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EXPOSURE TO AIRBORNE MICROORGANISMS AND ENDOTOXIN IN HERB PROCESSING PLANTS

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Abstract: Microbiological air sampling was performed in two herb processing plants located in eastern Poland. Air samples for determination of the levels of bacteria, fungi, dust and endotoxin were collected at 14 sites during cleaning, cutting, grinding, sieving, sorting and packing of 11 kinds of herbs (nettle, caraway, birch, celandine, marjoram, mint, peppermint, sage, St. John's wort, calamus, yarrow), used for production of medications, cosmetics and spices. It was found that processing of herbs was associated with a very high pollution of the air with bacteria, fungi, dust and endotoxin. The numbers of microorganisms (bacteria and fungi) in the air of herb processing plants ranged within 40.6–627.4 × 10^3 cfu/m^3 (mean ± S.D = 231.4 ± 181.0 × 10^3 cfu/m^3). The greatest concentrations were noted at the initial stages of production cycle, during cleaning, cutting and grinding of herbs. The numbers of airborne microorganisms were also significantly ($p < 0.0001$) related to the kind of processed herb, being the greatest at processing marjoram, nettle, yarrow and mint. The values of the respirable fraction of airborne microflora in the examined facilities varied within a fairly wide range and were between 14.7–67.7%. The dominant microorganisms in the air of herb processing plants were mesophilic bacteria, among which endospore-forming bacilli (*Bacillus* spp.) and actinomycetes of the species *Streptomyces albus* were most numerous. Among Gram-negative bacteria, the most common was endotoxin-producing species *Alcaligenes faecalis.* Altogether, 37 species or genera of bacteria and 23 species or genera of fungi were identified in the air of herb processing plants, of these, 11 and 10 species or genera respectively were reported as having allergenic and/or immunotoxic properties. The concentrations of dust and bacterial endotoxin in the air of herb processing plants were large with extremely high levels at some sampling sites. The concentrations of airborne dust ranged within 3.2-946.0 mg/m^3 (median 18.1 mg/m^3), exceeding at 13 out of 14 sampling sites the Polish OEL value of 4 mg/m^3. The concentrations of airborne endotoxin ranged within 0.2-2681.0 µg/m^3 (median 16.0 µg/m^3), exceeding at all sampling sites the suggested OEL value of 0.1 µg/m^3. In conclusion, the workers of herb processing plants could be exposed to large concentrations of airborne microorganisms, dust and endotoxin posing a risk of work-related respiratory disease.

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Key words: herb processing plants, occupational exposure, organic dust, bioaerosols, bacteria, fungi, endotoxin.

INTRODUCTION

Workers of agricultural industry engaged at processing of different plant materials may be exposed to the inhalation of large quantities of organic dusts containing allergenic and/or immunotoxic agents of plant and microbial origin. High levels of potentially hazardous bacteria, fungi and endotoxin were found in the air of grain stores and mills [9, 13, 26, 28, 54], animal feed industry facilities [9, 16, 52], tobacco processing plants

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[10, 22], and stores of horticulture seeds [11]. The agents may penetrate into lungs of exposed workers and evoke inflammatory reactions leading to respiratory disease, such as organic dust toxic syndrome (ODTS), allergic alveolitis, mycotoxicoses, asthma, mucous membrane irritation, and chronic bronchitis [5, 21, 26, 27, 48, 57-59].

To date, no comprehensive studies on exposure to potentially hazardous microbial agents associated with organic dusts have been conducted in herb processing facilities. Herbs obtained from numerous plant species, either cultivated or collected in nature, are widely used for the production of medications, cosmetics and spices. The herb processing industry is developing in many countries, including Poland, where about 2,500 workers are employed. People growing or processing herbs may be exposed to hazardous bioaerosols derived from the plants themselves or from epiphytic microorganisms associated with the plants. Mackiewicz *et al.* [35] described the case of allergic alveolitis in a herb growing farmer, following exposure to thyme dust.

The aim of the present work was to determine the levels of microorganisms, dust and endotoxin in the air of facilities processing different kinds of herbs.

MATERIALS AND METHODS

Examined facilities. Air sampling was performed in two big herb processing plants ("A" and "B") located in eastern Poland in which respectively 90 and 70 workers were employed. In these plants different kinds of herbs are processed, delivered either by herb growers or collectors of wild herbs. The processing of herbs, mostly with the use of specialized machinery, included the following stages: cleaning, cutting or grinding, sorting and/or sieving, and final packing in portions suitable for use as medicines, spices, or ingredients of cosmetics.

In plant "A" the air samples were taken at the following sites: cleaning of marjoram herb (*Majorana hortensis* Moench) with a machine "Schilbach" (A1); cutting of yarrow herb (*Achillea millefolium* L.) with a cutting machine (A2); cleaning of caraway (*Carum carvi* L.) seeds with a machine "Petkus" (A3); cleaning of caraway (*Carum carvi* L.) seeds with a grain screening machine (A4); cleaning of mint (*Mentha pulegium* L.) leaves with a screening machine "Allgaier" (A5); grinding of sage (*Salvia officinalis* L.) leaves with a machine "Alpine" (A6); cleaning of stinging nettle (*Urtica dioica* L.) leaves with a screening machine "Schilbach" (A7); automatic packing of ground leaves of stinging nettle (*Urtica dioica* L.) (A8); and manual sorting of celandine roots (*Chelidonium maius* L.) (A9).

In plant "B", the samples were taken at the following sites: manual packing of white warty birch (*Betula verrucosa* Erh.) leaves (B1); cutting of peppermint (*Mentha piperita* L.) herb with a cutting machine (B2); sieving of calamus (*Acorus calamus* L.) rhizome with a machine "Schilbach" (B3); cutting of St. John's wort (*Hypericum perforatum* L.) herb with a cutting machine (B4); and cutting of stinging nettle (*Urtica dioica* L.) herb with a cutting machine (B5).

The samples were always collected during uninterrupted work of machinery and full activity of all workers engaged at the workplace. At each site, a full series of 20 double samples for viable microorganisms (as described below) and samples for dust and endotoxin were collected on the same day.

Microbiological examination of the air. Air samples were taken in herb processing plants with a custom-designed particle-sizing slit sampler [8] enabling estimations of both total and respirable fractions of the microbial aerosol (Polish Patent 87612 assigned on 6 June 1977). Each air sample was a duplicate, taken at a flow rate of 20 l/min. This 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 another "b" sampled through a pre-selector (consisting of a system of glass tubes and regulated deposition disks covered with sticky substance) for the respirable fraction. The value of respirable fraction was expressed as a percent (%) of total count, calculated by division of the number(s) of cfu on plate(s) "b" through the number(s) of cfu on plate(s) "a" and multiplication by 100. The median cut point for the respirable fraction was 3.0 µm, approximating the recommendations of the American Conference of Governmental Industrial Hygienists [55]. 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 five double samples was taken on each of the following agar media: blood agar for total non-fastidious mesophilic Gram-negative and Gram-positive bacteria, whey agar for lactobacilli, half-strength tryptic soya agar for thermophilic actinomycetes, and malt agar for fungi. The blood agar plates were subsequently incubated for one day at 37ºC, then three days at 22ºC and finally three days at 4ºC. The malt agar plates were subsequently incubated for four days at 30ºC and four days at 22ºC [9]. The prolonged incubation at lower temperatures aimed to isolate as wide a spectrum of bacteria and fungi as possible. The whey agar plates were incubated the same as the blood agar plates and the tryptic soya agar plates were incubated for five days at 55ºC. The grown colonies were counted and differentiated and the data reported as cfu per one 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 non-fastidious mesophilic bacteria, lactobacilli, thermophilic actinomycetes and fungi. The percent composition of the total microflora of the air was then determined.

Bacterial isolates were identified by microscopic and biochemical methods, as recommended by Bergey's Manual [24, 53, 56] and Cowan & Steel [4]. Additionally,

the selected isolates were identified with microtests: API Systems 20E and NE (bioMérieux, Marcy l'Etoile, France) and BIOLOG System (Biolog, Inc., Hayward, CA, USA). Fungi were classified by microscopic methods, according to Barron [1], Larone [29], Litvinov [33], Ramirez [42], and Raper & Fennell [43].

For determination of dust and endotoxin concentrations, the air samples were collected on polyvinyl chloride filters by the use of an AS-50 one-stage sampler (TWOMET, Zgierz, Poland). Two samples were taken at each sampling site. The concentration of dust in the air was estimated gravimetrically. The concentration of bacterial endotoxin in the airborne dust was determined by the *Limulus* amebocyte lysate gel tube test (LAL) [31]. The filters were extracted for one 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 one 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 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 [41].

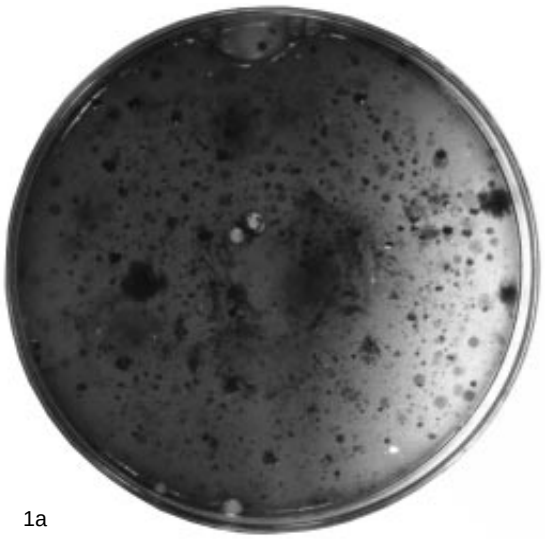
Microbiological examination of settled dust. Five samples of settled herb dusts were collected in sterile Erlenmayer flasks for microbiological analysis. In plant "A" the following three samples were collected: • dust from marjoram herb, collected under cleaning machine "Schilbach"; • dust from yarrow herb, collected under cutting machine; • dust from sage herb, collected under grinding machine "Alpine". In plant "B" the following two

Table 1. Microorganisms in the air of herb processing plant "A": concentrations and respirable fractions (Rf).

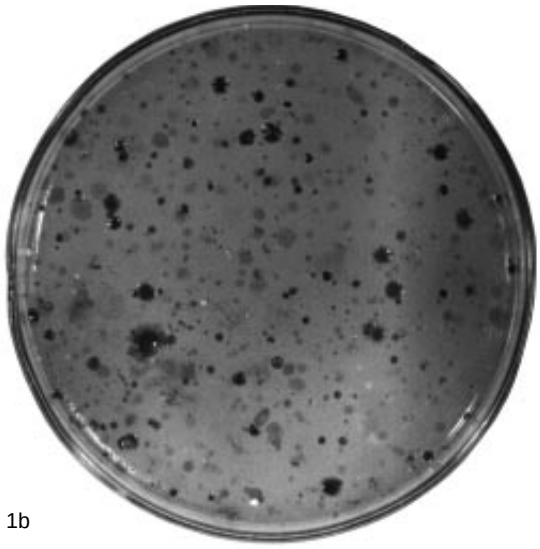
Plant, sampling site	Non-fastidious mesophilic bacteria (Blood agar)		Lactobacilli (Whey agar)		Thermophilic actinomycetes (Tryptic soya agar)		Fungi (Malt agar)		Total microorganisms	
	Concentration (mean ± S.D., cfu/m^3 × 10^3)	Rf (%)	Concentration (mean ± S.D., cfu/m^3 × 10^3)	Rf (%)	Concentration (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 (%)
A1. Cleaning of marjoram	466.0 ± 221.0	52.5	0	0	54.2 ± 19.1	98.6	107.2 ± 30.6	20.5	627.4 ± 191.5	51.0
A2. Cutting of yarrow	354.5 ± 80.1	28.8	0	0	22.0 ± 1.2	21.8	25.8 ± 6.6	62.3	402.3 ± 75.1	30.6
A3. Cleaning of caraway (1)	156.8 ± 59.1	13.4	0.3 ± 0.4	0	10.6 ± 1.8	11.3	14.6 ± 7.8	31.1	182.3 ± 54.4	14.7
A4. Cleaning of caraway (2)	57.7 ± 10.4	42.4	0.6 ± 0.8	60.0	2.8 ± 0.7	39.1	26.6 ± 3.6	43.7	87.7 ± 10.0	42.8
A5. Cleaning of mint	293.5 ± 84.1	56.8	1.8 ± 1.7	83.3	5.2 ± 0.3	72.8	40.9 ± 6.3	63.0	341.4 ± 84.2	58.0
A6. Grinding of sage	72.8 ± 17.5	41.5	1.0 ± 1.5	12.5	3.5 ± 2.5	27.6	16.7 ± 1.8	44.6	94.4 ± 15.2	41.3
A7. Cleaning of nettle	391.2 ± 169.4	74.6	0.2 ± 0.5	0	15.0 ± 12.5	34.4	73.9 ± 35.8	38.0	480.3 ± 158.9	67.7
A8. Packing of nettle	86.5 ± 29.3	30.6	0	0	3.2 ± 2.3	43.4	16.6 ± 6.5	22.5	106.3 ± 30.1	29.7
A9. Sorting of celandine root	148.7 ± 56.8	58.5	0	0	22.0 ± 5.1	23.0	13.2 ± 5.1	50.9	183.9 ± 48.5	53.7
Mean	225.3 ± 153.3	44.3	0.4 ± 0.6	31.2	15.4 ± 16.5	41.3	37.3 ± 32.5	41.8	278.4 ± 193.6	43.3

Table 2. Microorganisms in the air of herb processing plant "B": concentrations and respirable fractions (Rf).

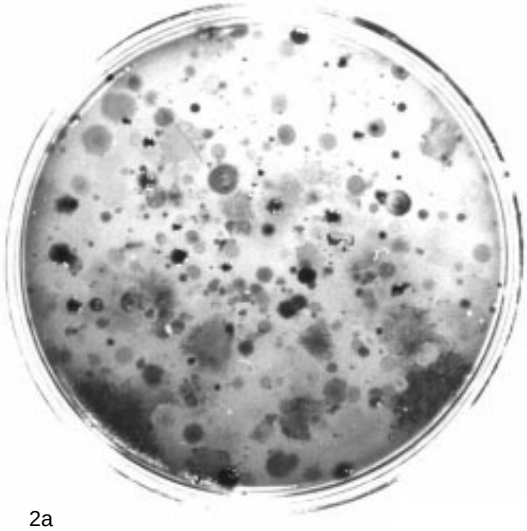
Plant, sampling site	Non-fastidious mesophilic bacteria (Blood agar)		Lactobacilli (Whey agar)		Thermophilic actinomycetes (Tryptic soya agar)		Fungi (Malt agar)		Total microorganisms	
	Concentration (mean ± S.D., cfu/m^3 × 10^3)	Rf (%)	Concentration (mean ± S.D., cfu/m^3 × 10^3)	Rf (%)	Concentration (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 (%)
B1. Packing of birch leaves	35.8 ± 4.4	26.8	0.1 ± 0.3	0	0.1 ± 0.3	100	4.6 ± 1.8	26.3	40.6 ± 4.7	26.9
B2. Cutting of peppermint	135.1 ± 87.7	37.6	2.5 ± 1.9	0	0.8 ± 1.1	0	21.4 ± 4.1	41.0	159.8 ± 77.0	37.2
B3. Sieving of calamus rhizome	73.2 ± 30.5	54.8	0.5 ± 0.8	25.0	1.7 ± 0.5	14.3	6.0 ± 0.6	34.0	81.4 ± 38.6	52.2
B4. Cutting of St. John's wort	37.2 ± 12.7	68.4	0.2 ± 0.5	0	0.4 ± 0.5	0	3.7 ± 1.5	38.7	41.5 ± 13.1	64.7
B5. Cutting of nettle	383.5 ± 58.5	48.9	0.7 ± 0.7	33.3	2.8 ± 1.2	17.4	23.8 ± 5.6	59.1	410.8 ± 54.6	49.3
Mean	133.0 ± 145.7	47.3	0.8 ± 1.0	11.7	1.2 ± 1.1	26.3	11.9 ± 9.8	39.8	146.9 ± 155.3	46.1



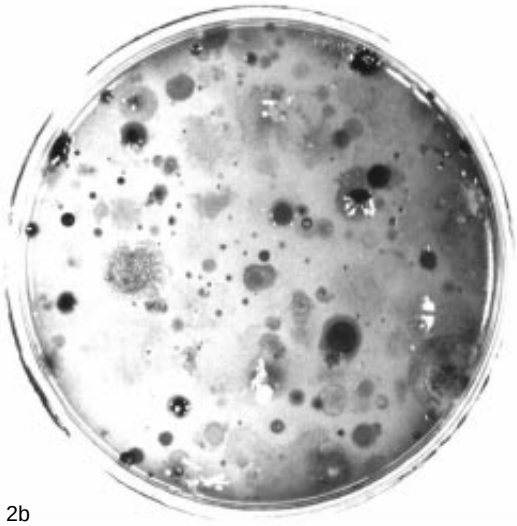
1a



1b



2a



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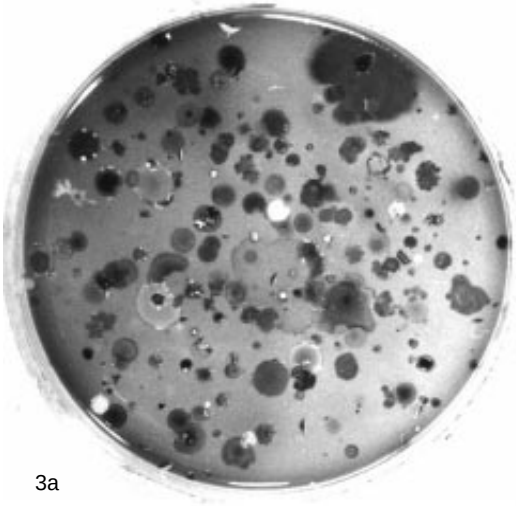

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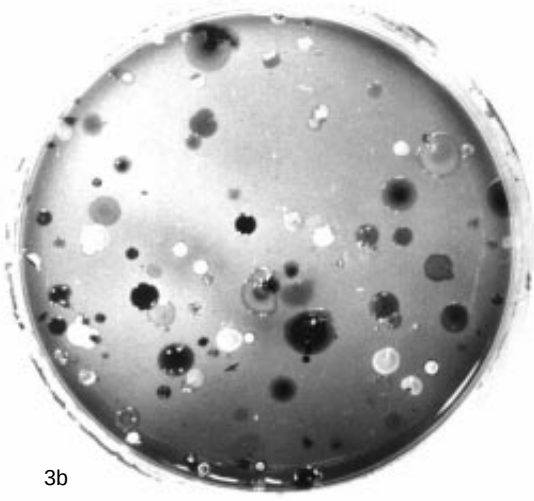

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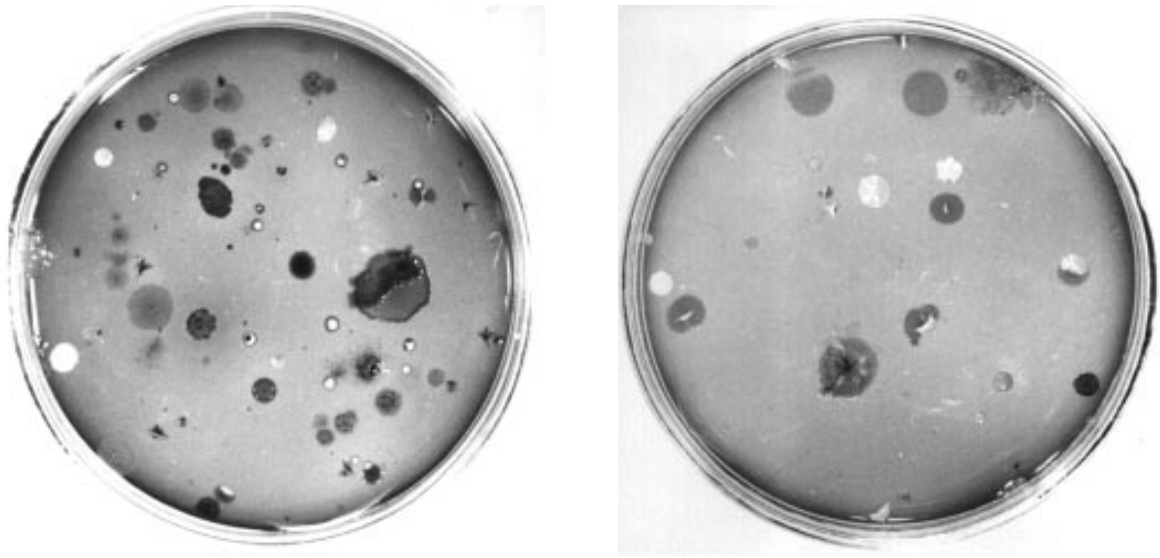

Figure 1. Photographs of air samples for mesophilic bacteria taken in herb processing plants, showing various degrees of microbial pollution of the air during processing of different herbs. The samples were collected at the following sites: 1a-1b - at cleaning of marjoram (plant "A", site A1); 2a-2b - at sorting of celandine roots (plant "A", site A9); 3a-3b - at sieving of calamus rhizome (plant "B", site B3); 4a-4b - at packing of birch leaves (plant "B", site B1). The samples were taken by the use of particle-sizing sampler on blood agar plates, each in volume of 1.667 l. The photographs 1a, 2a, 3a, 4a show total bacterial flora of the air, while photographs 1b, 2b, 3b, 4b show the respirable fraction. It may be seen that the concentration of bacteria in the air was very high at cleaning of marjoram (mean 466.0 cfu/m^3), high at sorting of celandine roots (mean 148.7 cfu/m^3), moderate at sieving of calamus rhizome (mean 73.2 cfu/m^3), and low at packing of birch leaves (mean 35.8 cfu/m^3). *Bacillus* strains dominated at processing of marjoram and celandine roots, forming respectively over 80% and over 50% of total isolates. The most common organisms at processing of calamus rhizome and birch leaves were *Bacillus* strains, *Streptomyces* strains, and corynebacteria, each forming 20-30% of total isolates.

samples were collected: • dust from birch leaves, collected at the site of packing the leaves; • dust from calamus rhizome, collected under sieving machine "Schilbach".

The concentration and species composition of bacteria and fungi in the collected samples was determined by dilution plating [37]. One gram of each sample was suspended in 100 ml of the sterile saline (0.85% NaCl) containing 0.1% (v/v) of Tween 80 and 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. The concentration of bacterial endotoxin in the samples of settled dust was determined by the *Limulus* amebocyte lysate gel tube test (LAL), also described above.

Statistical analysis. The results were analysed by Shapiro-Wilk test for distribution and chi-square test, using STATISTICA for Windows v. 4.5 package (Statsoft©, Inc., Tulsa, Oklahoma, USA).

The study was performed mostly during the years 1984-1986 and continued during 1997–2001. All the sampling and determinations of the concentration and species composition of the airborne microflora was completed in both facilities at all sites during the first phase of the study (1984–1986). In the second phase of the study (1997–2001), the detailed taxonomic studies of the bacterial and fungal isolates and statistical analyses of the results were carried out. Preliminary results of this work have been reported elsewhere [12-14, 16, 18, 20].

RESULTS

The concentrations of total microorganisms in the air of herb processing plants were large, ranging within 40.6-627.4 × 10^3 cfu/m^3 (Tables 1–2). At most sampling sites (9 out of 14), the concentrations exceeded the level of 10^5 cfu/m^3. The average number of microorganisms in the air of plant "A" (278.4 × 10^3 cfu/m^3) was almost twice as high as in plant "B" (146.9 × 10^3 cfu/m^3).

The levels of airborne microorganisms varied both with the kind of processed herb (chi-square test: $p < 0.0001$) and the stage of the production process (chi-square test: $p < 0.0001$). The greatest concentrations, exceeding 300 × 10^3 cfu/m^3, were recorded during processing of marjoram (Fig. 1), nettle, yarrow and mint. Microbial pollution of the air was, on average, greater during initial stages of the production cycle (cleaning, cutting, grinding) ranging within 41.5–627.4 × 10^3 cfu/m^3, than in final stages (sorting, sieving, packing), when it ranged within 40.6–183.9 × 10^3 cfu/m^3. This may be seen clearly in the example of nettle processing; the concentrations of airborne microorganisms during cleaning, cutting and packing of nettle herb were respectively 480.3 × 10^3 cfu/m^3, 410.8 × 10^3 cfu/m^3, and 106.3 × 10^3 cfu/m^3.

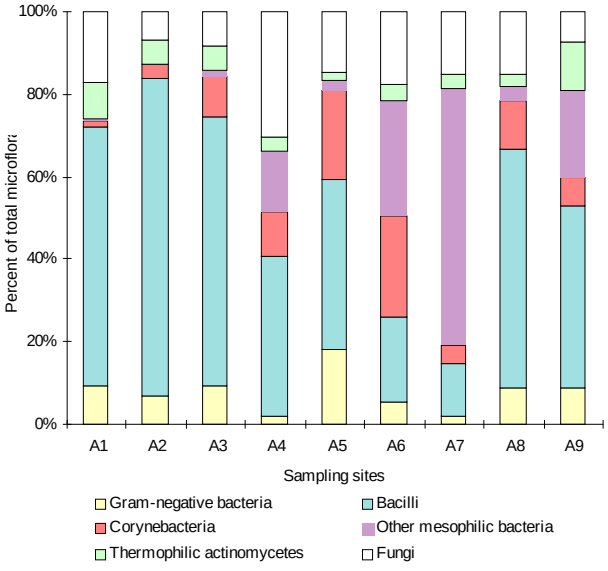

Figure 2. Composition of airborne microflora in herb processing plant "A" (total count, including mesophilic bacteria, thermophilic actinomycetes and fungi).

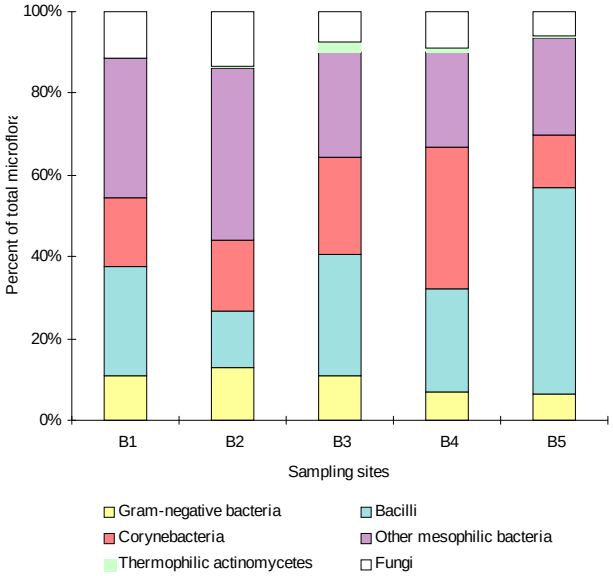

Figure 3. Composition of airborne microflora in herb processing plant "B" (total count, including mesophilic bacteria, thermophilic actinomycetes and fungi).

The composition of airborne microflora in herb processing plants is depicted in Figures 2–3. Mesophilic bacteria were dominant at all sampling sites. Endospore-forming bacilli (*Bacillus* spp.) distinctly prevailed at five sampling sites (cleaning of marjoram, cutting of yarrow, cleaning of caraway with a "Petkus" machine, packing of nettle, cutting of nettle), forming 50.3–76.9% of the total airborne microflora, and remained the most numerous microorganisms at the other four sites (cleaning of mint, cleaning of caraway with grain screening machine, sorting of celandine, sieving of calamus), constituting 29.5–44.2% of the total. Mesophilic actinomycetes of the species *Streptomyces albus* (included in Figures 2–3 in "other mesophilic bacteria") distinctly prevailed at one

Table 3. List of microbial species and genera identified in the samples of air from herb processing plants.

Gram-negative bacteria: ***Acinetobacter calcoaceticus****+ (A2, A6, A7, A9, B), ***Alcaligenes faecalis****+ (A, B)**,** *Neisseria flava* (B1, B2), ***Pantoea agglomerans****+ **(synonyms: *Erwinia herbicola, Enterobacter agglomerans*)** (A2, A3, A5-A9), *Pseudomonas fluorescens* (A8), *Pseudomonas* spp. (A1, A5, A9, B), ***Proteus vulgaris*** **+** (A4, A9, B3), *Sphingomonas sanguis* (A5), *Xanthomonas campestris* (A4), *Xanthomonas maltophilia* (A5).

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

Corynebacteria: ***Arthrobacter globiformis**** (A6, A7, A9, B2, B5), *Arthrobacter* spp. (A, B), *Brevibacterium helvolum* (A6-A8), ***Brevibacterium linens**** (A1-A3, A5, A6, A8, A9, B2, B4), *Corynebacterium* spp. (A, B), *Microbacterium lacticum* (A1-A3, A5-A9, B).

Other mesophilic bacteria: *Lactobacillus* spp. (A3-A7, B), *Micrococcus luteus* (A6), *Micrococcus roseus* (A6, A8), *Micrococcus* spp. (A, B), *Rhodococcus* spp. (A1-A3, A5-A9, B), *Staphylococcus epidermidis* (A4, A5, A7, A9, B2-B5), *Staphylococcus saprophyticus* (A4, A9, B2-B5), *Staphylococcus* spp. (A, B), ***Streptomyces albus**** (A2-A9, B), *Streptomyces* spp. (A, B1-B4).

Thermophilic actinomycetes: *Micromonospora* spp. (B1), ***Saccharomonospora viridis**** (A1, A2, A5-A7, A9), ***Saccharopolyspora rectivirgula**** **(synonyms: *Faenia rectivirgula, Micropolyspora faeni*)** (A4, A6, A7, A9), ***Thermoactinomyces vulgaris**** (A, B), *Thermomonospora fusca* (A4, A6, A7, A9).

Fungi: ***Alternaria alternata****+** (A1-A3, A5-A8, B), *Alternaria malvae* (A2, A3, A6, A8, B), *Alternaria* spp. (A6), *Aspergillus amstelodami* (B2), ***Aspergillus candidus****+ (A2, A6, B5), *Aspergillus chevalieri* (B1), ***Aspergillus fumigatus****+** (A1-A4, A6, A9, B1)**,** *Aspergillus mangini* (B2), *Aspergillus nidulans* (A4, A7, A9, B1, B5), ***Aspergillus niger**** + (A4, A6, A7, A9), *Aspergillus pseudoglaucus* (B4), *Aspergillus repens* (A2-A5, A7, A8, B1, B3-B5), ***Candida* spp.*** (A2, A8, B), *Fusidium terricola* (B1, B5), *Macrosporium commune* (B3-B5), ***Mucor* spp.*** (A1-A5, A7-A9, B3, B5), ***Penicillium citrinum****+ (A3, A5, B5), ***Penicillium* spp.*+** (A2, A3, A6, B)**,** *Prophytroma tubularis* (A2), ***Rhizopus nigricans*** * + (A2, A3, A5, A7, A9, B3, B4), *Scopulariopsis* spp. (B1, B5), *Trichoderma sympodianum* (B1), ***Trichoderma viride**** (A2, A5, A7, A9, B3).

Sites of isolation are given in parentheses. Quoting only the letter attributed to a particular plant ("A" or "B" without numbers) means that the species was isolated from all sampling sites within the plant. 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. *Proteus vulgaris* and *Aspergillus fumigatus* may cause infectious disease in man.

Table 4. Concentrations of dust and bacterial endotoxin in the air of herb processing plants.

Plant, sampling site	Concentration of dust (mg/m^3)	Concentration of endotoxin ($\mu g/m^3$)
Herb processing plant "A"		
A1. Cleaning of marjoram	946.0	756.8
A2. Cutting of yarrow	418.9	2,681.0
A3. Cleaning of caraway (1)	8.8	6.4
A4. Cleaning of caraway (2)	15.0	200.0
A5. Cleaning of mint	30.2	16.0
A6. Grinding of sage	8.0	4.0
A7. Cleaning of nettle	25.5	16.0
A8. Packing of nettle	3.2	0.8
A9. Sorting of celandine root	42.7	20.0
Herb processing plant "B"		
B1. Packing of birch leaves	21.1	3.6
B2. Cutting of peppermint	6.3	0.2
B3. Sieving of calamus rhizome	15.1	17.8
B4. Cutting of St. John's wort	4.1	0.2
B5 Cutting of nettle	58.9	71.5
Median	18.1	16.0

sampling site (cleaning of nettle) forming 61.7% of the total airborne microflora and were the most numerous microorganisms at the other three sites (grinding of sage, packing of birch leaves, cutting of peppermint), constituting 25.3–25.8% of the total. Corynebacteria were the most numerous microorganisms at cutting of the St. John's wort, forming 34.6% of the total airborne microflora.

The percentage of Gram-negative bacteria in the total airborne microflora ranged within 1.8–17.9%, the percentage of corynebacteria within 1.3–34.6%, the percentage of thermophilic actinomycetes within 0.3–11.9%, and the percentage of fungi within 5.9–30.5%. *Alcaligenes faecalis* was dominant among Gram-negative bacteria forming 78% of their total count, *Corynebacterium* spp. among corynebacteria (68% of the total), and *Thermoactinomyces vulgaris* among thermophilic actinomycetes (90% of the total). Among fungi, there were no distinctly dominant species; the most numerous were *Alternaria alternata* (28% of the total count), *Mucor* spp. (25%) and *Aspergillus fumigatus* (15%). Lactobacilli formed only a small fraction of the total airborne microflora in herb processing plants, ranging within 0–1.6%.

The values of the respirable fraction of airborne microflora in herb processing plants varied within a fairly wide range and were between 14.7–67.7% (on average 44.3 ± 15.2%) (Tab. 1–2).

In the air samples taken in the examined facilities, 37 species or genera of bacteria and 23 species or genera of fungi were identified, of these, 11 and 10 species or genera respectively were reported as having allergenic and/or immunotoxic properties [15, 19, 23, 26, 27, 37] (Tab. 3). These figures are certainly an underestimation, as a part of bacterial and fungal strains could be identified only to the generic level.

The concentrations of dust and endotoxin in the air of herb processing plants were large (Tab. 4). Values varied within wide limits, showing non-parametric distribution (Shapiro-Wilk test: $p < 0.00001$). The concentrations of airborne dust ranged within 3.2–946.0 mg/m^3 (median 18.1 mg/m^3), exceeding at 13 out of 14 sampling sites the Polish OEL value of 4 mg/m^3, at 9 out of 14 sites a level of 10.0 mg/m^3, and at two sites a very high level of 100.0 mg/m^3. The concentrations of airborne endotoxin ranged within 0.2–2681.0 $\mu g/m^3$ (median 16.0 $\mu g/m^3$), exceeding

Table 5. Concentrations of microorganisms and endotoxin in the samples of settled dusts collected in herb processing plants.

Sampling site	Non-fastidious mesophilic bacteria (Blood agar) $cfu \times 10^6/g$ (percent)				Thermophilic actinomycetes (Tryptic soya agar) $cfu \times 10^6/g$ (percent)	Fungi (Malt agar) $cfu \times 10^6/g$ (percent)	Total microorganisms $cfu \times 10^6/g$ (percent)	Endotoxin µg/g
	Gram-negative bacteria			Gram-positive bacteria				
	Alcaligenes faecalis	*Pantoea agglomerans*	Other species					
Dust from marjoram herb, collected in plant "A" under cleaning machine "Schilbach"	9,510.00 (99.866%)	1.70 (0.018%)	1.45 (0.015%)	9.40 (0.099%)	0.1 (0.001%)	0.066 (0.001%)	9,522.716 (100%)	400.0
Dust from yarrow herb, collected in plant "A" under cutting machine	174.15 (96.091%)	4.70 (2.593%)	0.4 (0.221%)	1.95 (1.076%)	0.01 (0.006%)	0.024 (0.013%)	181.234 (100%)	400.0
Dust from sage herb, collected in plant "A" under grinding machine "Alpine"	400.0 (99.365%)	0 0	1.5 (0.373)	1.0 (0.248)	0.01 (0.002%)	0.048 (0.012%)	402.558 (100%)	200.0
Dust from birch leaves, collected in plant "B" at the site of packing the leaves	0 0	0 0	0.0005 (4.76%)	0.0060 (57.14%)	0 0	0.0040 (38.10%)	0.0105 (100%)	2,000.0
Dust from calamus rhizome, collected in plant "B" under sieving machine "Schilbach"	0.0360 (6.92%)	0.3045 (58.50%)	0.0530 (10.18%)	0.1130 (21.71%)	0.001 (0.19%)	0.0130 (2.50%)	0.5205 (100%)	4,000.0

at 11 out of 14 sampling sites a level of 1.0 µg/m^3, at eight sites a high level of 10.0 µg/m^3, at three sites a very high level of 100.0 µg/m^3, and at one site an extraordinary high level of 1,000.0 µg/m^3.

The concentrations of total microorganisms in the samples of settled dust collected in plant "A" were large, ranging within 1.8×10^8 cfu/g - 9.5×10^9 cfu/g. The distinctly predominant microorganism was the Gram-negative species *Alcaligenes faecalis*, constituting 96.1–99.9% of the total microflora (Tab. 5). The microbial concentrations in the samples collected in plant "B" were much smaller, ranging within 1.0×10^4 cfu/g - 5.2×10^5 cfu/g. In one sample a Gram-negative species, *Pantoea agglomerans*, predominated, while in the other - Gram-positive bacteria (bacilli, corynebacteria and cocci) predominated (Tab. 5).

The concentration of bacterial endotoxin in the samples of settled dust was not correlated with the number of Gram-negative bacteria and other microorganisms. In the samples collected in plant "A" the endotoxin concentration ranged within 200.0–400.0 µg/g, while in the samples collected in plant "B" it ranged within 2,000.0–4,000.0 µg/g (Tab. 5).

DISCUSSION

The present study has demonstrated that the workers of herb processing plants are exposed to large concentrations of airborne microorganisms, dust and endotoxin posing an occupational hazard. Based on the obtained results, herb processing plants should be placed among the working environments with the highest bioaerosol pollution, such as: grain stores, seed stores, animal feed factories, pig farms, poultry farms, and waste composting facilities [5, 9, 15-18, 26-28, 32, 52, 54].

The concentrations of total airborne microorganisms in the examined plants were of the order 10^4–10^5 cfu/m^3. 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 (10^4) cfu/m^3 proposed by Malmros *et al.* [36] was exceeded at all sampling sites, whereas the OEL value of 100×10^3 (10^5) cfu/m^3 proposed by Dutkiewicz and Jabłoński [15, 18] was exceeded at nine sampling sites out of 14 examined. The OEL value of 20×10^3 cfu/m^3 proposed by Dutkiewicz and Jabłoński [15, 18] for thermophilic actinomycetes was exceeded at three sampling sites out of 14 examined, while the OEL value of 50×10^3 cfu/m^3 proposed by these authors for fungi was exceeded at two sampling sites. The concentration of Gram-negative bacteria (recovered on blood agar plates among other mesophilic bacteria) exceeded at all sampling sites the OEL value of 1×10^3 cfu/m^3 proposed by Clark [3] and Malmros *et al.* [36] while the OEL value of 20×10^3 cfu/m^3 proposed for these bacteria by Dutkiewicz and Jabłoński [15, 18] was exceeded at five sampling sites out of 14 examined. The risk of exposure to airborne microorganisms was greater at the initial stages of the production cycle (cleaning and cutting of herbs) and at the processing of some herbs (marjoram, nettle, yarrow, mint) which generated dust heavily contaminated with microorganisms.

The species composition of the airborne microflora of herb processing plants, which characterized itself by the prevalence of endospore-forming bacilli (*Bacillus* spp.), and, at some sites, also mesophilic actinomycete *Streptomyces albus*, was different from that found in the most of the hitherto investigated agricultural facilities where either corynebacteria and Gram-positive cocci or Gram-negative bacteria were dominant organisms [9, 15-18, 20]. Some similarities to the composition of the airborne microflora of herb processing plants could be found in a tobacco processing plant where *Bacillus* strains were abundant [10] and in grain processing plants where *Streptomyces albus* was common on some working stands [9, 10, 13, 18].

The risk of exposure to airborne microflora in the herb processing facilities is increased by the presence of numerous microbial species known as producers of allergens and/or toxins. *Alcaligenes faecalis*, the dominant species among Gram-negative bacteria, produces a biologically active endotoxin [51] and may evoke allergic alveolitis [37]. Even more potent endotoxic and allergenic properties are shown by *Pantoea agglomerans*, the other Gram-negative species isolated from the air of examined facilities [15, 16, 37, 51]. Among other bacteria occurring in the air of herb processing plants, numerous species of bacilli (*Bacillus subtilis*), corynebacteria (*Arthrobacter globiformis, Brevibacterium linens*) and actinomycetes (*Streptomyces albus, Saccharomonospora viridis, Saccharopolyspora rectivirgula, Thermoactinomyces vulgaris*) were reported as causative agents of allergic alveolitis [15, 23, 26, 27, 37]. Among fungi occurring in this environment, at least 10 species or genera were reported as potential agents of allergic and immunotoxic disease of the respiratory tract; considering both frequency of occurrence and pathogenic properties, the greatest respiratory risk is posed by *Aspergillus fumigatus*, *Alternaria alternata*, *Mucor* spp., and *Penicillium* spp. [19, 26, 27].

The concentrations of dust and bacterial endotoxin in the air of the herb processing plants were large, with extremely high levels at some sampling sites. The concentrations of dust were of the order 10^0-10^2 mg/m^3, exceeding at 13 out of 14 sampling sites the Polish OEL value of 4 mg/m^3 [45] by 1.03-236.5 times.

The concentrations of airborne endotoxin in the examined facilities were of the order 10^{-1}-10^3 µg/m^3, exceeding at all sampling sites the safe levels proposed by various authors [3, 7, 28, 36, 46]. The stated values exceeded 2-26,810 times the OEL values of 0.1 µg/m^3 proposed by Clark [3], Rylander [46] and Malmros *et al.*

[36]. These are even much higher than the OEL values of 25 ng/m^3 proposed by Laitinen [28] and the OEL value of 5 ng/m^3 proposed by DECOS [7]. The concentrations of airborne endotoxin exceeded at 12 out of 14 examined sampling sites the value of 0.2 µg/m^3 supposed to cause a decrease of lung function during workshifts [49], and at 11 sites the values of 1–2 µg/m^3 which are supposed to evoke ODTS symptoms [49]. This was in accordance with the fact that many of the exposed workers reported symptoms characteristic for ODTS [25].

The levels of airborne endotoxin found in the present work were distinctly higher compared to those found by numerous authors in various working environments [7, 32], and also to those found by our group in other agricultural environments [17, 20]. Accordingly, considering the possibility of an overestimation of real endotoxin levels, the results were critically analysed with regard to the conditions of the study and literature data.

Most of all, the high endotoxin levels were the direct function of extremely large concentrations of dust in the air of herb processing plants (up to 946.0 mg/m^3) which at most sampling sites contained endotoxin in the percent range 0.003–0.12%, conforming to the results of endotoxin determination in various vegetable dusts obtained by our group [15, 20] and other authors [2, 6, 38, 40]. At only two sampling sites, at cutting of yarrow and cleaning of caraway on a grain screening machine, the percentages of endotoxin in airborne dust were higher (0.64% and 1.33% respectively) which may suggest that the values of endotoxin concentration calculated for these sites, including the extraordinary high value at cutting yarrow (2,681 µg/m^3), are probably overestimates. One possible explanation for these findings is the presence in airborne dust of some herb derivatives which might unspecifically react with the *Limulus* reagent and thus cause false positive results.

Though most of the airborne endotoxin concentrations reported by earlier authors from various work environments are of the order 10^{-3}–10^0 µg/m^3 (10^0-10^3 ng/m^3), values of the order 10^1–10^2 µg/m^3 were also reported, mostly from farms and grain storing and processing facilities [28, 34, 39, 40, 44]. Rask-Andersen *et al.* [44] have not found any significant relationship between high endotoxin concentrations in farm air and the occurrence of symptoms in exposed subjects and therefore consider the possibility of false-positive *Limulus* reactions that may be caused by glucans, peptidoglycan and other microbial substances present in dust. However, one must keep in mind that because other microbial substances are less potent in *Limulus* test compared to endotoxin [7] and usually occur in organic dusts in smaller quantities [47], possible cross-reactions do not explain all the high concentrations of airborne endotoxin stated in the agricultural work environments. On the other hand, in the opinion of Rylander [50], the values of endotoxin concentrations detected by *Limulus* test are underestimated by 30–50 times due to masking of lipopolysaccharide molecules by other constituents of bacterial cell. Similarly, Larsson [30] expressed the view that the endotoxin concentration values determined by *Limulus* test may be underestimated by orders of magnitude. With respect to the above opinions, in the present work the procedure by which samples were pre-heated to 100ºC before performing the *Limulus* test may help to dissolve endotoxin, to inactivate interfering substances and to disclose active lipopolysaccharide molecules and thus may increase the reliability of the results.

To summarize, the concentrations of endotoxin in the air of herb processing plants determined in the present study are most probably not overestimates, with the exception of two sampling sites.

The concentration of microorganisms in the samples of settled dust collected at the sites of cleaning marjoram, cutting yarrow and grinding sage in plant "A" were much greater compared to those collected at the sites of packing birch leaves and sieving of calamus rhizome in plant "B". These findings are in accordance with the results of microbiological air sampling carried out at the same sites. *Alcaligenes faecalis* in the samples of settled dust from plant "A" not only was the dominant Gram-negative bacterium, similar to the air samples, but was also a distinctly dominant microorganism, forming over 95% of the total microflora. The much smaller contribution of this species to the total microflora of the air in herb processing plants can be explained by the vulnerability of these bacteria to desiccation when they become airborne. In the settled dust, Gram-negative bacteria may survive longer, but after an extended storage they die, whereas the thermostable endotoxin persists. This may explain the high levels of endotoxin in the samples of settled dust from plant "B", despite low concentrations of live Gram-negative bacteria found in these samples.

CONCLUSION

The workers of herb processing plants could be exposed 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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