ORIGINAL ARTICLES AAEM

Ann Agric Environ Med 2005, 12, 281–288

# EXPOSURE TO AIRBORNE MICROORGANISMS, DUST AND ENDOTOXIN DURING PROCESSING OF PEPPERMINT AND CHAMOMILE HERBS ON FARMS

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Skórska C, Sitkowska J, Krysińska-Traczyk E, Cholewa G, Dutkiewicz J: Exposure to airborne microorganisms, dust and endotoxin during processing of peppermint and chamomile herbs on farms. *Ann Agric Environ Med* 2005, **12**, 281–288.

**Abstract:** The aim of this study was to determine the levels of microorganisms, dust and endotoxin in the air during processing of peppermint (*Mentha piperita*) and chamomile (*Matricaria recutita*) by herb farmers, and to examine the species composition of airborne microflora. Air samples were collected on glass fibre filters by use of personal samplers on 13 farms owned by herb cultivating farmers, located in Lublin province (eastern Poland). The concentrations of total viable microorganisms (bacteria + fungi) in the farm air during processing of peppermint herb were large, within a range from 895.1-6,015.8 × $10^3$ cfu/$m^3$ (median 1,055.3 × $10^3$ cfu/$m^3$). During processing of chamomile herb they were much lower and varied within a range from 0.88-295.6 × $10^3$ cfu/$m^3$ (median 27.3 × $10^3$ cfu/$m^3$). Gram-negative bacteria distinctly prevailed during processing of peppermint leaves, forming 46.4-88.5% of the total airborne microflora. During processing of chamomile herb, Gram-negative bacteria were dominant at 3 out of 6 sampling sites forming 54.7-75.3% of total microflora, whereas at the remaining 3 sites the most common were fungi forming 46.2-99.9% of the total count. The species *Pantoea agglomerans* (synonyms: *Erwinia herbicola, Enterobacter agglomerans*), having strong allergenic and endotoxic properties, distinctly prevailed among Gram-negative isolates. Among fungi, the most common species was *Alternaria alternata*. The concentrations of airborne dust and endotoxin determined on the examined herb farms were large. The concentrations of airborne dust during peppermint and chamomile processing ranged from 86.7-958.9 mg/$m^3$, and from 1.1-499.2 mg/$m^3$, respectively (medians 552.3 mg/$m^3$ and 12.3 mg/$m^3$). The concentrations of airborne endotoxin determined during peppermint and chamomile processing were within a wide range 1.53-208.33 µg/$m^3$ and 0.005-2604.19 µg/$m^3$ respectively (medians 57.3 µg/$m^3$ and 0.96 µg/$m^3$). In conclusion, farmers cultivating peppermint are exposed during processing of this herb to large concentrations of airborne microorganisms, dust and endotoxin posing a risk of work-related respiratory disease. The exposure to bioaerosols during processing of chamomile is lower; nevertheless, peak values create a respiratory risk for exposed farmers.

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**Key words: herb farmers, peppermint, chamomile, processing, occupational exposure, organic dust, bioaerosols, bacteria, fungi, endotoxin.**

## INTRODUCTION

Occupational handling of plant materials (grain, hay, straw, flax, cotton and others) may be associated with exposure to large quantities of organic dust and bioaerosols causing allergic and/or immunotoxic reactions and respiratory disease in the workers [8, 12, 13, 22, 34, 35, 42, 43, 49]. So far, relatively little is known about a risk associated with handling of various herbs in spite of growing interest in cultivating and processing herbs for

Received: 26 August 2005
Accepted: 15 November 2005

medicinal, alimentary and cosmetic purposes [3, 25]. Dutkiewicz *et al.* [18] found that the workers of big herb processing plants were exposed to large concentrations of microorganisms ($10^4$-$10^5$ cfu/$m^3$) and endotoxin ($10^{-1}$-$10^3$ µg/$m^3$). Similarly, Krysińska-Traczyk *et al.* [31] recorded large concentrations of microorganisms ($10^4$-$10^5$ cfu/$m^3$) and endotoxin ($10^1$-$10^3$ µg/$m^3$) during cleaning of thyme by herb farmers. Recently, Skórska *et al.* [52] found that farmers processing valerian roots are exposed to concentrations of microorganisms and endotoxin varying within wide limits ($10^2$-$10^6$ cfu/$m^3$ and $10^{-3}$-$10^4$ µg/$m^3$, respectively).

People occupationally exposed to dust from herbs showed a high proportion of allergic reactions to the extracts of herbs and microorganisms associated with herb dust, and often reported work-related respiratory symptoms [19, 23, 24, 25, 26]. Accordingly, our group has extended the research on exposure to herb dust and its effects onto further species of medicinal herbs, including peppermint and chamomile.

During the last decade, the herbal market has grown fast in Europe and America [3], and only in Poland about 100,000 farmers are growing herbs for industrial purposes [26]. Both peppermint and chamomile belong to commonly grown herbs.

Peppermint (*Mentha piperita* L.) is an aromatic, easy growing perennial plant, belonging to the Lamiaceae family. Peppermint leaves are used as a herb for medicinal, culinary and cosmetic purposes. The extracts of leaves yield approximately 0.1–1.0% volatile oil which is an active component composed primarily of menthol and menthone. Peppermint is claimed to be antispasmodic, diaphoretic, digestive, carminative, antiseptic and slightly anesthetic [4].

Chamomile (*Matricaria recutita* L.) is a sweet-scented, annual plant belonging to the Asteraceae family, having daisy-like flowers with white petals and yellow central disks (heads). Dried flower heads are widely used as a herb for production of herbal teas, in the pharmaceutical and cosmetic industries, and in traditional medicine. Chamomile herb is claimed to be anti-inflammatory, antiseptic, antispasmodic, relaxant, carminative, antiallergenic, and a catalyst in wound healing [4].

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

## MATERIALS AND METHODS

**Examined farms.** Air sampling was performed on 13 farms owned by herb cultivating farmers, located in Lublin province (eastern Poland) on the territory of 6 villages, at the distance of 100-140 km from the city of Lublin. Samples were collected during processing of harvested and dried chamomile herb in May and June 2003, and during processing of harvested and dried peppermint herb in August 2004.

Peppermint (*Mentha piperita* L.) plants were machine harvested, dried for 10-14 days with the use of a hot air blower, threshed indoors with a machine or outdoor with a combine, and repeatedly cleaned indoor with a sieving machine for separating dried leaves which were finally sacked for disposal to herb processing facilities. Air samples during peppermint processing by farmers were taken at sites 1-4 on the following farms: 1) Farm 1: Machine threshing of dried peppermint plants; 2) Farm 2: Machine threshing of dried peppermint plants; 3) Farm 3: Combine threshing of dried peppermint plants; 4) Farm 4: Cleaning of dried peppermint herb with sieving machine.

Chamomile (*Matricaria recutita* L.) plants were machine harvested, dried for 10-14 days with the use of a hot air blower, threshed indoors with a machine, and repeatedly cleaned indoors with a sieving machine for separating dried flower heads which were finally sacked for disposal to herb processing facilities. Dried flower petals were also sacked as a by-product and delivered to herb processing facilities. Air samples during chamomile processing by farmers were taken at sites 5-13 on the following farms: 5) Farm 5: Machine threshing of dried chamomile plants; 6) Farm 6: Cleaning of dried chamomile herb with a sieving machine; 7) Farm 7: Cleaning of dried chamomile herb with a sieving machine; 8) Farm 8: Cleaning of dried chamomile herb with a sieving machine; 9) Farm 9: Shoveling of chamomile by-products (petals) into sacks; 10) Farm 10: Shoveling of chamomile flower heads into sacks; 11) Farm 11: Machine threshing of dried chamomile plants; 12) Farm 12: Machine threshing of dried chamomile plants; 13) Farm 13: Machine threshing of dried chamomile plants.

**Microbiological examination of the air.** Air samples were taken by use of an AP-2A personal sampler (TWOMET, Zgierz, Poland), at the flow rate of 2 l/min. Glass fibre filters, with 1 µm pore size and 37 mm diameter, were used. On each farm, 3 samples were collected in parallel using 3 samplers during 30 minutes: one for determination of the concentration and species composition of microorganisms, and the other two for determination of the concentration of dust and endotoxin. The concentration of dust in the air was determined gravimetrically from the difference between weight of the filter measured before and after sampling. The concentration of airborne dust estimated for each farm was a mean of 3 single determinations.

In addition, on each farm, 3 air samples were collected in parallel on the polyvinyl chloride filters by use of 3 stationary AS-50 samplers (TWOMET, Zgierz, Poland), at the flow rate of 50 l/min, for determination of the dust and endotoxin concentrations. The concentration of airborne dust, estimated gravimetrically for each farm, was a mean of 3 single determinations.

The concentration and species composition of microorganisms in collected air samples were determined by dilution plating. The filters were extracted in 3 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 4 agar media: blood agar for estimation of total mesophilic Gram-negative and Gram-positive bacteria, eosin methylene blue (EMB) agar (Merck, Darmstadt, Germany) for estimation of Gram-negative bacteria, half-strength tryptic soya agar (Sigma, St. Louis, MO, USA) for estimation of thermophilic actinomycetes, and malt agar (Difco, Detroit, MI, USA) for estimation of fungi. The blood agar plates and EMB 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 [11]. The prolonged incubation at lower temperatures aimed to isolate as wide a spectrum of bacteria and fungi as possible. 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 metre of air (cfu/m$^3$). The total concentration of viable microorganisms in the air was obtained by the addition of the concentrations of total mesophilic bacteria (grown on blood agar medium), thermophilic actinomycetes and fungi. The percent composition of the total microflora of the air was then determined. For technical reasons, the concentration of microorganisms in the air was not determined on the farms 11-13.

Bacterial isolates were identified with microscopic and biochemical methods, as recommended by Bergey's Manual [30, 53, 56] and Cowan & Steel [6]. 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], Raper & Fennell [44] and Samson *et al.* [51].

The concentration of bacterial endotoxin in the airborne dust was determined by the *Limulus* amebocyte lysate gel tube test (LAL) [37]. 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 Cod, Inc., Falmouth, MA, USA). The test was incubated for 1 hour in a water bath at 37ºC, using pyrogen-free water as a negative control and the standard lipopolysaccharide (endotoxin) of *Escherichia coli* 0113:H10 (Difco) as positive control. The formation of a stable clot was regarded as a positive result. The estimated concentration of endotoxin in dust (ng/mg) was multiplied per estimated concentration of dust in the air (mg/m$^3$) and the results were 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 nanograms was multiplied by 10.

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

## RESULTS

The concentrations of total viable microorganisms in the farm air during processing peppermint herb were large, within a range from 895.1-6,015.8 × 10$^3$ cfu/m$^3$ (median 1,055.3 × 10$^3$ cfu/m$^3$). During processing chamomile herb they were much lower and varied within a range from 0.88-295.6 × 10$^3$ cfu/m$^3$ (median 27.3 × 10$^3$ cfu/m$^3$) (Tab. 1). Mesophilic bacteria occurred in abundant quantities and proved to be the most common organisms at most sites. Fungi were less numerous and thermophilic actinomycetes formed only small portion of total microflora.

Gram-negative bacteria distinctly prevailed during processing of peppermint leaves, forming 46.4-88.5% of the total airborne microflora. During processing of chamomile herb, Gram-negative bacteria were dominant at 3 of the 6 sampling sites forming 54.7-75.3% of total microflora, whereas at remaining 3 sites the most common were fungi forming 46.2-99.9% of the total count (Fig. 1). Endospore-forming bacilli formed 0.5-11.2% of total count during processing of peppermint leaves and 0-34.5% during processing of chamomile herb. Corynebacteria, Gram-positive cocci, mesophilic actinomycetes and thermophilic actinomycetes were less numerous (Fig. 1).

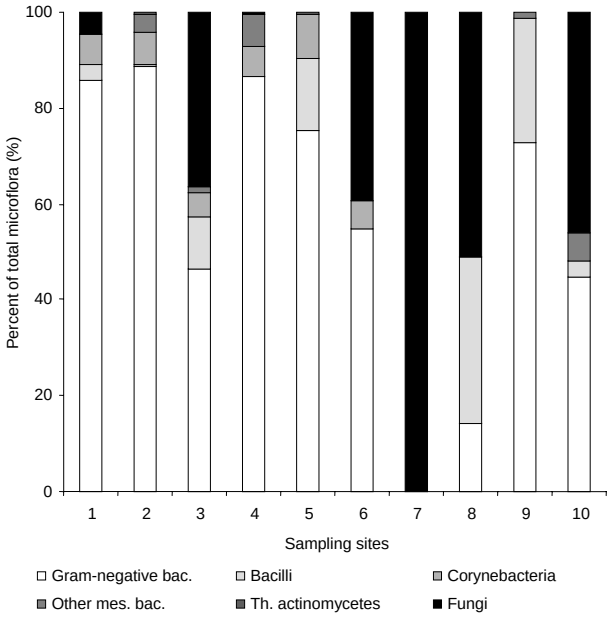


**Figure 1.** Composition of airborne microflora on farms during particular activities at processing of peppermint and chamomile herbs: total count, including mesophilic bacteria, thermophilic actinomycetes and fungi. The "other mesophilic bacteria" comprise mesophilic actinomycetes (*Streptomyces* spp.) and cocci (*Staphylococcus* spp., *Enterococcus* spp.).

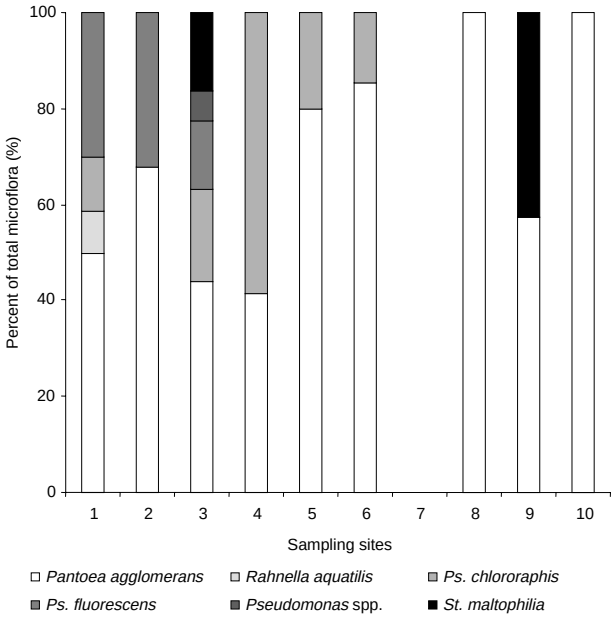


**Figure 2.** Composition of airborne Gram-negative bacteria on farms during particular activities at processing of peppermint and chamomile herbs.

The concentrations of airborne Gram-negative bacteria recovered on EMB agar were large, up to the level $10^6$ cfu/m$^3$ (Tab. 1), although smaller compared to those recovered on blood agar. The species *Pantoea agglomerans* (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*) distinctly prevailed among Gram-negative bacteria isolated on EMB agar, forming 41.6-100% of the total count (Fig. 2). The remaining part of Gram-negative flora consisted mostly of the rods belonging to the family Pseudomonadaceae (*Pseudomonas chlororaphis*, *Pseudomonas fluorescens*, *Stenotrophomonas maltophilia*). *Ps. fluorescens* was isolated only during processing of peppermint and *St. maltophilia* during processing of chamomile (Fig. 2).

The concentrations of airborne thermophilic actinomycetes were very low, ranging from 0-5.0 × $10^2$ cfu/m$^3$. *Thermoactinomyces* strains (*Th. thalpophilus*, *Th. vulgaris*) distinctly prevailed at all but one sampling sites (Fig. 3).

The concentrations of airborne fungi found during processing of peppermint and chamomile herbs showed a marked variation, being of the order $10^1$-$10^5$ cfu/m$^3$ (Tab. 1). The species *Alternaria alternata* distinctly predominated at 5 sampling sites, forming 75-100% of the total fungal count. At the remaining sites, yeast prevailed (2 sites) and *Cladosporium* spp., *Oidiodendron* spp. and *Penicillium* spp. (1 site each) (Fig. 4).

In the air samples taken on the examined farms, 24 species or genera of bacteria and 11 species or genera of fungi were identified, of these, 8 and 7 species or genera respectively were reported as having allergenic and/or immunotoxic properties [2, 13, 21, 29, 34, 35, 49] (Tab. 2). These figures are certainly an underestimation, as a part of bacterial and fungal strains could be identified only to generic level.

The concentrations of airborne dust determined on the examined farms during peppermint and chamomile processing with the use of personal sampling were large and ranged from 86.7-958.9 mg/m$^3$, and from 1.1-499.2 mg/m$^3$, respectively (medians 552.3 mg/m$^3$ and 12.3

**Table 1.** Concentrations of microorganisms (cfu/m$^3$ × $10^3$) in the farm air during processing of peppermint and chamomile plants.

| Sampling Site No. | Activity | Total mesophilic bacteria (Blood agar) | Gram-negative bacteria (EMB agar) | Thermophilic actinomycetes (Tryptic soya agar) | Fungi (Malt agar) | Total microorganisms* |
|---|---|---|---|---|---|---|
| Processing of peppermint plants | | | | | | |
| 1 | Farm 1: Machine threshing of dried peppermint plants | 5,750.0 | 1,010.0 | 0.2 | 265.6 | 6,015.8 |
| 2 | Farm 2: Machine threshing of dried peppermint plants | 1,065.0 | 290.0 | 0.36 | 2.1 | 1,067.46 |
| 3 | Farm 3: Combine threshing of dried peppermint plants | 570.0 | 480.0 | 0.1 | 325.0 | 895.1 |
| 4 | Farm 4: Cleaning of dried peppermint herb with a sieving machine | 1,040. 0 | 385.0 | 0.15 | 2.9 | 1,043.05 |
| Median | | 1,052.5 | 432.5 | 0.18 | 134.2 | 1,055.3 |
| Processing of chamomile plants | | | | | | |
| 5 | Farm 5: Machine threshing of dried chamomile plants | 295.0 | 62.5 | 0.5 | 0.1 | 295.6 |
| 6 | Farm 6: Cleaning of dried chamomile herb with a sieving machine | 123.5 | 10.3 | 0.025 | 80.0 | 203.525 |
| 7 | Farm 7: Cleaning of dried chamomile herb with a sieving machine | 0.02 | 0 | 0.025 | 35.0 | 35.045 |
| 8 | Farm 8: Cleaning of dried chamomile herb with a sieving machine | 0.43 | 0.6 | 0 | 0.45 | 0.88 |
| 9 | Farm 9: Shoveling of chamomile by-products (petals) into sacks | 19.5 | 10.0 | 0 | 0.025 | 19.525 |
| 10 | Farm 10: Shoveling of chamomile flower heads into sacks | 8.5 | 2.6 | 0 | 7.3 | 15.8 |
| Median | | 14.0 | 6.3 | 0.013 | 3.9 | 27.3 |

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

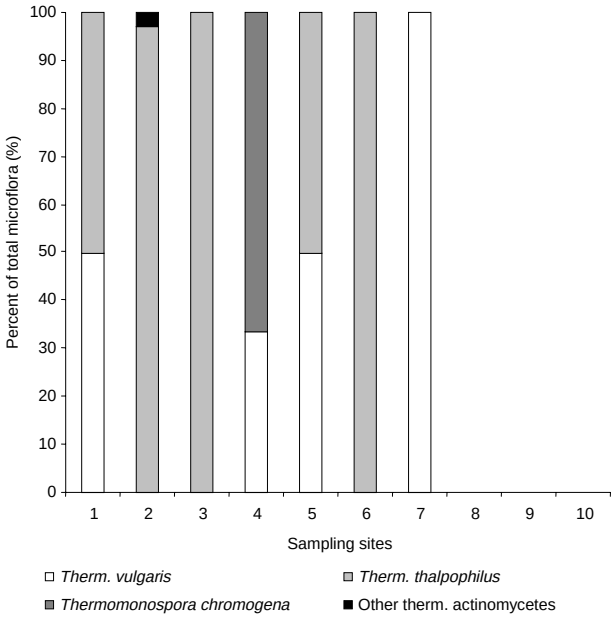


**Figure 3.** Composition of airborne thermophilic actinomycetes on farms during particular activities at processing of peppermint and chamomile herbs.

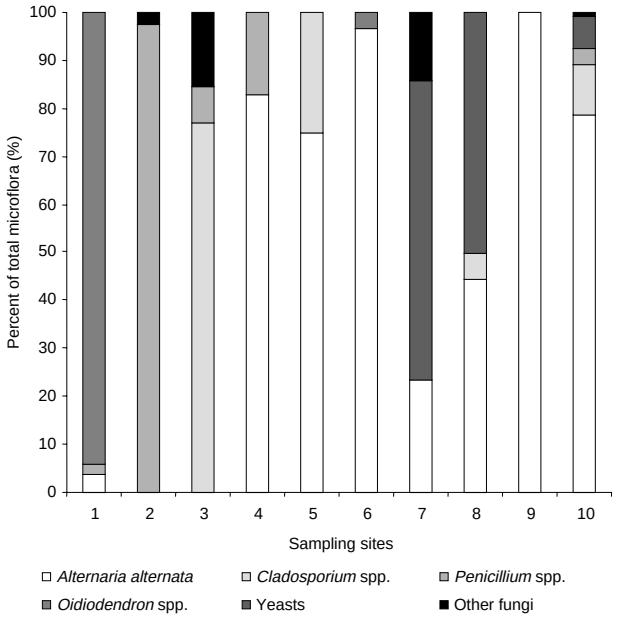


**Figure 4.** Composition of airborne fungi on farms during particular activities at processing of peppermint and chamomile herbs.

mg/m$^3$). The dust concentrations determined parallelly with the use of stationary sampling were insignificantly lower ($p>0.05$) and ranged from 40.8-246.7 mg/m$^3$, and from 0.8-104.9 mg/m$^3$, respectively (medians 104.3 mg/m$^3$ and 8.2 mg/m$^3$) (Tab. 3).

The concentrations of airborne endotoxin determined during peppermint and chamomile processing with the use of personal sampling were within a range 1.53-208.33 µg/m$^3$ and 0.005-2604.19 µg/m$^3$ respectively (medians 57.3 µg/m$^3$ and 0.96 µg/m$^3$) and were significantly smaller ($p<0.05$) compared to stationary sampling. The concentrations of airborne endotoxin determined with the use of stationary sampling during peppermint and chamomile processing were within a range 6.25-624.99 µg/m$^3$ and 0.06-3125.0 µg/m$^3$ respectively (medians 312.5 µg/m$^3$ and 3.13 µg/m$^3$) (Tab. 3).

The values of airborne dust and endotoxin showed a significant correlation, both when determined with the use of personal sampling ($p<0.001$) and with the use of stationary sampling ($p<0.01$).

## DISCUSSION

The concentrations of airborne microorganisms recorded during processing of peppermint were very large (median $1.06 \times 10^6$ cfu/m$^3$), similar to those reported for working environments with the highest bioaerosol pollution, such as: grain stores, seed stores, animal feed factories, malt houses, herb processing plants, pig farms, poultry farms, flax farms and waste composting facilities [8, 11, 14, 15, 16, 18, 22, 32, 34, 38, 42, 54]. The concentrations of airborne microorganisms found during processing of chamomile were distinctly lower and showed a great variability, similar to that observed at processing valerian roots [52]. The median concentration of airborne microorganisms recorded during processing

**Table 2.** List of microbial species and genera identified in the samples of farm air during processing of peppermint and chamomile plants.

**Gram-negative bacteria:** ***Pantoea agglomerans****+ (synonyms: ***Erwinia herbicola***, ***Enterobacter agglomerans***) (1-6, 8-10), *Pseudomonas chlororaphis* (1, 3-6), ***Pseudomonas fluorescens***+* (1-3), *Pseudomonas* spp. (3), ***Rahnella aquatilis***+ (1), *Stenotrophomonas maltophilia* (3, 9).

**Bacilli:** *Bacillus cereus* (5, 8), *Bacillus megaterium* (9), ***Bacillus subtilis**** (3, 8, 10), *Bacillus* spp. (1-3, 5, 8-10).

**Corynebacteria:** *Curtobacterium pusillum* (5), *Gordona* sp. (1-4), *Sanguibacter keddieii* (5, 6).

**Gram-positive cocci:** *Enterococcus faecium* (6), *Staphylococcus lentus* (2, 4), *Staphylococcus pasteuri* (5), *Staphylococcus sciuri* (10), *Staphylococcus* spp. (2-4, 10).

**Mesophilic actinomycetes:** ***Streptomyces albus**** (2, 9), *Streptomyces* spp. (2, 3, 10).

**Thermophilic actinomycetes:** ***Thermoactinomyces thalpophilus**** (1-3, 5, 6), ***Thermoactinomyces vulgaris**** (1, 4, 5, 7), ***Thermomonospora chromogena**** (4), *Streptomyces* spp. (2).

**Fungi:** ***Alternaria alternata****+ (1, 4-10), ***Aspergillus fumigatus****+ (10), ***Candida*** spp.* (7, 8), ***Cladosporium cladosporioides**** (5, 8, 10), ***Cladosporium herbarum**** (3), ***Fusarium* spp.**+ (1, 2, 7), *Geotrichum cardianum* (3), *Oidiodendron rhodogenum* (6), *Oidiodendron* spp. (1), ***Penicillium* spp.** (1-4, 10), *Rhodotorula rubra* (7, 10)

Sites of isolation are given in parentheses. The names of the species reported as having allergenic and/or immunotoxic properties (see text) are in bold and marked as follows: * allergenic species; + immunotoxic species.

**Table 3.** Concentrations of dust and bacterial endotoxin in the farm air during processing of peppermint and chamomile plants.

| Sampling site No. | Activity | Personal sampling | | Stationary sampling | |
|---|---|---|---|---|---|
| | | Dust (mg/m$^3$) Mean ± SD | Endotoxin (µg/m$^3$) | Dust (mg/m$^3$) Mean ± SD | Endotoxin (µg/m$^3$) |
| Processing of peppermint plants | | | | | |
| 1 | Farm 1: Machine threshing of dried peppermint plants | 958.9 ± 125.1 | 10.42 | 246.7 ± 124.2 | 624.99 |
| 2 | Farm 2: Machine threshing of dried peppermint plants | 86.7 ± 34.0 | 208.33 | 163.6 ± 45.7 | 618.2 |
| 3 | Farm 3: Combine threshing of dried peppermint plants | 418.9 ± 356.2 | 1.53 | 44.4 ± 18.9 | 6.77 |
| 4 | Farm 4: Cleaning of dried peppermint herb with a sieving machine | 685.6 ± 288.6 | 104.17 | 40.8 ± 23.7 | 6.25 |
| Median | | 552.3 | 57.3 | 104.3 | 312.5 |
| Processing of chamomile plants | | | | | |
| 5 | Farm 5: Machine threshing of dried chamomile plants | 14.5 ± 2.2 | 1.06 | 37.6 ± 2.7 | 66.0 |
| 6 | Farm 6: Cleaning of dried chamomile herb with a sieving machine | 4.8 ± 2.0 | 0.52 | 3.9 ± 2.7 | 0.63 |
| 7 | Farm 7: Cleaning of dried chamomile herb with a sieving machine | 1.1 ± 0.1 | 0.005 | 0.8 ± 0.2 | 0.06 |
| 8 | Farm 8: Cleaning of dried chamomile herb with a sieving machine | 12.3 ± 4.2 | 0.96 | 1.9 ± 0.6 | 0.16 |
| 9 | Farm 9: Shoveling of chamomile by-products (petals) into sacks | 2.7 ± 0.2 | 0.52 | 10.5 ± 0 | 3.13 |
| 10 | Farm 10: Shoveling of chamomile flower heads into sacks | 1.6 ± 0.4 | 0.05 | 8.2 ± 1.5 | 0.62 |
| 11 | Farm 11: Machine threshing of dried chamomile plants | 16.7 ± 11.7 | 26.5 | 27.8 ± 9.5 | 156.25 |
| 12 | Farm 12: Machine threshing of dried chamomile plants | 499.2 ± 92.5 | 2604.19 | 104.9 ± 19.4 | 3125.0 |
| 13 | Farm 13: Machine threshing of dried chamomile plants | 27.2 ± 14.3 | 153.0 | 4.2 ± 2.2 | 1562.5 |
| Median | | 12.3 | 0.96 | 8.2 | 3.13 |

chamomile ($1.4 \times 10^4$ cfu/m$^3$) approximated values found in sawmills [17] and at processing of hops [27] and potatoes [20]. Nevertheless, peak values exceeded the level $10^5$ cfu/m$^3$ and created a respiratory risk for exposed farmers.

As, so far, there are no internationally recognised Occupational Exposure Limit (OEL) values for bioaerosols, the results obtained in the present work could be compared only to the proposals raised by particular authors. As regards total viable airborne microorganisms, the OEL values proposed by Malmros *et al.* ($10 \times 10^3$ cfu/m$^3$) [40], and by Dutkiewicz & Jabłoński ($100 \times 10^3$ cfu/m$^3$) [13] were exceeded on all farms processing peppermint, and respectively on 5 and 2 of the 6 farms processing chamomile. The OEL values for airborne Gram-negative bacteria proposed by Clark [5] and Malmros *et al.* [40] ($1 \times 10^3$ cfu/m$^3$), and by Dutkiewicz & Jabłoński [13] and Górny & Dutkiewicz [28] ($20 \times 10^3$ cfu/m$^3$) were exceeded on all farms processing peppermint, and respectively on 4 and 1 of the 6 farms processing chamomile. The OEL value proposed by Dutkiewicz & Jabłoński [13] and Górny & Dutkiewicz [28] for airborne fungi ($50 \times 10^3$ cfu/m$^3$) was exceeded on 2 of the 4 farms processing peppermint, and on 1 of the 6 farms processing chamomile, while nowhere was the OEL value proposed by these authors for airborne thermophilic actinomycetes ($20 \times 10^3$ cfu/m$^3$) exceeded.

Gram-negative bacteria prevailed among airborne microorganisms at all sampling sites during processing peppermint and at half of the sampling sites during processing chamomile. The dominant species was the epiphytic bacterium *Pantoea agglomerans* (synonyms: *Erwinia herbicola, Enterobacter agglomerans*) possessing strong endotoxic and allergenic properties [10, 12, 33, 41, 46, 50]. It has been documented that *Pantoea agglomerans* evokes strong immunologic response in herb processing workers [19, 25] and could be a cause of allergic alveolitis in a herb farmer [39]. The results of the present work confirm the potential role of this bacterium as an occupational allergen in herb dust.

Farmers processing peppermint and chamomile were also exposed to Gram-negative bacteria of the family Pseudomonadaceae. Although so far only a little is known about the allergenic and immunotoxic properties of these bacteria, they have been identified as common constituents of oil mist in metallurgic industry facilities [7, 55] and implicated as causative agents of allergic alveolitis in exposed workers [2]. They should be also considered as a source of environmental endotoxin.

Fungi were dominant airborne microorganisms at half of the sampling sites during processing chamomile. Among them, the most common species was *Alternaria alternata*, known as a cause of allergic diseases of the upper respiratory tract [34]. *Penicillium* and *Cladosporium* species, which were found to be common at particular sampling sites, also reveal allergenic properties [13, 34].

The concentrations of dust and bacterial endotoxin in the farm air recorded during processing of peppermint and chamomile attained very high levels. The concentrations of dust during processing peppermint were of the order $10^1$-$10^2$ mg/m$^3$, exceeding on all farms the Polish OEL value of 4 mg/m$^3$ [45] by 22-240 times. The concentrations of dust during chamomile processing were of the order $10^0$-$10^2$ mg/m$^3$, exceeding on 6 of the 9 farms the Polish OEL value by 1.2-125 times.

The levels of bacterial endotoxin at particular sampling sites, mostly at herb threshing, reached very high values, posing a risk of respiratory disease in exposed farmers. Altogether, on 8 of the 13 examined herb farms, airborne endotoxin occurred in 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 [48]. The concentrations of airborne endotoxin during processing peppermint exceeded on all farms the OEL values proposed by Clark [5] (0.1 µg/m$^3$), Rylander [47] (0.1-0.2 µg/m$^3$), Malmros *et al.* [40] (0.1 µg/m$^3$), Górny & Dutkiewicz [28] (0.2 µg/m$^3$), Laitinen *et al.* [36] (0.025 µg/m$^3$), and by Dutch Expert Committee on Occupational Standards (DECOS) [9] (0.005 µg/m$^3$). The concentrations of airborne endotoxin during chamomile processing exceeded on 7 of the 9 farms the OEL values proposed by Clark [5], Rylander [47], Malmros *et al.* [40], and Górny & Dutkiewicz [28], and on 8 of the 9 farms the OEL values proposed by Laitinen *et al.* [36], and by Dutch Expert Committee on Occupational Standards (DECOS) [9].

## CONCLUSION

Farmers cultivating peppermint are exposed during the processing of this herb to large concentrations of airborne microorganisms, dust and endotoxin posing a risk of work-related respiratory disease. The exposure to bioaerosols during processing of chamomile is lower; nevertheless, peak values create a respiratory risk for exposed farmers.

**Acknowledgements**

The skillful assistance of Ms. Wiesława Lisowska and Ms. Halina Wójtowicz at performing the study is gratefully acknowledged.

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