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EXPOSURE TO BIOAEROSOLS IN THE SELECTED AGRICULTURAL FACILITIES OF THE UKRAINE AND POLAND – A REVIEW

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Abstract: The aim of this work was to review the studies on bioaerosols which were carried out in the years 1972–2009 in following branches of agricultural industry in the Ukraine and Poland: animal farms for cows and pigs, animal feed facilities, production of biofuel from rape, herb farms and herb processing facilities. In all facilities were determined: concentration of dust and microorganisms in the air and species composition of microflora. Moreover, in Polish animal farms, herb farms and herb processing facilities, as well as in the Ukrainian animal feed facilities, was determined the concentration of bacterial endotoxin in the air. Dust concentrations in animal farms located in the Ukraine and Poland ranged from 6–200 mg/m^3 and from 0.25–14.05 mg/m^3, respectively, while in animal feed facilities they ranged from 35–306 mg/m^3 and from 3.8–405 mg/m^3, respectively. Dust concentrations in the facilities producing biofuel from rape in the Ukraine were in the range 3.6–28 mg/m^3, whereas on herb farms and in herb processing facilities in Poland they were in the range 0.8–1,319.6 mg/m^3, and 2.2–946 mg/m^3, respectively. The determined values exceeded in most cases the maximal acceptable concentration (MAC) which in the Ukraine and Poland is equal to 4 mg/m^3. The concentrations of microorganisms on animal farms located in the Ukraine and Poland ranged from 5.5×10^4–1.9×10^7 cfu/m^3 and from 4.7×10^4–1.5×10^6 cfu/m^3, respectively, while in animal feed facilities they ranged from 2.7×10^4–2.6×10^9 cfu/m^3 and from 1.7×10^3–2.0×10^6 cfu/m^3, respectively. Concentrations of microorganisms in the facilities producing biofuel from rape in the Ukraine were in the range 1.5×10^3–5.7×10^6 cfu/m^3, whereas on herb farms and in herb processing facilities in Poland they were in the ranges of 8.8×10^2–8.0×10^6 cfu/m^3, and 9.7×10^3–6.3×10^5 cfu/m^3, respectively. The determined values exceeded in most cases the maximal acceptable concentration (MAC) which in the Ukraine is equal to 5.0×10^4 cfu/m^3. The concentrations of endotoxin in Polish animal houses were in the range 0.00125–75.0 µg/m^3, whereas on herb farms and herb processing facilities they were in the ranges of 0.0045–2,448.8 µg/m^3 and 0.2–681.0 µg/m^3, respectively, and in the Ukrainian animal feed facilities were within the range 0.008–240.0 µg/m^3. They exceeded in most cases the level of 0.2 µg/m^3 proposed as a threshold. In the air microflora of examined facilities prevailed Gram-positive bacteria (corynebacteria, cocci, spore-forming bacilli, actinomycetes) of which some (*Arthrobacter* spp., thermophilic actinomycetes) could be a cause of allergic alveolitis (hypersensitivity pneumonitis). Among Gram-negative bacteria isolated from the air of agricultural settings dominated the epiphytic species *Pantoea agglomerans*, possessing potent allergenic and endotoxic properties. Fungi were abundant in the air of the Ukrainian agricultural settings and comprised species able to produce harmful mycotoxins. In conclusion, the airborne biological factors in stated concentrations may exert harmful effects on the state of the health of exposed workers. Formation of the bioaerosol depends on the specificity of the setting, kind of technological operations, degree of mechanization, properties of processed materials, temperature and humidity, and concentration of dust in the air.

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INTRODUCTION

In the Ukraine and Poland, there is a steady increase in the number of agricultural facilities in which bioaerosols are the main occupational hazards. These facilities comprise both small farms and big industrial complexes such as animal feed factories. Recently, in the Ukraine a number of facilities processing genetically modified plants have been established, in particular for the production of biofuel from rape [60, 63, 68, 69, 70].

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The list of hazardous biological agents is also increasing, due to natural processes, such as mutations of microorganisms, and to human activity in creating new transgenic cultures of plants and animals and genetically modified microorganisms. Formation of bioaerosols in agricultural facilities depends on working conditions, production technology, processed materials, and presence of additives (such as pesticides).

At present, best known are the constituents of agricultural bioaerosols which cause infectious diseases: anthrax, Q fever, ornithosis, tularaemia, brucellosis, salmonellosis, aspergillosis, and many other diseases [15]. However, the majority of the authors point out that less known "saprophytic" microorganisms, which are prevailing constituents of agricultural bioaerosols and until now had not been recognized as pathogens, may exert a negative impact on the health of exposed workers. Pathogenic properties of these "saprophytic" bacteria and fungi are associated with their ability to produce allergens and toxins (endotoxins, mycotoxins). They play an important role in the etiology and pathogenesis of different work-related respiratory diseases of man, such as extrinsic allergic alveolitis (hypersensitivity pneumonitis), allergic rhinitis, bronchial asthma, organic dust toxic syndrome (ODTS, toxic pneumonitis), and pulmonary mycoses [14, 15, 24, 31, 36, 48, 50, 63, 64, 65, 66].

Accordingly, the aim of the present work was to review the studies on bioaerosols which have been undertaken in some important branches of agricultural industry in the Ukraine and Poland.

EXPOSURE TO AIRBORNE DUST AND MICROORGANISMS IN THE UKRAINIAN AGRICULTURAL SETTINGS

Objects and methods. The aerobiological studies were performed between the years 1980–2009 on the territory of Ukraine in 10 animal houses (4 industrial cattle-breeding settings, 4 milk farms, 2 piggeries) 5 animal feed facilities, and 2 factories producing biofuel from rape [5, 6, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69]

The hygienic estimation of microclimate and dust was conducted accordingly to the State Standard GOST 12.1.005-88 [58] issued in the past by the Soviet Union and adopted by the Ukraine. Dust concentration was determined by the gravimetric method. A total of 850 dust samples were examined.

The microbiological samples were collected by the method of impaction on agar media using a Krotov impactor type 818 OJSC, produced by Krasnogvardeyec, Saint Petersburg, Russia. Bacterial isolates were identified with microscopic and biochemical methods, as recommended by Bergey's Manual [33, 57, 71]. Fungi were determined with microscopic and biochemical methods, according to Bilay & Pidoplitchko [3], and Pidoplitchko [44]. The concentration of bacterial endotoxin in the air was determined by the *Limulus* test.

The microbiological pollution of air was evaluated basing on the former Soviet norm GOST 12.1.005-88 [59] which has been adopted in the Ukraine. A total of 2,354 microbiological samples were analysed.

For study of the exposure to bioaerosols in agricultural facilities in the Ukraine and conditions of their formation, there was performed a comparative analysis of the data obtained in the following branches of agricultural production: • animal farms harbouring cattle and pigs, • animal feed factories, • and facilities processing rape, in which extracted oil is used for production of biofuel, while waste is used as animal food.

Technological processes. Investigations of the technological processes in the examined facilities showed that they consist of similar operations: transportation, unloading and loading, weighing and storing of materials and final products. In animal farms, the essential operations are feeding and sweeping the rooms, while in animal feed facilities and other facilities processing plant materials the operations include drying, sieving, grinding, and granulation of the product.

Microclimate. In the formation of bioaerosols, a significant role is played by the temperature and relative humidity of air in production rooms and outside environment. It was found that the dynamics of the microclimate parameters in all examined facilities showed similar trends and were distinguished by marked fluctuations depending on production specificity, construction failures, effectiveness of the ventilation system and season of the year.

In animal houses, mean temperatures of air varied in the cold period between 8–18ºC and in the warm period between 15–27ºC. Relative humidity was between 52–83% [60].

At the processing of plant materials for biofuel, temperatures of air in the cold period were between 15–19ºC and in the warm period between 20–30ºC. Relative humidity of air varied between 60–75%. High temperatures up to 30ºC were recorded at the drying of plant material [68].

In animal feed facilities, temperatures of air varied in the cold period between 15–20ºC and in the warm period between 22–35ºC, Relative humidity was between 60–80%. High air temperatures occurred in the machine rooms for thermal processing, grinding and granulating the plant material [5, 62].

Dust. In agricultural settings, most of technological operations are associated with the release of dust into the breathing zone of workers. This is organic dust consisting of many components. It contains silica, biologically active plant and animal substances possessing allergenic properties, a wide spectrum of microorganisms and their products (toxins, enzymes), and other biological agents (such as particles of mites and insects). All these components form a bioaerosol of the agricultural facility.

It has been found that the concentration of dust in animal houses ranged between 6–200 mg/m^3 of air. High concentrations of dust were found at loading of fodder, feeding animals and removing manure. The maximum acceptable concentration (MAC) of dust in the air (4 mg/m^3) was exceeded up to 50 times [60].

In facilities processing rape for biofuel, the concentration of dust in the air ranged between 3.6–28 mg/m^3. High concentrations of dust were recorded at transportation, sieving and drying of rape. The MAC was exceeded up to 7 times, less than in other agricultural settings. The relatively low dust concentrations could be explained by the enhanced humidity of processed plant material, which significantly decreased the dustiness.

In the animal feed producing facilities, the dust concentrations in the air were between 35–306 mg/m^3. High concentrations of dust were recorded at grinding and granulating of feed and at distribution of the ready product. The MAC was exceeded 8–75 times.

In all examined facilities, release of dust depended on the level of mechanization, tightness of the equipment, proportion of the operations taken manually, and of the properties and degree of dustiness of the materials being processed.

Organic dust appears to be a favourable medium for the persistence of the life function of numerous species of bacteria and microscopic fungi, which may release allergens and toxins, depending on the temperature and humidity of air [6].

In all the examined facilities a direct positive correlation was found between the concentrations of dust and microorganisms in the air of the working zone. The correlation coefficients (r) ranged from 0.7–0.8, at $p<0.01$.

Microorganisms. The concentrations of microorganisms in the air of the working zone in animal houses ranged from $5.5 \times 10^4 - 1.9 \times 10^7$ cfu/m^3. The maximum acceptable concentration (MAC, equal to 5.0×10^4 cfu/m^3) was exceeded, at the maximum concentrations, up to 380 times. The greatest concentrations were noted at the unloading of plant materials, at feeding the animals, and at cleaning of the animal rooms.

At processing of rape for biofuel, the concentrations of microorganisms in the air ranged from $1.5 \times 10^3 - 5.7 \times 10^6$ cfu/m^3. The greatest concentrations were found at loading, transportation and drying the rape [70].

In animal feed facilities, the concentrations of microorganisms in the air ranged from $2.7 \times 10^4 - 2.6 \times 10^9$ cfu/m^3. The marked exceedings of the MAC value were noted at unloading, grinding, drying, and granulation of the raw material and at distribution of the ready product [6].

In these facilities, the dynamics of microbial pollution was studied in the course of the work shift, by determining the concentrations of microorganisms in the washings from the technological equipment, the protection clothes, and the skin of the hands of workers (Tab. 1).

Table 1. The concentrations of microorganisms in the washings from the technological equipment, the protection clothes and the skin of the hands of workers, determined in the course of work shift in animal feed facilities.

Stage of technological process	Microbial concentration (cfu/m^2)	
	Start of work shift	Middle of work shift
Central steering room		
Technological equipment	2.7×10^4–4.3×10^4	3.5×10^4–8.6×10^5
Protective clothes	1.2×10^4–2.8×10^4	3.7×10^4–7.1×10^5
Skin of hands of workers	1.6×10^3–1.8×10^4	6.5×10^4–1.3×10^5
Grinding Departament		
Technological equipment	1.7×10^5–2.6×10^5	4.3×10^5–6.1×10^6
Protective clothes	6.8×10^4–7.2×10^4	5.8×10^5–7.4×10^5
Skin of hands of workers	2.8×10^4–3.5×10^4	6.8×10^4–9.7×10^5
Granulation Departament		
Technological equipment	1.6×10^5–3.8×10^5	7.2×10^5–1.2×10^6
Protective clothes	1.2×10^5–4.1×10^5	4.9×10^5–3.4×10^6
Skin of hands of workers	1.8×10^4–2.0×10^5	3.1×10^5–7.5×10^5
Manual loading of raw material		
Technological equipment	Not applicable	Not applicable
Protective clothes	4.8×10^4–2.5×10^5	1.7×10^6–8.3×10^6
Skin of hands of workers	2.3×10^4–3.0×10^5	1.2×10^6–6.5×10^6
Loading of green mass		
Technological equipment	3.5×10^4–7.8×10^4	8.0×10^4–9.7×10^4
Protective clothes	2.7×10^4–4.1×10^4	6.2×10^4–8.1×10^4
Skin of hands of workers	1.8×10^4–5.0×10^4	6.9×10^4–2.0×10^5

The concentrations of microorganisms in the washings from technological equipment, protection clothes and hands of the workers were distinctly greater in the middle of the work shift than at the start of shift (Tab. 1). The species composition of microflora isolated from washings showed a close identity with that determined in the air samples taken in the animal feed facilities. The concentrations of microorganisms in the samples washed off from the surface of skin showed a significant correlation with the concentrations of dust in all production departments ($r=0.7$, $p<0.05$), and in non-production rooms ($r=0.9$, $p<0.01$).

Sources of microbial pollution of the air. For determination of the sources of microbial pollution of the working zone, numerous samples were taken from various raw materials and animal foods. It was found that all kinds of raw materials, as well as animal foods and ready products, were abundantly contaminated with a wide spectrum of microorganisms. All these materials represent a good nutrient medium for the preservation of life functions of microorganisms and their propagation. A species composition of microflora contaminating these materials corresponds to that found in the air of agricultural facilities, and in the washings from equipment, clothes and hands of the workers.

Thus, the bulk of microorganisms contaminating the air of the breathing zone and other elements of working environment, originates from the processed raw materials. In animal houses, an additional source of microbial contamination are the animals themselves and products of their metabolism (faeces, urine, saliva, milk).

Effects of the season of the year on airborne microorganisms. No relationship could be found between the season of the year and concentration of airborne microorganisms in animal feed facilities and at the processing of rape for fuel. In animal farms, an increase of the concentration of microorganisms was noted in the spring/summer period. In this period, a distinct growth of the Gram-positive cocci *Staphylococcus aureus* and *S. saprophyticus* was observed, more rarely that of *Streptococcus faecalis* and *S. faecium*. In spring/summer period there markedly increased also the numbers of Gram-negative bacteria of the genera *Escherichia*, *Pseudomonas* and *Klebsiella*, more rarely those of the genera *Citrobacter* and *Acinetobacter*.

Species composition of airborne microflora. Analysis of the species composition of microflora isolated from the air of agricultural settings showed that bioaerosol in all these settings contained a wide variety of microorganisms. In these settings were recorded great concentrations of Gram-positive spore-forming bacilli of the genus *Bacillus*. These bacteria formed 10–24% of total microbial isolates in animal farms, 12–66% in animal feed facilities and 0.2–12% in facilities processing rape for biofuel. The most often isolated species were: *Bacillus subtilis*, *B. cereus*, *B. alvei*, *B. laterosporus*, *B. circulans*, *B. megaterium*, *B. pumilus*, *B. stearothermophilus*, *Clostridium tetani*. According to some reports, these bacteria may be hazardous for the human respiratory system. It has been shown that aerosol containing spores of *B. subtilis* and *B. licheniformis* may cause extrinsic allergic alveolitis [32].

The other Gram-positive bacteria, mostly cocci, formed 5–35% of total microbial isolates in animal farms, 0.2–14% in animal feed facilities and 0.5–8% in facilities processing rape for fuel. The cocci most often isolated were: *Staphylococcus aureus*, *S. epidermidis*, *S. saprophyticus*, *Streptococcus faecalis*, *S. faecium*, *S. mitis*. These species may cause infections and inflammatory processes. Other Gram-positive bacteria isolated from the examined settings were thermophilic actinomycetes, producing small spores which may penetrate into deep parts of the lungs and cause extrinsic allergic alveolitis. Of these bacteria, were isolated: *Saccharopolyspora rectivirgula*, *Thermoactinomyces vulgaris*, *T. thalpophilus* and *Saccharomonospora viridis*.

The other group of potentially pathogenic microorganisms are Gram-negative bacteria. They formed 16–45% of total microbial isolates in animal farms, 4.4–23.1% in animal feed facilities and 0.2–5% in facilities processing rape for fuel. The prevailing species were: *Proteus vulgaris*, *P. mirabilis*, *Pantoea agglomerans* (synonyms: *Erwinia herbicola*, *Enterobacter agglomerans*), *Escherichia coli*, *Citrobacter* spp., *Pseudomonas* spp., *Klebsiella* spp., *Alcaligenes* spp., *Acinetobacter* spp.

Table 2. Species of microfungi known as mycotoxin-producers, isolated from the air of animal feed facilities.

No.	Name of isolated species
1	*Alternaria alternata* (Fr.) Keissl
2	*Aspergillus candidus* Gk.
3	*Asp. flavus* Gk.
4	*Asp. fumigatus* Fres.
5	*Botrytis cinerea* Pers.
6	*Cladosporium cladosporioides* (Fres.) de Vries
7	*Fusarium sporotrichiella* v. *tricintum* (Cda.) Bilai
8	*Penicillium citrinum* Thom
9	*P. viridicatum* West.
10	*P. verrucosum* v. *cyclopium* Saneson et Hadlock
11	*Trichothecium roseum* Gink

Altogether, 15 genera and 39 species of microfungi were isolated.

Microscopic fungi formed a large part of the microflora isolated from the air of agricultural settings. They formed 12–28% of total microbial isolates in animal farms, 20–44% in animal feed facilities and 15–85% in facilities processing rape for fuel.

The spectrum of the isolated fungal species was wide. The prevailing genera were: *Mucor*, *Aspergillus*, *Penicillium*, *Fusarium*. From air samples taken in animal food facilities were isolated 39 fungal species, of which 11 are potential producers of mycotoxins (Tab. 2).

Endotoxin. The concentration of endotoxin in the air was determined in animal feed facilities [2]. The concentration of airborne endotoxin was between 0.008–240.0 $\mu g/m^3$, and at most sites exceeded the proposed threshold value of 200 ng/m^3 (0.2 $\mu g/m^3$) [30, 47].

EXPOSURE TO AIRBORNE DUST, MICROORGANISMS AND ENDOTOXIN IN POLISH AGRICULTURAL SETTINGS

Objects and methods. The aerobiological studies were performed between the years 1972–2009 on the territory of eastern Poland in 14 animal houses (4 cow barns and 10 piggeries), in one big animal feed facility (at 16 sites), on 31 herb cultivating farms [6 cultivating thyme (*Thymus vulgaris*), 15 cultivating valerian (*Valeriana officinalis* L.) and 10 cultivating peppermint (*Mentha piperita* L.) and chamomile (*Matricaria recutita* L.)], and in 3 big herb processing facilities (at 18 sampling sites), of which 2 facilities (“A” and “B”) were examined in 1984–1986, and one facility (“P”) in 2007–2009 [10, 13, 17, 19, 20, 22, 30, 34, 40, 52, 54, 56]. The concentration of microorganisms was determined by culture methods (determining of cfu/m^3). The air samples were taken: • by impaction, in an animal feed facility using Bourdillon slit sampler, and in animal houses, herb farms cultivating thyme, and herb processing facilities “A” and “B” using originally designed particlesizing slit sampler enabling determination of the total and respirable fraction of microbial aerosol [8]; • and by filtration, on herb farms cultivating peppermint, chamomile, and valerian and in herb processing facility "P", taking samples on glass fibre filters using an AS-50 Sampler (TWOMED, Poland), and determining the microbial concentration by dilution plating. Bacterial isolates were identified with microscopic and biochemical methods, as recommended by Bergey's Manual [32, 55, 69]. Fungi were classified with microscopic methods, according to Barron [1], Litvinov [38], Ramirez [45], and Raper & Fennell [46].

The dust concentration was determined by gravimetric method. The concentration of bacterial endotoxin was determined by the *Limulus* clot method in all the settings except for the animal feed facility.

Dust. The concentration of dust in cow barns ranged between 0.25–0.80 mg/m^3 of air and never exceeded the Polish maximal acceptable concentration (MAC) of dust in the air equal to 4 mg/m^3 [43]. In piggeries, the concentrations of dust were higher and ranged between 3.03–14.05 mg/m^3 of air, exceeding the MAC at 6 out of 10 examined houses by 1.3–3.5 times [17, 19, 20, 40].

In the examined animal feed factory, the concentration of dust ranged from 3.8–405.0 mg/m^3 of air, exceeding the MAC at 15 out of 16 sites by 1.2–101.2 times [56].

On the herb farms cultivating thyme the concentration of dust recorded during handling herbs was in the range 317.8–1,319.6 mg/m^3 of air, on the farms cultivating peppermint and chamomile it was in the range of 0.8–246.7 mg/m^3 of air, while on the farms cultivating valerian it was in the range of 1.5–158.9 mg/m^3 of air [34, 52, 54]. In the big herb processing plants "A" and "B", the concentration of dust ranged from 3.2–946 mg/m^3 of air, whereas in recently examined plant "P" it ranged from 2.2–68.3 mg/m^3 of air [22, 30]. The MAC value was exceeded on all 6 thyme farms by 79.5–330 times, on 7 out of 10 peppermint and chamomile farms by 2.1–61.7 times, on 13 out of 15 valerian farms by 4.3–39.7 times, at 13 out of 14 sampling sites in herb processing plants "A" and "B" by 1.03–236.5 times, and at 3 out 4 sampling sites in herb processing plant "P" by 1.06–17.1 times [22, 30, 34, 52, 54].

Microorganisms. The concentrations of microorganisms in the air of breathing zone in cow barns ranged from $4.7 \times 10^4 - 2.9 \times 10^5$ cfu/m^3 [17, 20]. As in Poland there is no obligatory MAC value for bioaerosols (proposed value is 1.0×10^5 cfu/m^3 for mesophilic bacteria [30]), the results were compared with the Ukrainian MAC value of 5.0×10^4 cfu/m^3. This value was exceeded at 3 out of 4 sampling sites by 3.6 to 5.8 times. The concentrations of airborne microorganisms in piggeries were significantly greater compared to cow barns ($p<0.05$), being in the range of $6.0 \times 10^5 - 1.5 \times 10^6$ cfu/m^3 [19, 20, 40]. and exceeded at all sampling sites the Ukrainian MAC value by 12–30 times. The respirable fraction of microbial aerosol was 38.5–75.7% of the total count in cow barns and 40.5–49.2% of the total count in piggeries.

The concentration of airborne microorganisms in a big animal feed facility ranged from $1.7 \times 10^3 - 2.0 \times 10^6$ cfu/m^3 [10, 56], and exceeded the Ukrainian MAC value at 13 out of 16 sampling sites by 1.2 to 40.0 times.

The concentrations of airborne microorganisms on the herb farms cultivating thyme were in the range of $9.04 \times 10^4 - 5.9 \times 10^5$ cfu/m^3, and the respirable fraction was within high values of 62.6–92.1%. The Ukrainian MAC value was there exceeded on all 6 farms by 1.8 to 11.9 times [34]. On the farms cultivating peppermint and chamomile the concentrations of microorganisms in the air were in the broad range $8.8 \times 10^2 - 6.0 \times 10^6$ cfu/m^3 [54], exceeding the Ukrainian MAC value on 6 out of 10 examined farms by 4.1–120.3 times. On valerian farms, the range of the concentrations of airborne microorganisms was similarly broad, being in the range of $9.5 \times 10^2 - 8.0 \times 10^6$ cfu/m^3 [52]. The Ukrainian MAC value was there exceeded on 5 out of 15 examined farms by 1.5 to 160 times. In the big herb processing facilities "A" and "B", the concentrations of microorganisms in the air ranged from $4.1 \times 10^4 - 6.3 \times 10^5$ cfu/m^3, exceeding the Ukrainian MAC value on 12 out of 14 sampling sites by 1.6 to 12.5 times. The respirable fraction of microbial aerosol formed 14.7–67.7% of the total count. Microbial pollution of the air was, on average, greater during initial stages of the production cycle (cleaning, cutting, grinding) than in final stages (sorting, sieving, packing) ($p<0.001$) [13, 22]. In the modern herb processing facility "P", the concentrations of microorganisms in the air ranged from $9.7 \times 10^3 - 7.7 \times 10^4$ cfu/m^3, exceeding the Ukrainian MAC value at 1 out of 4 sampling sites by 1.5 times [30].

Species composition of airborne microflora. Gram-positive bacteria distinctly predominated in the air of cow barns and piggeries, forming, respectively, 87.6–97.0% and 92.5–98.5% of the total microbial isolates. Gram-negative bacteria formed, respectively, 0.5–6.6% and 1.4–5.1% of the total microbial isolates, while fungi formed, respectively, 0.4–5.8% and 0.1–5.2% of the total microbial isolates. Among Gram-positive bacteria isolated from the air of animal houses, the most numerous were corynebacteria (*Arthrobacter* spp., *Corynebacterium* spp.). The second most numerous were coagulase-negative staphylococci (*Staphylococcus epidermidis*, *S. saprophyticus*). Thermophilic actinomycetes usually did not exceed 1% of the total microflora except for one calf barn, where they accounted for 68.3% of total (1.73×10^5 cfu/m^3) with *Thermoactinomyces thalpophilus* being a prevailing species. The other thermophilic actinomycetes isolated from the air of animal houses were: *Saccharopolyspora rectivirgula*, *Thermoactinomyces vulgaris*, *Saccharomonospora viridis*, and *Thermomonospora fusca*. Among Gram-negative bacteria, *Acinetobacter calcoaceticus*, *Pseudomonas* spp. and *Pantoea agglomerans* prevailed in the air of cow barns, while *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *P. agglomerans*, *Enterobacter cloacae* and *A. calcoaceticus* prevailed in the air of piggeries. Among fungi, *Alternaria alternata*, *Aspergillus fumigatus*, *A. restrictus*, *Penicillium* spp. and yeasts prevailed in the air of cow barns, while yeasts, *A. fumigatus*, and *Penicillium* spp. prevailed in the air of piggeries [17, 19, 20, 40].

Gram-positive bacteria prevailed also in the air of animal feed facility, forming 56.6–97.0% of the total isolates. Gram-negative bacteria formed 2.2–39.0% of the total count, and fungi formed 0.4–25.5% of the total count. Among Gram-positive bacteria, the most numerous were coagulase-negative strains of *Staphylococcus* (*S. epidermidis*, *S. saprophyticus*) which formed, on average, nearly half of microorganisms isolated from the air of animal feed facility. The next in number were corynebacteria (*Arthrobacter* spp., *Corynebacterium* spp.), followed by spore-forming bacilli of the genus *Bacillus* and mesophilic actinomycetes (mostly *Streptomyces* spp.). Among the Gram-negative bacteria, the most common was the species *Pantoea agglomerans*, followed by *Acinetobacter calcoaceticus*. Among fungi, the most common were strains of *Rhizopus* spp., followed by yeasts, *Penicillium* spp. and *Aspergillus* spp. [10, 56].

In the air of the farms cultivating thyme, Gram-positive bacteria formed 74.8–94.5%, Gram-negative bacteria formed 0–2.6%, and fungi formed 4.0–25.0%. The *Bacillus* spp. strains predominated in the total microflora. Among fungi, prevailed *Alternaria alternata* and *Aspergillus candidus* [34].

In the air of the farms cultivating peppermint and chamomile, Gram-positive bacteria formed 0.06–72.4%, Gram-negative bacteria formed 0–68.1%, and fungi formed 0.13–99.9%. The most common species in total microflora was the Gram-negative bacterium *Pantoea agglomerans*. Among fungi, prevailed *Alternaria alternata* [54].

In the air of the farms cultivating valerian, Gram-positive bacteria formed 0.3–87.4%, Gram-negative bacteria formed 0–56.9%, and fungi formed 0.02–99.6%. The most common were Gram-negative bacterial species *Pseudomonas* spp., *Stenotrophomonas* spp. and *Pantoea agglomerans*. Among fungi, prevailed *Penicillium* spp. and *Aspergillus* spp. [52].

In the air of the big herb processing facilities "A" and "B", there distinctly prevailed Gram-positive bacteria which formed 65.3–85.6% of the total microbial isolates. Gram-negative bacteria formed 1.8–17.9% of the total count and fungi formed 5.9–30.5% of the total count. Among the Gram-positive bacteria the most common were spore-forming strains of *Bacillus* spp., followed by the mesophilic actinomycete *Streptomyces albus* and corynebacteria. Among Gram-negative bacteria, the most common were strains of *Alcaligenes faecalis*, forming 78% of their total count [12, 22]. Gram-positive bacteria prevailed also in the air of the big herb processing facility "P", forming 68.4–92.8% of the total microbial isolates. Gram-negative bacteria formed there 1.0–24.7% of the total count and fungi formed 2.5–15.2% of the total count. Among Gram-positive bacteria the most common were staphylococci and corynebacteria, while among Gram-negative bacteria the commonest species was *Pantoea agglomerans* [30].

Endotoxin. The concentration of bacterial endotoxin in the air of cow barns was in the range of 0.00125–0.0125 μg/m^3, and nowhere exceeded the value of 0.2 μg/m^3 (200 ng/m^3) proposed as a threshold level [30, 47]. By contrast, the concentration of endotoxin in the air of piggeries was in the range of 1.88–75.0 μg/m^3, and everywhere exceeded the threshold value by 9.4–375 times [17, 19, 20, 40], as well as the values of 1–2 μg/m^3 which are supposed to evoke ODTS symptoms [48].

The concentration of airborne endotoxin on herb farms cultivating thyme ranged from 37.4–2,448.8 μg/m^3, and on all 6 farms exceeded the threshold value by 187–12,244 times [34]. The concentration of endotoxin in the air of herb farms cultivating peppermint and chamomile ranged from 0.06–625.0 μg/m^3, and on 8 out of 10 farms exceeded the threshold value by 3.1–3,125.0 times [54]. The concentration of airborne endotoxin on herb farms cultivating valerian ranged from 0.0045–981.0 μg/m^3, and on 13 out of 15 farms exceeded the threshold value by 17.3–4,905 times [52]. In big herb processing facilities "A" and "B", the concentration of airborne endotoxin was within the range 0.2–2,681.0 μg/m^3, and at 12 out of 14 sampling sites exceeded the threshold value by 4.0–13,405 times [22]. In the big herb processing facility "P" the concentration of endotoxin in the air was within a narrow range 41.7–62.5 μg/m^3, and everywhere exceeded the threshold value by 208.5–312.5 times [30]. In most cases, the concentrations of endotoxins measured at handling herbs exceeded the values of 1–2 μg/m^3, which are supposed to evoke ODTS symptoms [48].

DISCUSSION

The presented results show that in the various branches of the agricultural industry in the Ukraine and Poland the levels of airborne dust, microorganisms and endotoxin, in the majority of cases, exceed the safe levels and may be a potential cause of work-related respiratory diseases.

The large exposure of piggery workers to airborne bacteria in Poland resulted in a high incidence (58.5%) of work-related symptoms and a high immunologic response to microbial allergens present in the work environment [40]. Similarly, the harmful effects of exposure to bioaerosols from herbs in Poland were confirmed by the epidemiological examinations of herb farmers and herb processing workers. As many as 30.7–76.5% of people exposed to herb dust reported work-related respiratory and general symptoms [23, 25, 26, 53, 55]. They also showed a significant post-shift drop of spirometric values [28], and a high immunologic reactivity to antigens of *Pantoea agglomerans* and other microorganisms occurring in the work environment [23, 26, 27, 53, 55].

Although bioaerosols remain a major occupational hazard in the agricultural settings of Central and Eastern Europe, there are some indications that the technological improvements introduced after economic transformation since 1990, steadily decrease the exposure. Thus, the maximal concentrations of airborne dust, microorganisms and endotoxin in the recently examined herb processing facility were, respectively, 14, 8, and 43 times lower than in corresponding facilities before 20–25 years [13, 22, 29]. A similar drop, by 2.6–50.2 times, was noted at the comparison of the recently obtained values of the concentration of microorganisms in the grain elevators and mills with those from 1978 [10, 29].

The airborne microflora of agricultural settings consists of Gram-positive bacteria, Gram-negative bacteria and fungi. On average, Gram-positive bacteria, composed of corynebacteria, cocci, bacilli and actinomycetes, form a prevailing part of the microflora, both in the Ukraine and in Poland. These bacteria produce peptidoglycan, a cell wall component having proinflammatory properties [29], and some species (such as thermophilic actinomycetes, *Arthrobacter* spp.) could be a cause of allergic alveolitis [42]. Gram-negative bacteria were less numerous but were still present in the air.

Among Gram-negative bacteria, the most harmful is an epiphytic species *Pantoea agglomerans* which has been isolated from most places examined in the present study. This bacterium occurs commonly on plants and is released into the air in large quantities during processing of grain and other plant materials [9]. This species may be a cause of allergic alveolitis [11, 42, 41] and produces a biologically potent endotoxin [9] which is a high molecular weight lipopolysaccharide (LPS), resistant to high temperatures [39]. Endotoxin is produced in the outer membrane of Gram-negative bacteria as a heteropolymer of LPS with proteins and phospholipids, and is released in large quantities into dust in the form of discoid particles measuring 30–50 nm in diameter [16, 18]. When inhaled by workers, endotoxin activates alveolar macrophages and induces inflammatory reactions in the lung.

The concentration of bacterial endotoxin in the air polluted with herb dust was in the range 10^{-3}–10^{3} μg/m^3, and was comparable to concentrations found in organic dusts from grain, cotton, grasses and other plant materials [21, 47]. In animal farms, the endotoxin concentration was within range of 10^{-3}–10^{1} μg/m^3 [20, 40], and in animal feed facilities it was in the range 10^{-3}–10^{2} μg/m^3 [2, 35]. In most cases the threshold limit value of 2×10^{-1} μg/m^3 was exceeded.

Fungi occurred in the air of agricultural settings both in the Ukraine and in Poland, but their concentration and proportion in the total microflora were distinctly greater in the Ukraine, which creates a potential hazard for the exposed workers. It has been documented by numerous studies that fungi classified previously as "non-pathogenic saprophytes", in fact, may cause allergic diseases of the lungs, allergic rhinitis, allergic conjunctivitis, allergic dermatitis and mycoses [31, 36, 61]. The concentrations of fungal spores and hyphal fragments in the air of various agricultural settings may attain large concentrations; for example, in animal feed facilities the concentrations were 10^{6}–10^{9} cfu/m^3 of air.

In the cell wall of fungi occur β-glucans that show a strong biological activity. β-glucans are glucose polymers linked by β-glycosidic bonds. They may stimulate the reticuloendothelial system and evoke various biological effects, mainly activation of macrophages. It is assumed that (1→3)-β-D-glucans which are present in organic dusts may cause chronic respiratory diseases in exposed workers [39, 50].

Microscopic fungi may produce mycotoxins, the low-molecular secondary metabolites that cause severe diseases in humans and animals. To the important producers of mycotoxins belong the genera *Aspergillus*, *Penicillium*. *Fusarium*, *Cladosporium*, *Alternaria*, *Candida*, *Helminthosporium*. The list of mycotoxin-producing fungi is steadily increasing [7].

The fungal species identified in the air of animal feed facilities in the Ukraine may produce such mycotoxins as: aflatoxins, ochratoxins, sterigmatocystin, trichothecens, and a large complex of metabolites produced by *Alternaria* spp. Among these mycotoxins there are carcinogens, mutagens, substances inhibiting protein synthesis and substances exerting harmful effects on the neural system. It is assumed in the literature that mycotoxins may be a potential cause of malignant neoplasms in exposed workers [51, 72].

In conclusion, the results of microbiological investigations performed in various kinds of agricultural settings in the Ukraine and Poland clearly indicate that the airborne biological factors play in these settings a primary role as occupational risk factors, exerting harmful effects on the state of the health of exposed workers. Even though all the results are not fully comparable because of the differences in methodology of sampling, there is no doubt about the final conclusion on the work-related hazard presented by bioaerosol. Formation of the harmful bioaerosol depends on the specificity of the setting, kind of technological operations, degree of mechanization, and properties of processed materials

Formation of bioaerosol depends also on the temperature and humidity and is highly proportional to the concentration of dust in the air. The concentration and species composition of bioaerosol is related to the microflora of the processed materials, or to microflora released by bred animals. The presence in the work environment of large concentrations and wide spectrum of microorganisms showing infectious, allergenic, toxic and carcinogenic effects may be a cause of work-related diseases in the exposed agricultural workers [26, 40, 55, 70].

Thus, technological progress in cleaning the indoor air and monitoring of biological factors in agricultural settings are necessary steps for creating hygienic recommendations aiming to improve the working conditions and to protect the workers from perilous health hazards. In this monitoring it could be helpful to establish in the countries of the European Union the maximal acceptable concentration (MAC) values for airborne microorganisms and endotoxin, such as that established in the Ukraine for the concentration of microorganisms in the air (5.0×10^4 cfu/m^3) [59] which enables the comparison of aerobiological results to the level recognized as safe [4]. Even if such a value was to be higher than in the Ukraine, and at first introduced only as a reference (non obligatory) value, it would be certainly better than none.

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