentrations and respirable fractions (Rf).

Sampling site (Number, name)	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 (%)
1. Unloading potatoes from trucks	51.8 ± 8.4	75.8	0.1 ± 0.1	100	0	0	2.2 ± 0.6	13.5	54.0 ± 8.5	73.2
2. Washing potatoes	26.9 ± 8.8	29.7	4.9 ± 1.8	43.9	0	0	1.4 ± 1.3	0	28.3 ± 10.1	28.2
3. Peeling potatoes	38.6 ± 6.8	25.2	1.3 ± 0.5	33.3	0	0	0.5 ± 0.5	37.5	39.1 ± 6.6	25.3
4. Cutting potatoes and blanching potato pulp	55.1 ± 12.0	45.0	0.2 ± 0.2	50.0	0	0	2.1 ± 1.2	68.6	57.2 ± 11.9	45.7
5. Milling dried potatoes	28.3 ± 6.4	25.0	0.1 ± 0.1	0	0.1 ± 0.1	0	10.4 ± 2.3	51.7	38.8 ± 5.3	32.1
6. Sacking potato meal	60.4 ± 15.9	36.4	0	0	0	0	12.5 ± 4.9	92.8	72.9 ± 14.4	46.1
7. Pouring potato starch for syrup	78.5 ± 6.8	38.9	0	0	0.1 ± 0.1	0	14.5 ± 6.0	100	93.1 ± 7.0	51.2
8. Pouring corn starch for syrup	71.5 ± 15.2	55.6	0.2 ± 0.3	100	0	0	14.3 ± 2.7	16.7	85.8 ± 15.6	49.1
Mean	51.4 ± 21.0	41.5	0.9 ± 1.7	40.9	0.02 ± 0.06	0	7.3 ± 6.7	47.6	58.7 ± 24.6	43.9

France) and BIOLOG System (Biolog, Inc., Hayward, CA, USA). Fungi were classified with microscopic methods, according to Barron [2], Larone [37], Litvinov [39], Ramirez [49], and Raper & Fennell [50].

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 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) [38]. The filters were extracted for 1 hour in 10 ml of pyrogen-free water at room temperature, heated to 100ºC 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 1 hour in a water bath at 37ºC, using pyrogen-free water as a negative control and the commercial lipopolysaccharide (endotoxin) of *Escherichia coli* 0111:B4 (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/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), the value in nanograms was multiplied by 1.2 [48].

Microbiological examinations of potatoes. In order to obtain more information about the origin of particular microorganisms recovered from the air of a potato processing plant, the following samples derived with the use of a sterile scalpel from potato tubers before or during processing were collected in sterile Erlenmeyer flasks for microbiological analysis:

- scrapings from potato tuber collected at unloading station (sampling site 1),
- epidermis peeled off potato tuber collected at unloading station (sampling site 1),
- a piece of cube from peeled potato tuber collected from cutting machine before blanching (sampling site 4),
- a piece of cube from peeled potato tuber collected from the conveyor after blanching and washing (sampling site 4).

The concentration and species composition of bacteria and fungi in the collected samples was determined by dilution plating [18]. After triturating in a glass homogenizer, 1 gm of each sample was suspended in 100 ml of the sterile saline (0.85% NaCl) containing 0.1% (v/v) of Tween 80; after vigorous shaking, serial 10-fold dilutions in saline were made up to 10^{-10}. The 0.1 ml aliquots of each dilution were spread on duplicate sets of the following media: blood agar plates for total mesophilic bacteria, half-strength tryptic soya agar for thermophilic actinomycetes and malt agar for fungi. The incubation conditions and identification methods were the same as described above for air samples.

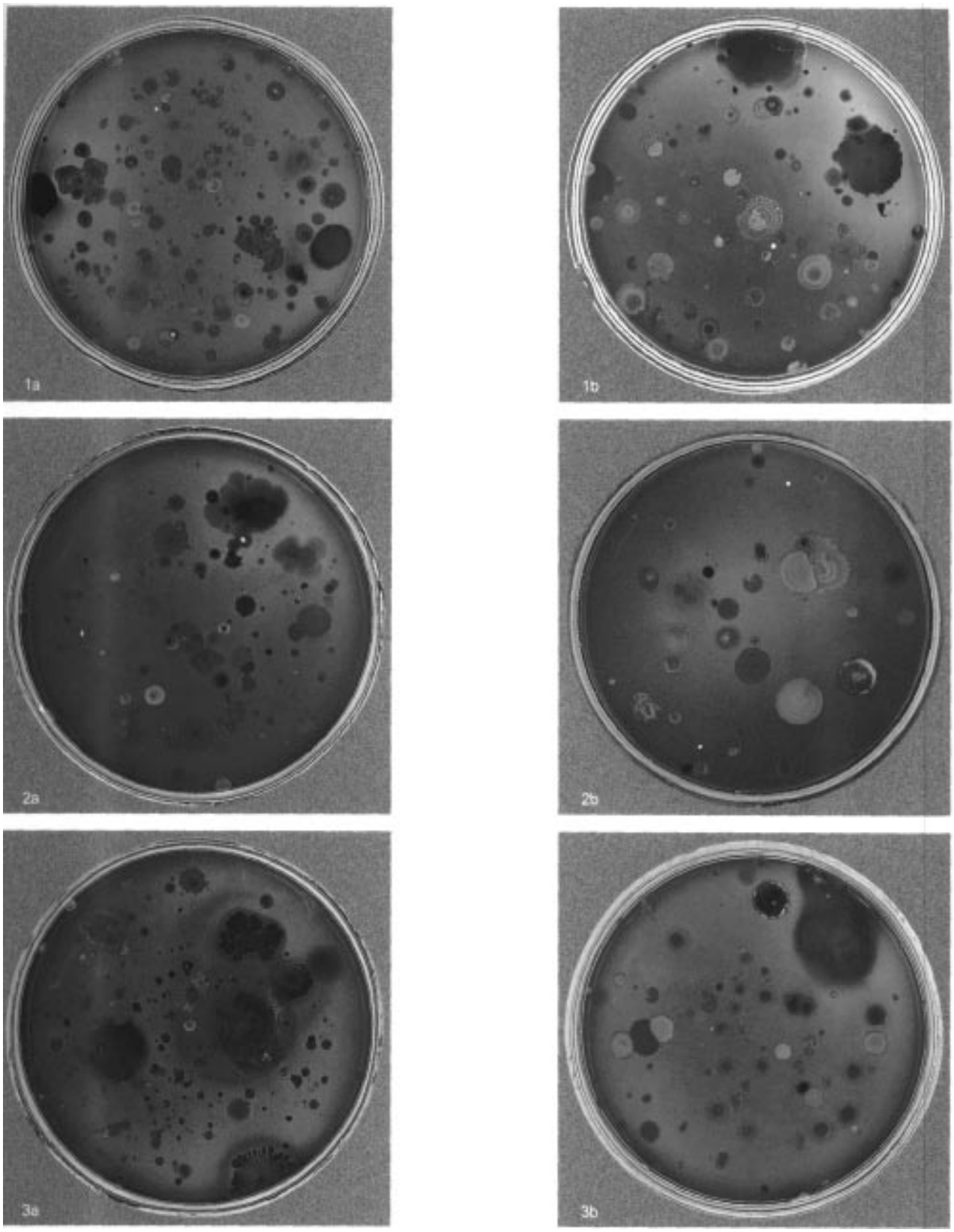

Figure 1. Photographs of air samples for mesophilic bacteria taken in the potato processing plant at following sites: 1a-1b - unloading potatoes from trucks (site 1); 2a-2b - peeling potatoes (site 3); 3a-3b - sacking potato meal (site 6). The samples were taken using particle-sizing sampler on blood agar plates, each in volume of 3.33 l. Photographs 1a, 2a, 3a show total bacterial flora of the air, while photographs 1b, 2b, 3b show the respirable fraction. It may be seen that the levels of airborne bacteria did not vary much throughout the production process, at all sites being of the order 10^4 cfu/m^3. The prevailing organisms were corynebacteria (forming 54–81% of total isolates), followed by Gram-positive cocci, endospore-forming bacilli and Gram-negative bacteria.

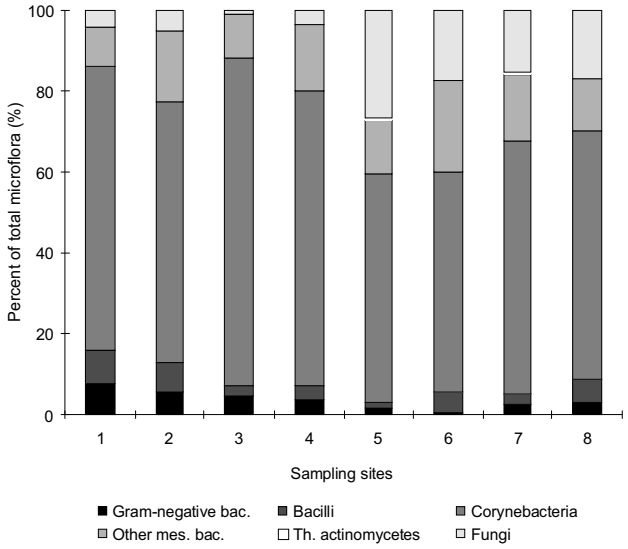

Figure 2. Composition of airborne microflora in the potato processing plant: total count, including mesophilic bacteria, thermophilic actinomycetes and fungi.

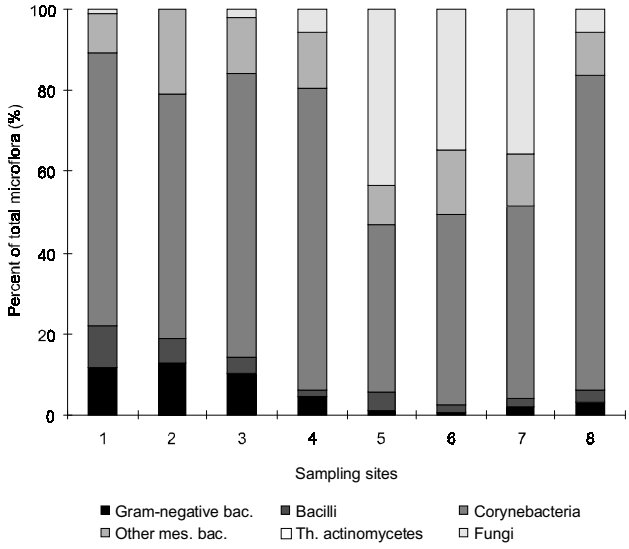

Figure 3. Composition of airborne microflora in the potato processing plant: respirable fraction, including mesophilic bacteria, thermophilic actinomycetes and fungi.

Statistical analysis. The results were analysed by Shapiro-Wilk test for distribution and Spearman correlation test, using STATISTICA for Windows v. 5.0 package (Statsoft©, Inc., Tulsa, Oklahoma, USA).

The study was performed mostly during the years 1989–1991 and continued during 1999–2002. Preliminary results of this work have been reported elsewhere [15, 16, 17].

RESULTS

The concentrations of total microorganisms in the air of a potato processing plant were in the moderately large order of 10^4 cfu/m^3 at all sampling sites, ranging from 28.3–93.1 × 10^3 cfu/m^3 (Tab. 1). In the division producing potato flakes and meal, the initial concentration of airborne microorganisms at unloading potatoes from trucks was above 50.0 × 10^3 cfu/m^3, then dropped below this level during washing and peeling, but raised again at cutting and blanching potatoes (Fig. 1). In the division producing potato syrup from starch, relatively large values above 85.0 × 10^3 cfu/m^3 were noted at both sampling sites during pouring of potato and corn starch (Tab. 1).

The composition of the total airborne microflora in a potato processing plant is depicted in Figure 2, and the composition of the respirable fraction of airborne microflora in Figure 3. Mesophilic bacteria were dominant at all sampling sites, forming 73.1–98.8% of the total airborne microflora and 56.7–100% of the respirable fraction of airborne microflora.

Among mesophilic bacteria, corynebacteria (irregular Gram-positive rods) distinctly prevailed and accounted for 54.3–81.1% of the total airborne microflora (Fig. 2) and 41.1–77.9% of the respirable fraction (Fig. 3). The most common were strains of *Corynebacterium* spp. (forming 26.1–49.2% of the total airborne microflora), followed by strains of *Arthrobacter* spp., *Microbacterium* spp., and *Agromyces ramosus*. The latter species deserves particular attention as to date it has not been reported in the air of occupational environments. *Agromyces ramosus* is a fastidious soil bacterium classified among irregular, nonsporing Gram-positive rods [27, 30]. It grows on blood agar in the form of small hemolytic colonies composed of branched, filamentous elements which subsequently undergo fragmentation into coccoid or club-shaped cells (Fig. 4). In the air of the examined potato processing plant, *Agromyces ramosus* was not detected during unloading of potatoes or during pouring of starch for syrup. Its airborne concentration, however, increased rapidly after peeling of potatoes, and attained maximal values at cutting and blanching of potatoes, and at sacking of potato meal (Fig. 5), forming respectively 9.1% and 8.2% of the total airborne microflora. These figures indicate that this particular bacterial species develops inside potato tubers, which has been confirmed by the results of bacteriological investigation of various samples of potato tubers collected in the examined plant (Tab. 2). It was demonstrated that *Agromyces ramosus* was absent in the scrapings from potato tuber, appeared in a small quantity of 2.0 × 10^5 cfu/g in the epidermis of the potato tuber (in both cases other corynebacterial species prevailed in the total count of mesophilic bacteria), and occurred in a large concentration of 4.4 × 10^6 cfu/g in a cube of peeled potato tuber collected from the cutting machine, forming 76.4% of the total count of mesophilic bacteria. The process of blanching, including steaming and sulfuration of potato pulp, caused over 100-fold decrease in the concentration of *A. ramosus*, but these bacteria still formed 52.5% of the total count (Tab. 2). These data clearly show that *Agromyces ramosus* abundantly develops in the parenchyma of potato tubers and is disseminated into the air of potato processing plants, together with fine particles of potato pulp.

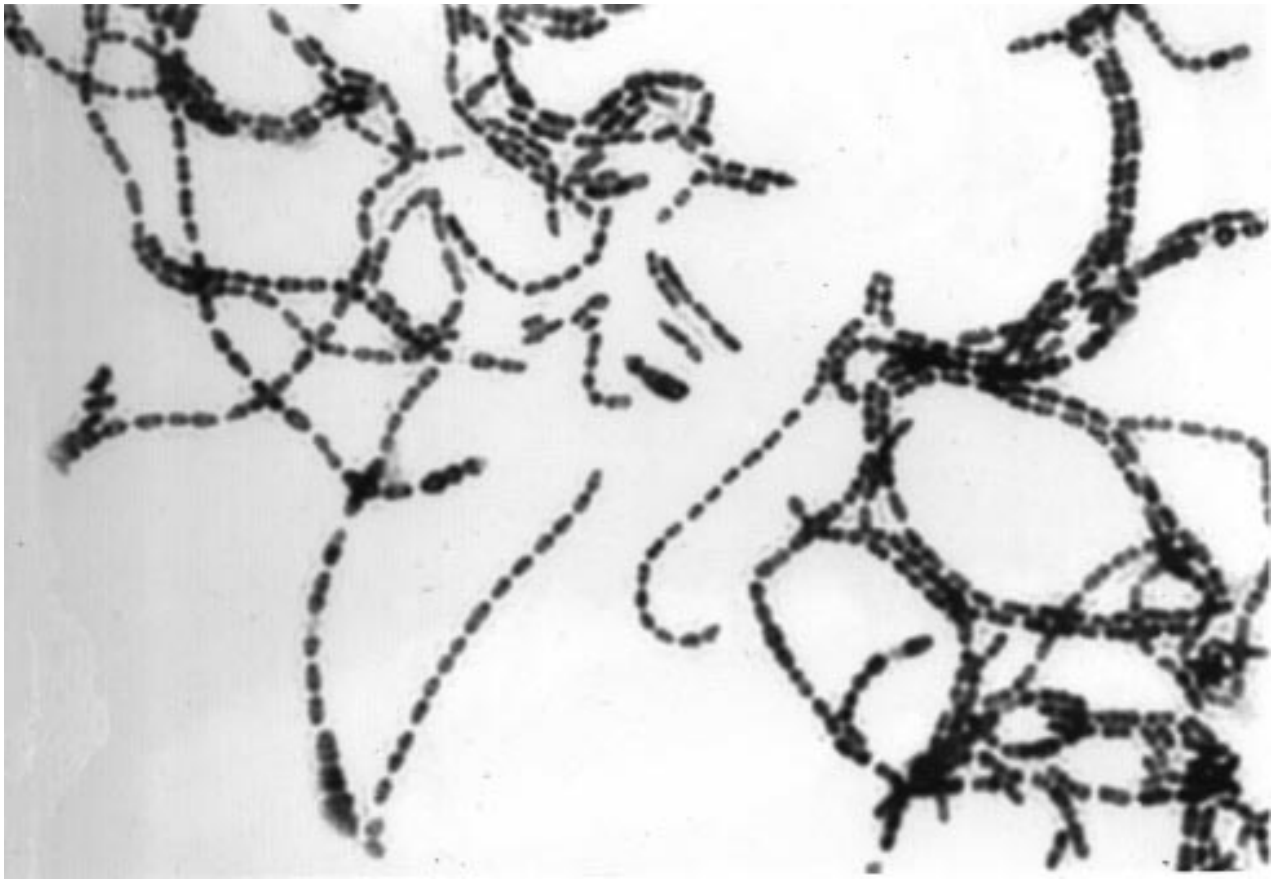

Figure 4. *Agromyces ramosus*, strain ZM-61 isolated from parenchyma of peeled potato cube, collected in the potato processing plant. Note branched, filamentous elements fragmenting into coryneform or coccoid cells. Gram-stained preparation, × 4,000.

The proportion of Gram-negative bacteria in the total airborne microflora (Fig. 2) was low (0.6–7.6%), and a little higher (0.4–12.8%) in the respirable fraction of the microflora (Fig. 3). The highest percentages of Gram-negatives were noted at the initial stages of potato processing, e.g. at unloading, washing and peeling: respectively, 7.6%, 5.7%, 4.5% of total microflora, and 11.9%, 12.8%, 10.4% of respirable microflora (Figs 2 and 3). These results were confirmed by examination of the samples of potato tubers collected in the examined plant, which proved that Gram-negative bacteria were associated with the outer stratum of potato tuber; their concentration in epidermis was 1.6×10^6 cfu/g (33.3% of the total count) while in the parenchyma it was about 10-fold lower (Tab. 2). Among Gram-negative bacteria occurring in the air of a potato processing plant, the most common taxa were *Alcaligenes faecalis*, *Pseudomonas* spp., and *Acinetobacter calcoaceticus*.

Endospore-forming bacilli (*Bacillus* spp.) constituted 2.0–8.1% of the total count (Fig. 2) and 1.6–9.9% of the respirable count (Fig. 3). A group described as "other mesophilic bacteria" which consisted mostly of Gram-positive cocci (*Micrococcus* spp., *Staphylococcus* spp.) formed 9.6–29.0% of the total count (Fig. 2) and 9.7–21.1% of the respirable count (Fig. 3). Thermophilic actinomycetes were found only at 2 sampling sites in trace quantities (Fig. 2).

Fungi constituted 1.2–26.9% of the total airborne microflora and 0–43.3% of the respirable fraction of airborne microflora (Figs 2 and 3). It is noteworthy that at 3 sampling sites (milling dried potatoes, sacking potato meal, pouring potato starch for syrup) the percentages of fungi in the respirable fraction were about twice as high compared to those in total airborne microflora, and were even comparable to the percentages of corynebacteria which distinctly prevailed in the microflora of the examined plant (Fig. 3). The fungal flora of the air in the potato processing plant was fully dominated by *Aspergillus niger* which accounted for 99.8% of the total airborne fungi, and 98.9% of the respirable fraction of airborne mycoflora.

The values of the respirable fraction of airborne microflora in the potato processing plant varied within a fairly wide range and were between 25.3–73.2% (on average 43.9 ± 15.5%) (Tab. 1).

In the air samples taken in the examined facility, 39 species or genera of bacteria and 7 species or genera of fungi were identified, of these, 8 and 6 species or genera respectively were reported as having allergenic and/or immunotoxic properties [14, 19, 29, 34, 35, 44] (Tab. 3). These figures are certainly an underestimation, as a part of bacterial and fungal strains could be identified only to generic level.

The concentrations of dust in the air of the division producing potato flakes and meal were moderate (1.4–6.7

Table 2. Concentrations of mesophilic bacteria in the samples of potatoes collected in a potato processing plant (cfu× 10^3/gram, percent of the total count).

Sample	Gram-negative bacteria	Gram-positive cocci	*Bacilllus* spp.	Coryneform bacteria		*Streptomyces* spp.	Total mesophilic bacteria
				Agromyces ramosus	Other species		
Scrapings from potato tuber collected at unloading station (sampling site 1)	1102.2 (16.7 %)	699.6 (10.6%)	198.0 (3.0%)	0 (0)	3300.0 (50.0%)	1300.2 (19.7%)	6600.0 (100%)
Epidermis peeled off potato tuber collected at unloading station (sampling site 1)	1598.4 (33.3%)	100.8 (2.1%)	0 (0)	201.6 (4.2%)	2798.4 (58.3%)	100.8 (2.1%)	4800.0 (100%)
Cube of peeled potato tuber collected from cutting machine before blanching (sampling site 4)	199.5 (3.5%)	347.7 (6.1%)	0 (0)	4354.8 (76.4%)	798.0 (14.0%)	0 (0)	5700.0 (100%)
Cube of peeled potato tuber collected from the conveyor after blanching and washing (sampling site 4)	9.0 (14.7%)	7.0 (11.5%)	3.0 (4.9%)	32.0 (52.5%)	10.0 (16.4%)	0 (0)	61.0 (100%)

mg/m^3) in the first phase of the production cycle and attained a higher level of 17.0–26.6 mg/m^3 in the second phase, during processing of dried flakes and meal (Tab. 4). The workers of the division producing potato syrup were exposed during pouring of potato and corn starch to very large concentrations of airborne dust in the range of 114.9–200.5 mg/m^3 (Tab. 4).

The concentrations of airborne endotoxin were low during the initial stages of potato processing (unloading, washing, peeling), being within the range of 0.011–0.089 µg/m^3. A drastic, over 5,500–fold increase in the concentration of airborne endotoxin, up to the level of 125.0 µg/m^3, occurred after the process of blanching, comprising steaming and sulfuration of potato pulp (Tab. 4). At the following stage of milling dried potatoes, there was noted a further rise in the endotoxin concentration up to the extraordinarily high value of 1893.9 µg/m^3, which dropped at the next stage of sacking potato meal to the distinctly lower but still very high level of 45.9 µg/m^3. At pouring of starch for syrup, the concentrations of airborne endotoxin were much lower, being within the range of 0.029–0.156 µg/m^3.

A significant correlation was found between the concentration of dust and the concentrations of fungi and total microorganisms, and between the concentration of mesophilic bacteria and fungi ($p < 0.05$). No other significant relationships could be found between the examined aerosol components.

DISCUSSION

The present study has demonstrated that the workers of potato processing plants could be exposed to large concentrations of airborne microorganisms, dust and endotoxin posing an occupational hazard. The concentrations of total airborne microorganisms in the examined plant

Table 3. List of microbial species and genera identified in samples of air from a potato processing plant.

Gram-negative bacteria: ***Acinetobacter calcoaceticus**+** (2, 7), ***Alcaligenes faecalis**+** (1-8)**,** *Citrobacter freundii* (4), ***Pantoea agglomerans**+ (synonyms: *Erwinia herbicola, Enterobacter agglomerans*)** (1-4, 7, 8), *Pseudomonas* spp. (1-8), *Pseudomonas vesicularis* (1, 8), *Serratia liquefaciens* (1, 4, 7, 8).

Bacilli: *Bacillus cereus* (1, 2, 4, 6-8), *Bacillus megaterium* (1-8), ***Bacillus subtilis**** (1-8), *Bacillus pumilus* (1, 4, 6-8), *Bacillus* spp. (1-8).

Corynebacteria: *Agromyces ramosus* (2-6), ***Arthrobacter globiformis**** (1-8), *Arthrobacter* spp. (1-8), *Aureobacterium saperdae* (2), *Brevibacterium casei* (2, 4), *Brevibacterium helvolum* (8), ***Brevibacterium linens**** (1-8), *Corynebacterium* spp. (1-8), *Corynebacterium pseudodiphteriticum* (6), *Corynebacterium xerosis* (2, 3, 5, 7), *Corynebacterium thomssenii* (5), *Microbacterium lacticum* (1-8).

Other mesophilic bacteria: *Diplococcus pneumoniae* (6), *Lactobacillus* spp. (1-3), *Micrococcus luteus* (1-8), *Micrococcus roseus* (1-8), *Micrococcus varians* (1-8), *Micrococcus* spp. (1-8), *Staphylococcus epidermidis* (1-8), *Staphylococcus saprophyticus* (2, 4, 6-8), *Staphylococcus warneri* (2), *Staphylococcus* spp. (1-8), *Streptococcus* spp. (2-6), ***Streptomyces albus**** (1-3, 5-8), *Streptomyces griseus* (2, 4), *Streptomyces* spp. (1-3, 7).

Thermophilic actinomycetes: ***Thermoactinomyces vulgaris**** (5, 7).

Fungi: ***Alternaria alternata**+** (1), ***Aspergillus fumigatus**+** (1)**,** ***Aspergillus niger****+ (1-8), ***Candida* spp.*** (1), *Geotrichum candidum* (1), ***Mucor* spp**.* (7), ***Rhizopus nigricans*** * + (1, 7).

Sites of isolation are given in parentheses. Names of species reported as having allergenic and/or immunotoxic properties (see text) are in bold and marked as follows: * allergenic species; + immunotoxic species. *Bacillus cereus*, *Diplococcus pneumoniae*, *Aspergillus fumigatus* and *Aspergillus niger* may cause infectious disease in man.

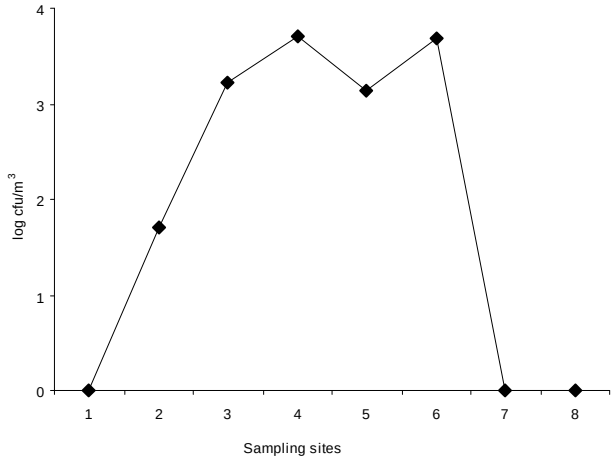

Figure 5. Concentration of *Agromyces ramosus* in the air of the potato processing plant in subsequent stages of production process.

Table 4. Concentrations of dust and bacterial endotoxin in air of a potato processing plant.

Sampling site (Number, name)	Concentration of dust (mg/m^3)	Concentration of endotoxin ($\mu g/m^3$)
1. Unloading potatoes from trucks	1.4	0.089
2. Washing potatoes	6.7	0.011
3. Peeling potatoes	2.3	0.022
4. Cutting potatoes and blanching potato pulp	6.0	125.000
5. Milling dried potatoes	26.6	1893.900
6. Sacking potato meal	17.0	45.900
7. Pouring potato starch for syrup	114.9	0.029
8. Pouring corn starch for syrup	200.5	0.156
Median	11.8	0.122

were of the order 10^4 cfu/m^3, approximating those reported by Zock [70] and Zock *et al.* [68] from the Dutch potato processing facilities, and by Forster *et al.* [22] from an English sugar beet refinery. These were greater by 1–3 orders of magnitude compared to microbial concentrations in dwellings and offices [23] but smaller compared to 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 [7, 12, 14, 16, 17, 18, 20, 34, 35, 61, 64].

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 airborne microorganisms, the OEL value of 10×10^3 cfu/m^3 proposed by Malmros *et al.* [42] was exceeded at all 8 sampling sites, whereas the OEL value proposed by Dutkiewicz and Jabłoński (50×10^3 cfu/m^3 at the value of respirable fraction equal to or above 50%, 100×10^3 cfu/m^3 at the value of respirable fraction below 50%) [14, 16] was exceeded at 2 sites (unloading potatoes, pouring potato starch for syrup). The OEL value of 1×10^3 cfu/m^3 proposed by Clark [5] and Malmros *et al.* [42] for airborne Gram-negative bacteria was exceeded at 2 sites (washing and peeling potatoes). Nowhere were the OEL values exceeded proposed by Dutkiewicz and Jabłoński [14, 16] for airborne Gram-negative bacteria, thermophilic actinomycetes and fungi (respectively 10×10^3 cfu/m^3, 10×10^3 cfu/m^3, and 25×10^3 cfu/m^3 at the value of respirable fraction equal to or above 50%, and 20×10^3 cfu/m^3, 20×10^3 cfu/m^3, and 50×10^3 cfu/m^3

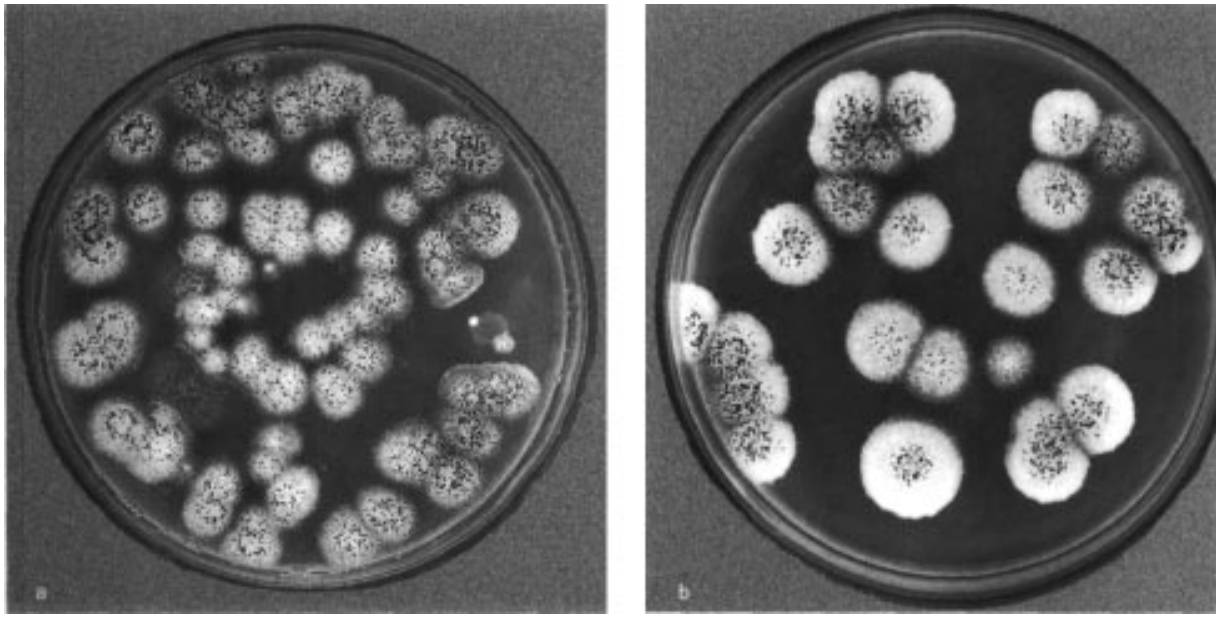

Figure 6. Photographs of air samples for fungi taken in the potato processing plant at sacking potato meal (sampling site 6). Samples were taken using particle-sizing sampler on malt agar plates (a - total airborne fungi; b - respirable fraction of airborne fungi) each in volume of 3.33 l. Note abundant growth of *Aspergillus niger* - the only fungal species recovered from the air at this site.

at the value of respirable fraction below 50%). On average, the concentrations of airborne Gram-negative bacteria found in this study were smaller than those reported by Zock *et al.* [68] from Dutch potato processing facilities, whereas the concentrations of fungi were similar.

The airborne microflora of the examined potato processing plant was clearly dominated by corynebacteria which at all sampling sites formed over 50% of the total count. These bacteria are commonly associated with organic dusts [44] and were isolated in large quantities from the air of agricultural settings at threshing of grain or flax [17] and tending farm animals [12, 14]. To date, there are only a few reports on corynebacteria associated with potatoes. *Corynebacterium sepedonicum* was isolated from raw and processed potatoes [8], while *Corynebacterium*–like and *Curtobacterium*–like bacteria were isolated from samples of waste water taken in a potato processing plant [21]. Surprisingly, the *Agromyces ramosus* that in this study was proved to be prevalent bacterium in potato parenchyma and common in the air polluted with particles of potato pulp, has not been reported so far neither from potatoes [8] nor from potato pulp [43]. The most probable explanation for this is that the isolation media used by earlier authors did not support the growth requirements of this fastidious bacterium. *Agromyces ramosus* grows well on media containing blood constituents [30] and hence the use of sheep blood agar in the present study as an isolation medium enabled abundant recovery of this organism from potato parenchyma and from the air of the potato processing plant. This indicates the need for further studies on this puzzling bacterium, including its possible implications for human health.

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 [44] 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 necessary.

Among the species of Gram-negative bacteria isolated from the air of the examined potato processing plant, *Alcaligenes faecalis* and *Acinetobacter calcoaceticus* were proved to possess allergenic and endotoxic properties [44, 59, 60]. Both species commonly occur in air polluted with organic dusts of plant and animal origin [12, 14, 16, 17]. *Acinetobacter* strains were isolated from potato pulp [43] and from samples of waste water taken in the potato processing plant [21]. According to Zock [70], and Ewers and Tapp [21], transport water, process water, and waste water are important sources of the endotoxin-producing Gram-negative bacteria in potato processing plants.

Though the fungal flora of air in the examined potato processing plant occurred in a relatively low concentration, the fact that it consisted almost entirely of *Aspergillus niger*, a potentially pathogenic species, poses an occupational hazard for exposed workers. This fungus reveals allergenic and immunotoxic properties and may evoke occupational asthma and allergic alveolitis in the workers of citric acid factories when used in the fermentation process [4, 28, 33, 66]. *Aspergillus niger* was also implicated in causing occupational asthma in the worker of a sugar beet processing facility [53] and its enzymes were identified as a cause of asthma in pharmacy workers and bakers [3, 40, 47]. *A. niger* can also cause pulmonary aspergillosis and ear infection [24, 31] and produces toxic metabolites: malformins, naphtoquinones and nigragillin [1]. Sorenson *et al.* [63] found that conidia of *A. niger* activate rat alveolar macrophages causing the release of inflammatory mediators. Based on these results, the authors suggested that the inhalation of large numbers of *A. niger* conidia could lead to pulmonary inflammation.

The concentrations of dust and bacterial endotoxin in the air of the examined potato processing plant varied within a wide range with extremely high levels at some sampling sites. The concentrations of dust were of the order 10^{o}–10^{2} mg/m^3, exceeding at 6 out of 8 sampling sites the Polish OEL value of 4 mg/m^3 [54] by 1.5–50.1 times. On average, the concentrations of airborne dust found in the present study were by 1-2 orders of magnitude greater compared to those reported from potato processing facilities in the Netherlands [68, 70] and the United States [21].

The concentrations of airborne endotoxin in the examined facility were of the order 10^{-2}–10^{3} µg/m^3. At 3 sampling sites at the first phase of potato processing (unloading, washing, peeling) and at 2 sampling sites at pouring of starch for syrup, the concentrations of airborne endotoxin were within the range of 0.011–0.156 µg/m^3, being comparable with the values reported from potato processing facilities in the Netherlands [68, 70, 72] and the United States [21]. Out of these 5 sampling sites, at 1 site the endotoxin concentration exceeded the occupational exposure limit (OEL) value of 0.1 µg/m^3 proposed by Clark [5], Rylander [56] and Malmros *et al.* [42], at 3 sites the OEL value of 25 ng/m^3 suggested by Laitinen *et al.* [36], and at all sites the OEL value of 5 ng/m^3 proposed by the Dutch Expert Committee on Occupational Standards (DECOS) [10]. At no site did the concentrations of airborne endotoxin exceed the value of 0.2 µg/m^3 supposed to cause decrease of lung function across a work shift [57] and the values of 1–2 µg/m^3 supposed to evoke ODTS symptoms [57].

A drastic rise in the concentration of airborne endotoxin up to the levels of 45.9–1893.9 µg/m^3 has been observed after the process of blanching. The very high values recorded at this work position and at the positions of milling and sacking dried potatoes posed a substantial hazard for workers engaged in the final phase of the production of dried potato flakes and meal. They exceeded

many times the proposed OEL values [5, 10, 36, 42] and the values supposed to cause decrease of lung function across a work shift and ODTS symptoms [57]. This rise of airborne endotoxin could be the result of steaming potatoes during the process of blanching, as it is known that heating might enhance the biological activity of the endotoxin by changing its physical structure [52, 56]. This suggests the possibility of a particular respiratory risk that might arise when endotoxin-containing organic materials are steamed, roasted or burnt in the course of various production or heating processes.

A possibility of unspecific, false-positive *Limulus* reactions must be also considered to explain the high endotoxin levels in this phase of potato processing, at least with regard to the extraordinary large value of 1893.9 µg/m^3 found at milling dried potatoes. While the concentrations of airborne endotoxin of the order 10^1–10^2 µg/m^3 were detected on farms and at harvesting and processing of grain and herbs [17, 18, 36, 41, 51], the concentrations of the order 10^3 µg/m^3 are extremely rare and should always be critically evaluated as a possible overestimate, as it was in relation to such a result obtained by our group in a herb processing plant [18]. It cannot be excluded that the extraordinary high result obtained in the present study might be attributed, at least in part, to an unspecific *Limulus* reaction with unknown constituents of potato pulp, or to an *Agromyces ramosus* bacterium that had been changed by steaming and sulfuration in the process of blanching. A non-specific reaction with glucans suggested by Rask-Andersen *et al.* [51] to explain the false-positive *Limulus* reactions are, in this case, less probable as Douwes *et al.* [9] found only small concentrations of glucans in samples of potatoes and potato starch.

Generally, the obtained results confirm the view expressed by Zock [70], and Ewers and Tapp [21] on the important role of airborne endotoxin in evoking work-related symptoms in potato processing workers.

CONCLUSION

The workers in potato processing plants could be exposed to large concentrations of airborne microorganisms, dust and endotoxin, posing a risk of work-related respiratory disease. The particular risk is created by a high exposure to airborne endotoxin at processing of dried potatoes after blanching.

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