

Exposure of agricultural workers to airborne microorganisms and endotoxin during handling of various vegetable products

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Abstract

Airborne concentrations of viable microorganisms and bacterial endotoxin were determined during 10 intramural agricultural activities including handling or processing of 6 kinds of vegetable products: grain, hay, horticulture seeds, herbs, flax and potatoes. The median values of the concentration of total viable microorganisms (bacteria + fungi) were within a range from 43.2×10^3 CFU/m³ at potato processing to 1240.0×10^3 CFU/m³ in small granaries, exceeding in 9 out of 10 cases the level of 10^5 CFU/m³ suggested as an occupational exposure limit (OEL). Many of the isolated bacteria and fungi were reported as allergenic and/or immunotoxic agents. The median values of the endotoxin concentration ranged from $0.0125 \mu\text{g}/\text{m}^3$ at handling hay to $54.9 \mu\text{g}/\text{m}^3$ at crushing grain, exceeding in 5 out of 7 cases the suggested OEL of $0.1 \mu\text{g}/\text{m}^3$. The high levels of exposure to airborne microorganisms and endotoxin found by this study indicate a potential risk of occupational respiratory disorders among agricultural workers, mostly in those handling grain.

1. Introduction

Farm workers and workers in agricultural industry could be exposed at work to organic dusts containing large quantities of microorganisms of plant and animal origin and microbial products, such as bacterial endotoxin, fungal glucans and mycotoxins. Inhalation of these agents may lead to allergic and/or immunotoxic processes in the lungs and consequently to respiratory disorders, such as allergic alveolitis, asthma and organic dust toxic syndrome (Lacey and Dutkiewicz, 1994; Crook and Olenchock, 1995; Eduard, 1997). Due to the large variation in cultivated crops, the degree of exposure and respiratory hazard associated with handling particular vegetable products is not fully known.

The objective of this work was to determine airborne concentrations of viable microorganisms and bacterial endotoxin during handling or processing of

6 kinds of vegetable products: grain, hay, horticulture seeds, herbs, flax and potatoes.

2. Materials and methods

Air sampling was performed during 10 intramural agricultural activities: grain threshing in barns, unloading grain in small granaries, crushing grain in a mill, feeding calves with hay, sacking ryegrass, cleaning parsley, processing nettle, processing mint, threshing flax, and processing potatoes (unloading, peeling), altogether at 36 sites in eastern Poland. Air samples for viable bacteria and fungi were taken on sheep blood agar plates (for mesophilic bacteria), half-strength tryptic soya agar plates (for thermophilic actinomycetes) and malt agar plates (for fungi) with a custom-designed particle-sizing slit sampler, enabling estimations of the total and respirable fractions of

the microbial aerosol (Dutkiewicz and Kwapiszewski, 1975). Each air sample was a duplicate, consisting of two parallelly exposed agar plates: one "A" sampled directly for total organisms 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 median cut point for the respirable fraction was $3.0\ \mu\text{m}$, approximating the recommendations of the American Conference of Governmental Industrial Hygienists (Threshold Limit Values, 1993–1994, ACGIH). The used sampler enables the determinations of the concentrations of microorganisms in the air in the range of 10^0 – 10^8 CFU/m³. The variation coefficient measured at the series of 10 samples was 15%. The instrument was patented in Poland (Polish Patent 87612 assigned on 6 June 1977).

In order to isolate as wide a spectrum of mesophilic bacteria as possible, exposed blood agar plates were incubated for one day at 37 °C, then three days at 22 °C and finally three days at 4 °C. Similarly for fungi, malt agar plates were incubated for four days at 30 °C and then four days at 22 °C (Dutkiewicz, 1978). Tryptic soya agar plates were incubated for five days at 55 °C. Colonies were then counted and identified and the total concentration of microorganisms in the air was calculated on the basis of the sum of counts from the three media used. The data were reported as colony forming units (CFU) per 1 m³ of air. The respirable fraction was expressed as a percentage of the total count.

Air samples for the determination of dust and endotoxin concentrations were collected in duplicate on preweighed polyvinyl chloride filters using a one-stage sampler (AS-50, TWOMET, Zgierz, Poland). Dust concentrations in the air were determined gravimetrically and expressed in mg/m³. Concentrations of bacterial endotoxin in the airborne dust were determined in these samples by the *Limulus* amoebocyte lysate (LAL) test, using the procedure applied by Clark et al., 1983. LAL reagent and standard endotoxin of *Escherichia coli* EC-5 used as a positive control were produced by Cape Cod Associates, Woods Hole, USA. The results were expressed as microgram equivalents of the endotoxin of *E. coli* EC-5 per 1 m³ of air. For technical reasons, the concentrations of dust and endotoxin were not determined at unloading grain in small granaries, at sacking ryegrass seeds, and at cleaning parsley seeds in a store.

Eight samples of threshing dust from grain and flax were examined by electron microscopy (EM) for the

presence of endotoxin-containing membrane vesicles (ECMV) released from the outer membrane of the bacteria (Dutkiewicz et al., 1992). Small portions of dust were pre-fixed in 2% glutaraldehyde in phosphate buffer at pH 7.3, and post-fixed in 1% buffered osmium tetroxide. After dehydration in ethanol, the samples were embedded in Low Viscosity (by dr Spurr), thin sectioned (silver colour) and post-stained with 2% uranyl acetate and lead citrate. The micrographs were taken with a Philips EM 300 electron microscope operating at 80 KV.

The statistical analysis was performed by the Mann-Whitney test for non-parametric distribution, with the aid of software package STATISTICA for Windows, release 4.5, StatSoft[®], Inc. 1993.

3. Results

The median values of the concentration of total viable microorganisms (bacteria + fungi) were within a range of 43.2×10^3 CFU/m³ at potato processing (unloading, flushing, peeling) to 1240.0×10^3 CFU/m³ at unloading grain in small granaries (Table 1). The concentration of microorganisms at unloading grain was significantly higher ($P < 0.001$) compared to all other activities, whereas the concentration determined at potato processing was significantly lower ($P < 0.001$) compared to all other activities (Table 1). The values of the respirable fraction were high and made up 41.9–80.2% of the total count.

Corynebacteria were found to be the most common organisms at threshing grain and flax and at potato processing, staphylococci at handling horticulture seeds, *Streptomyces albus* at crushing grain and handling nettle, Gram-negative bacteria (mostly *Pantoea agglomerans*) in small granaries, thermophilic actinomycetes at handling hay and *Bacillus* spp. at handling mint. Fungi made up only 0.4–29.8% of the total count (Figure 1). Many of the species of bacteria and fungi which have been identified as common constituents of the air polluted with particular vegetable dusts are known as allergenic and/or immunotoxic agents (Table 2).

The median values of the endotoxin concentration were smallest at handling hay ($0.0125\ \mu\text{g}/\text{m}^3$) and potatoes ($0.022\ \mu\text{g}/\text{m}^3$), and largest at grain handling (50.0 – $54.9\ \mu\text{g}/\text{m}^3$) (Table 3). The values determined at three other activities ranged from $8.09\ \mu\text{g}/\text{m}^3$ at mint processing to $30.3\ \mu\text{g}/\text{m}^3$ at flax threshing. The concentration of endotoxin at processing potatoes was

Table 1. Microflora of the air during handling of various vegetable products: Concentration, respirable fraction.

Activity	Number of sites (samples)	Concentration of micro-organisms (CFU $\times 10^3/\text{m}^3$) Median (range)	Respirable fraction (%)
Grain threshing in barns	12 (60)	351.5 (30.0–1006.0)	80.2
Unloading grain in small granaries	5 (40)	1240.0 ^a (460.7–2898.7)	56.8
Crushing grain in a mill	1 (5)	127.8 (124.2–129.9)	41.9
Feeding calves with hay in a barn	1 (5)	158.4 (152.0–478.2)	74.2
Potato processing (unloading, flushing, peeling)	3 (15)	43.2 ^b (14.4–67.2)	42.2
Flax threshing in a barn	5 (25)	379.0 ^c (53.0–935.0)	67.7
Sacking ryegrass seeds in a store	2 (10)	491.1 ^d (310.2–830.4)	65.2
Cleaning parsley seeds in a store	2 (10)	306.9 (258.6–421.8)	60.5
Processing nettle leaves	3 (15)	367.2 (71.6–674.4)	48.9
Processing mint leaves	2 (10)	245.1 (99.0–336.0)	47.6

^a Concentration significantly higher compared to all other activities ($P < 0.001$).

^b Concentration significantly lower compared to all other activities ($P < 0.001$).

^c Concentration significantly higher compared to processing mint leaves ($P < 0.01$).

^d Concentration significantly higher compared to cleaning parsley ($P < 0.01$), processing nettle leaves ($P < 0.05$), and processing mint leaves ($P < 0.001$).

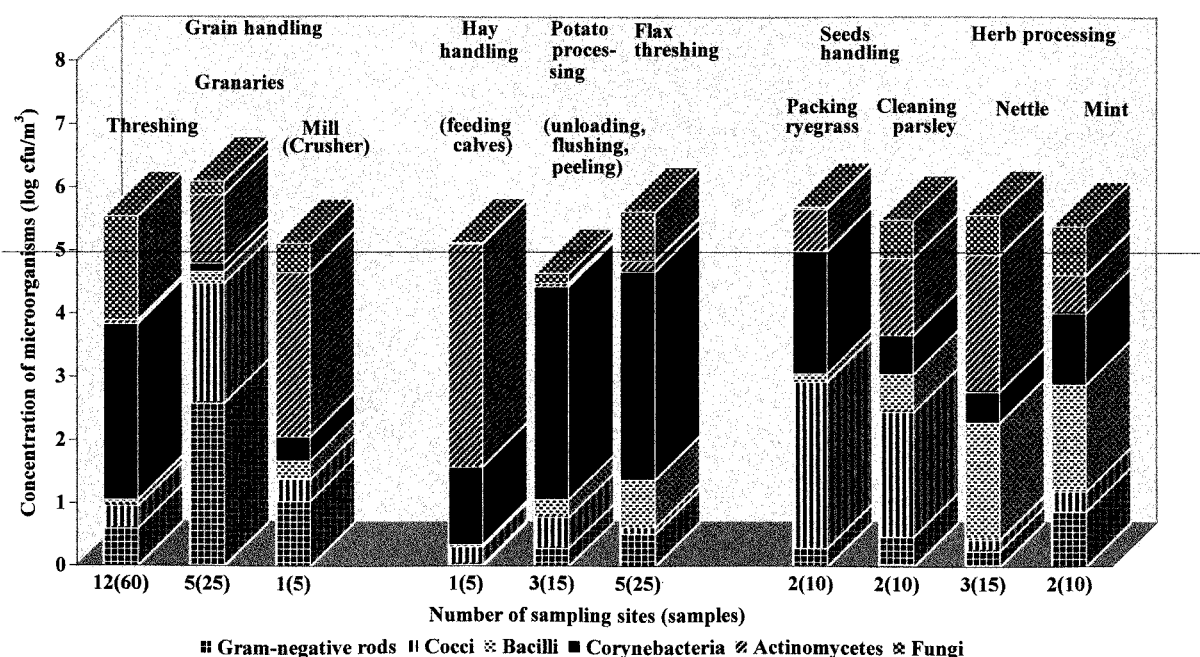


Figure 1. Relative abundance of the particular groups of microorganisms found in air during handling of various vegetable products, shown as percentages of the total count. Bar lengths are proportional to the logarithmic values of the concentration ($\log_{10}$ CFU/ m^3). Horizontal line indicates proposed occupational exposure limit (OEL) value for concentration of total microorganisms in air (1×10^5 CFU/ m^3).

Table 2. Microorganisms common in air polluted with vegetable dusts during performing of various agricultural activities (each species listed in the table formed on the average 5–75% of the total microflora).

Activity	Common species of bacteria and fungi
Grain threshing in barns	<i>Pantoea agglomerans</i> ^{*,+} (synonyms: <i>Erwinia herbicola</i> , <i>Enterobacter agglomerans</i>), <i>Xanthomonas maltophilia</i> , <i>Arthrobacter globiformis</i> [*] , <i>Corynebacterium</i> spp., <i>Alternaria alternata</i> ^{*,+} , <i>Cladosporium fulvum</i> [*]
Unloading grain in small granaries	<i>Pantoea agglomerans</i> ^{*,+} , <i>Pseudomonas fluorescens</i> , <i>Streptomyces albus</i> [*] , <i>Staphylococcus epidermidis</i> , <i>Aspergillus fumigatus</i> ^{*,+} , <i>Aspergillus repens</i>
Crushing grain in a mill	<i>Pantoea agglomerans</i> ^{*,+} , <i>Streptomyces albus</i> [*] , <i>Penicillium</i> spp. ^{*,+}
Feeding calves with hay in a barn	<i>Arthrobacter globiformis</i> [*] , <i>Thermoactinomyces thalpophilus</i> [*] , <i>Th. vulgaris</i> [*] , <i>Saccharopolyspora rectivirgula</i> [*] (synonyms: <i>Faenia rectivirgula</i> , <i>Micropolyspora faeni</i>)
Potato processing (unloading, flushing, peeling)	<i>Alcaligenes faecalis</i> ^{*,+} , <i>Acinetobacter calcoaceticus</i> ^{*,+} , <i>Corynebacterium</i> spp., <i>Agromyces ramosus</i> , <i>Aspergillus niger</i> [*]
Flax threshing in a barn	<i>Pantoea agglomerans</i> ^{*,+} , <i>Corynebacterium xerosis</i> , <i>Arthrobacter globiformis</i> [*] , <i>Alternaria alternata</i> ^{*,+}
Sacking ryegrass seeds in a store	<i>Staphylococcus</i> spp., <i>Micrococcus</i> spp., <i>Corynebacterium</i> spp., <i>Arthrobacter globiformis</i> [*] , <i>Streptomyces albus</i> [*] , <i>Thermoactinomyces vulgaris</i> [*] , <i>Aspergillus fumigatus</i> ^{*,+}
Cleaning parsley seeds in a store	<i>Pantoea agglomerans</i> ^{*,+} , <i>Staphylococcus epidermidis</i> , <i>Streptomyces albus</i> [*]
Processing nettle leaves	<i>Alcaligenes faecalis</i> ^{*,+} , <i>Streptomyces albus</i> [*] , <i>Bacillus megaterium</i> , <i>Alternaria alternata</i> ^{*,+} , <i>Aspergillus nidulans</i>
Processing mint leaves	<i>Alcaligenes faecalis</i> ^{*,+} , <i>Bacillus</i> spp., <i>Corynebacterium</i> spp., <i>Streptomyces albus</i> [*] , <i>Alternaria alternata</i> ^{*,+}

The names of allergenic and/or immunotoxic species are in bold

* Allergenic species

+ Immunotoxic species

Table 3. Concentration of dust and bacterial endotoxin in air during handling of various vegetable products.

Activity	Number of sites (samples)	Concentration of dust in air (mg/m ³) Median (range)	Concentration of endotoxin in air (μg/m ³) Median (range)
Grain threshing in barns	12 (24)	45.40 (0.6–118.6)	50.00 (0.05–993.9)
Crushing grain in a mill	1 (2)	112.50 (90.4–134.6)	54.90 (40.2–69.6)
Feeding calves with hay in a barn	1 (2)	0.25 (0.18–0.32)	0.0125 (0.0095–0.0155)
Potato processing (unloading, flushing, peeling)	3 (6)	2.30 (1.4–6.0)	0.022 ^a (0.011–0.090)
Flax threshing in a barn	5 (10)	93.60 (43.7–648.1)	30.03 (16.9–172.1)
Processing nettle leaves	3 (6)	25.50 (3.2–58.9)	16.00 (0.8–71.5)
Processing mint leaves	2 (4)	18.30 (6.3–30.2)	8.09 (0.18–16.0)

^a Concentration significantly lower compared to grain threshing, flax threshing, and processing nettle leaves ($P < 0.05$).

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The results of *Limulus* test were confirmed by the electron microscopy examination which showed the abundant presence of the endotoxin-containing membrane vesicles, measuring 30–120 nm, in the grain and flax dusts (Figures 2–3).

4. Discussion

The concentrations of culturable microorganisms found in the air during handling of various vegetable products by agricultural workers were very high,

exceeding in 9 out of 10 cases the level of 10^5 CFU/m³ suggested in Poland as the occupational exposure limit (OEL) (Dutkiewicz and Jabłoński, 1989). The risk was increased by the high values of respirable fraction able to reach alveoli, and by the presence of bacterial and fungal species reported as causative agents of allergic and immunotoxic diseases (Dutkiewicz and Jabłoński, 1989; Lacey and Dutkiewicz, 1994; Crook and Olenchock, 1995).

In the majority of examined activities, the concentrations of bacterial endotoxin in the air were large, posing a risk of non-specific lung inflammation in exposed workers. In 5 out of 7 cases, the concentration of endotoxin exceeded that suggested by Rylander



Figure 2. Thin sectioned sample of grain threshing dust from site 2. Numerous endotoxin-containing membrane vesicles (some marked with arrows) are seen between bacterial cells. EM, bar equal to 0.2 μm .

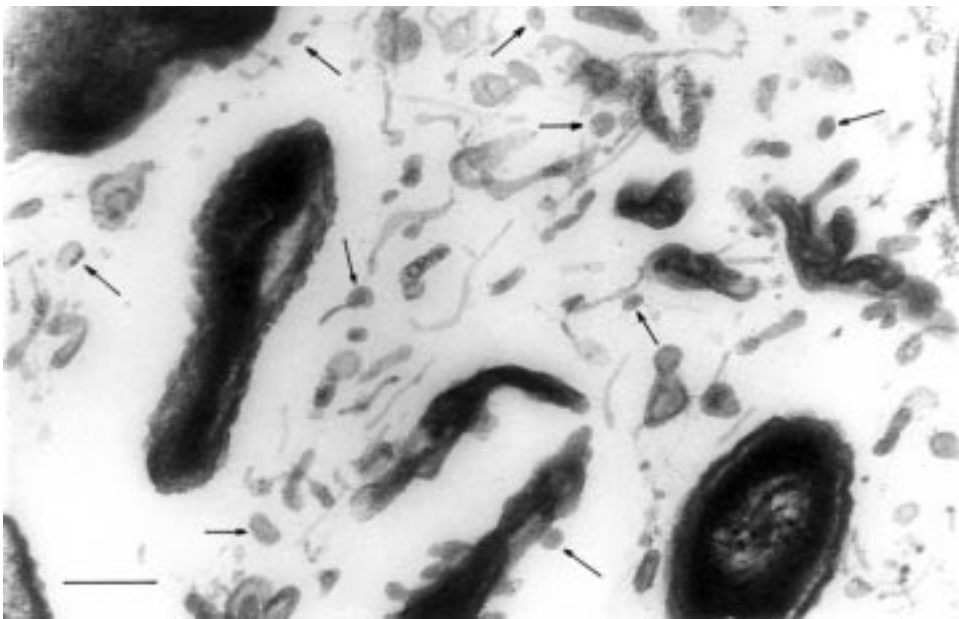


Figure 3. Thin sectioned sample of flax threshing dust from site 4. Abundant endotoxin-containing membrane vesicles (some marked with arrows), free or budding from outer membrane of bacteria, are seen. EM, bar equal to 0.2 μm .

(1987) OEL of $0.1 \mu\text{g}/\text{m}^3$. The highest levels of endotoxin, exceeding by more than 500 times the proposed OEL, were found in the air polluted with grain dust. The risk is enhanced by the fact that a large part of endotoxin occurs in the dust in the form of very small membrane vesicles (30–120 nm) which may easily penetrate into the deepest parts of the lung. It has been proved in animal experiment that the microvesicle-bound endotoxin shows a strong biological activity, stimulating an abundant production of inflammatory mediators (Skórska et al., 1996).

5. Conclusions

The high levels of exposure to airborne microorganisms and endotoxin found by this study indicate a potential risk of occupational respiratory disorders among the agricultural workers handling or processing vegetable products, mostly in those handling grain.

The risk is increased by the presence of allergenic and/or immunotoxic microbial species, mostly belonging to Gram-negative bacteria, actinomycetes and filamentous fungi.

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