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Original Research

Microbiological Air Contamination in Farming Environment

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Abstract

The aim of this study was to evaluate microbiological contamination levels of indoor air in some farm settings, including barns and dairy objects. The level of microorganism emissions from farm buildings into atmospheric air was also estimated. In the research a MAS-100 air sampler (MERCK) was used.

It was revealed that the number of microorganisms in barns ranged between $1.7 \cdot 10^3$ - $8.8 \cdot 10^4$ for mesophilic bacteria, $3.5 \cdot 10^1$ - $8.3 \cdot 10^2$ for hemolytic bacteria, $1.5 \cdot 10^3$ -$4.6 \cdot 10^4$ for staphylococci, $5 \cdot 10^0$ -$2 \cdot 10^2$ for coli-group bacteria and $1.7 \cdot 10^2$ -$2.4 \cdot 10^4$ for moulds (mainly from the genera *Penicillium*, *Aspergillus*, *Alternaria*, *Cladosporium*, *Mucor* and *Rhizopus*). There were no significant differences concerning microbiological air contamination between buildings of old and modern types.

In one of the dairy stores, high numbers of mesophilic bacteria and staphylococci occurred. Farming objects were confirmed to be strong emitters of bioaerosols.

Keywords: farm settings, indoor air, microbiological air contamination, airborne microorganisms, emission of bioaerosols

Introduction

One of the most important contaminants in the farming environment are bioaerosols. Animals and farm workers are exposed to high concentrations of bacteria and fungi as well as endotoxins and mycotoxins produced by them [1-4]. Airborne microorganisms may cause various negative effects, especially infectious, allergenic and immunotoxic diseases. Fungal conidia present in the air contain extremely high amounts of mycotoxins. According to Buchmiet and Żakowska [5], inhalation of mycotoxins may be more dangerous than their consumption with contaminated food. Animal feed is often contaminated by fungi, mainly of genera *Aspergillus*, *Penicillium* and *Fusarium*. Their conidia emitted into air may be a reason of occurrence of such animal illnesses like animal asthma, horse chronic pneumonia, bird aspergillosis, cattle miscarriage and others [6].

The concentration and kind of microorganisms in the indoor air depends on technical factors (i.e. type and age of a building), number of inhabitants (people or animals), the type of heating and ventilation systems, and microclimatic conditions: temperature, humidity, concentration of gases, lighting or dust concentration [7-9]. Improper working methods and hygenic conditions may be causes of considerable microbial air pollution [1].

To estimate the health risk of microbiological air contamination it's necessary to evaluate the number of bacteria and fungi, including potentially pathogenic strains. For this paper the number of mesophilic, hemolytic and coli-group bacteria, mannitol+ staphylococci and moulds in farming environment were determined.

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Materials and Methods

The research work covered the evaluation of microbiological air contamination in some barns (cow sheds, pigsties, poultry houses) and dairy buildings. The emission levels of bioaerosols from chosen farming objects into atmospheric air was also estimated. Air sampling was carried out in rural areas in Podlasie in January and February.

Two kinds of barns were taken into account:

— objects of "modern" type — exploited for less then 10 years, with mechanical ventilation, improved feeding systems, without or with thin-layer bedding (cow sheds I and II, pigsty IV)

— "conventional" objects — older ones, without ventilation systems (only natural ventilation), with traditional bedding and feeding methods (cow shed III, pigsty V, poultry houses VI and VII).

Tested dairy buildings were local milk collection stores.

Levels of bioaerosol emission were determined in the vicinity of cow sheds and a large pigsty.

In this study the determination of the total number of mesophilic bacteria, mannitol+ staphylococci, coli-group bacteria, hemolytic bacteria and moulds was accomplished. Mannitol+ (mannitol using) staphylococci were chosen as indicator, because the ability of mannitol utilization is characteristic for coagulase -positive, potentially pathogenic staphylococci.

Air samples were taken using a MAS-100 air sampler (Merck), based on the principle of the Andersen air sampler (corresponding to its 5^{th} stage, which guarantees that all particles > 1μm were collected). Air volumes were 50-250 liters (depending on expected contamination level) and the sampling rate was 100 l/min.

Two parallel samples for each group of bacteria were taken at the central point of each building, 1.2m from ground level in the case of indoor air. The emission level from farming objects was determined similarly, with sampling points situated 15 m from the emitter and the background level at the point placed 100 m from the farming buildings, taking into account the direction of the wind. The total number of samples was 10 at each sampling point (i. e. 120 air samples total).

Bacteria and fungi were collected and grown on standard culture media [10]: MPA nutrient agar for mesophilic bacteria, Chapman agar for staphylococci, Endo agar for coli-group bacteria, blood agar for hemolytic bacteria. Fungi were grown on Martin agar (Rose-Bengal Chloramphenicol Agar), containing (g/l) 5.0 mycological peptone, 10.0 glucose, 1.0 dipotassium hydrogen phosphate, 0.5 magnesium sulfate, 0.05 rose-bengal, 0.1 chloramphenicol, and 15.5 agar-agar.

Colonies were counted after 48h of incubation at 37°C for bacteria and after 6 days in 26°C for moulds. The average number of bacteria and fungi was calculated as colony-forming units in 1 m^3 (CFU/m^3). Total microbial count was corrected, using the conversion formula devised by Feller [11]:

$$Pr= N\ [1/N + 1/N\text{-}1 + 1/N\text{-}2+....+1/N\text{-}r+1]$$

where:

N= 400 (number of holes in perforated lid of the sampler)

r — number of CFU counted on Petri dish

Pr — statistically corrected total count of bacteria in tested air volume

For two types of farm buildings (of old and modern type), the statistical analysis using χ^2 test was accomplished, in order to check the statistical significance of the differences in microbiological contamination levels.

Based on morphological criteria and literature data [12-14], the identification of predominating fungal strains was accomplished.

Simultaneously with microbiological analyses, temperature and relative humidity of the air were determined.

Results

The research work was carried out in January and February. Temperature of atmospheric air ranged between -2°C and +1°C; temperatures inside barns and dairy objects was 10-12°C and 7-13°C, respectively. Relative indoor air humidity was about 80-90%, and of atmospheric air — 37%. The results of microbiological analyses in barns are presented in Fig. 1.

It has been stated, that the number of microorganisms (as CFU/m^3) in barns ranged between $1.7\cdot10^3$ -$8.8\cdot10^4$ for mesophilic bacteria, $3.5\cdot10^1$ -$8.3\cdot10^2$ for hemolytic bacteria, $1.5\cdot10^3$ -$4.6\cdot10^4$ for staphylococci, $5\cdot10^0$ -$2\cdot10^2$ for coli-group bacteria and $1.7\cdot10^2$ -$2.4\cdot10^4$ for moulds.

The most significant microbiological contamination has been detected at sampling point number IV (a modern pigsty). High amounts of mannitol+ staphylococci occurred in pigsties (IV, V), cow shed (II) and poultry house (VII). In some cases (II, V, VII) the number of staphylococci was higher than the number of mesophilic bacteria on MPA agar. Two cow-sheds (I, II), one pigsty (IV) and one poultry house (VII) were strongly contaminated with moulds. In animal houses, predominating strains of moulds were:

— cow sheds — *Penicillium sp*., *Penicillium piceum*, *Alternaria sp*., *Monilia sp*., *Rhizopus sp.*, moulds from genus *Aspergillus* (*A. niger*, *A. flavipes*, *A. nidulans.*, *A. versicolor*)

— pigsties — *Penicillium sp.*, *Penicillium brevicompactum*, *Aspergillus niger*, *Aspergillus sp.*, *Mucor sp.*, *Rhizopus sp.*, *Alternaria sp.*, *Cladosporium sp*.

— poultry houses — moulds from genus *Aspergillus* (*A. niger*, *A. nidulans*, *A. ochraceus*), *Penicillium notatum*, *Penicillium sp.*, *Cladosporium sp.*, *Alternaria sp*)

The average numbers of microorganisms in old and

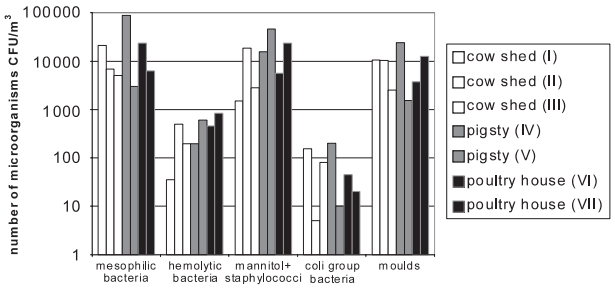

Fig 1. Number of microorganisms in various barns.

modern buildings was compared. Results are presented in Table 1.

The numbers of bacteria and moulds revealed a large spread of results. It should be noticed that in the case of microorganisms the order of magnitude of microbial number may be the more proper factor than the number of bacteria or fungi itself. Because of this, statistical analysis with χ^2 test was accomplished based on values calculated for each group of bacteria as log x_n, where x_n was average number of microorganisms. Statistical calculation was accomplished in parallel for all microbial groups and revealed that there was no statistically significant difference in microbiological contamination levels between old and modern buldings (χ^2 = 0.3, comparing with tabular χ^2 value of 9.488, with 4 degrees of freedom and $p<0.05$).

Table 1. Average number of microorganisms in old and modern farming objects.

Microorganisms	Average number of microorganisms (CFU /m^3)	
	Old-type buildings	Modern–type buildings
Mesophilic bacteria	(9.5±5.7)x10^3	(3.9±2.6)x10^4
Mannitol+ staphylococci	(1.9±1.2)x10^4	(1.2±0.5)x10^4
Hemolytic bacteria	(5.2±1.6)x10^2	(2.4±1.4)x10^2
Coli-group bacteria	(3.9±1.9)x10^1	(1.2±0.6)x10^2
Moulds	(5.1±2.9)x10^3	(1.5±0.5)x10^4

Number of microorganisms (as CFU/m^3) at two dairy buildings (milk collection points) was, respectively: 160 and 1662 for mesophilic bacteria, 15 and 344 for staphylococci, 31 and 17 for hemolytic bacteria, and 167 and 311 for moulds. Coli-group bacteria were not detected. The contamination level was high in one of the dairy objects, especially in the case of mannitol+ staphylococci and mesophilic bacteria.

The bioaerosol emission level was estimated near farm buildings — cow sheds and pigsties. Results of microbial number determination are presented in Fig. 2.

We found that farming objects are emitters of considerable amounts of microbiological contaminants into atmospheric air. Main components of bioaerosol at both locations were moulds (belonging to genera *Alternaria*, *Botrytis*, *Penicillium*, *Thamnidium*, *Aspergillus*, *Rhizopus* and *Cladosporium*) and — near cow sheds — mesophilic bacteria. The number of bacteria in atmospheric air near farm buildings was much higher than the background level as well as the number and variability of moulds (only two genera of fungi — *Cladosporium* and *Penicillium* — were detected in the case of the background sample).

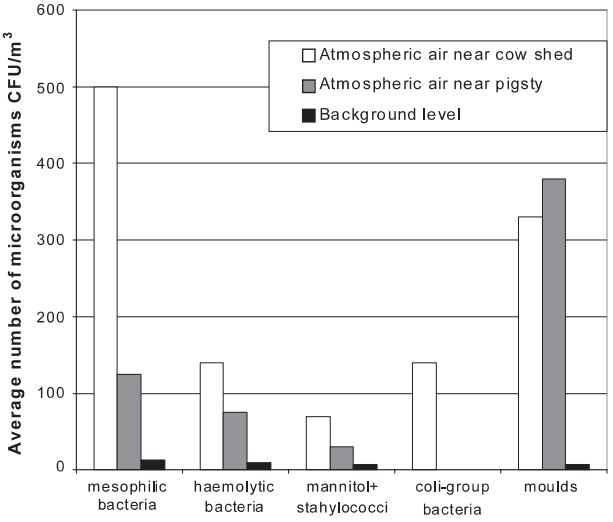

Fig. 2. Emission of microorganisms from farm settings into atmospheric air.

Discussion

In Poland there are no standard regulations concerning the permitted number of bacteria and fungi in indoor air, including farming accommodations [15]. In case of farming objects, Krzysztofik's proposals of permitted numbers of bacteria and fungi (CFU/m^3) are, respectively: less than $1.5\cdot10^5$ and $5\cdot10^3$ in cow sheds, $2\cdot10^5$ and 10^4 in pigsties, and 10^5 and $2\cdot10^3$ in poultry houses [10]. According to guidelines of the Institute of Rural Medicine [16] the total number of microorganisms (with more than 50% of microorganisms causing allergic threat) should not exceed $5\cdot10^4$ CFU/m^3.

Air quality in farming objects has been described by Gustaffson [17], Lange et al.[8], Barabasz and Jaśkowska [1], Andersson [15], Kluczek [7] and others. According to the results of various research works, the number of bacteria in animal houses ranged from $10^3\cdot$ -10^9 CFU/m^3 and number of fungi — from $2.5\cdot10^1$ to $4.9\cdot10^6$ CFU/m^3. This research work proved the considerably high level of air contamination in farming environments. High concentrations of bioaerosols are noticed not only in animal houses, but in other objects in rural areas (i.e. dairy stores) and in surrounding atmospheric air. Comparing these results with guidelines of the Institute of Rural Medicine [16], the number of microorganisms

doesn't comply with proposed standards. According to Krzysztofik's standards proposal [10], the number of bacteria revealed in this study was not high, but number of fungi often was exceeded. The presence of high amounts of potentially pathogenic staphylococci should be emphasized as a negative phenomenon. It must also be noticed that the actual total number of bacteria may be up to 5 times higher than that of culturable microorganisms, evaluated as CFU/m^3 [18]. Moreover, according to literature data [19], the microorganisms number in the air varies and differs for individual objects. Also in the case of this research work, differences in contamination levels in various objects were observed (Fig 1.) They might also be due to relative short sampling time and location of sampling point. However, it should be stressed that the sampling time was chosen according to expected contamination levels and equipment demands.

As a cause of high air contamination levels, Lange [8] indicated an improper functioning of ventilation systems, storage moisture of feed rations, kinds of work practice and climatic conditions. Gustaffson [17] said that the function and location of air inlets and outlets may have a strong influence on the spreading and concentration of pollutants in the indoor air. Accordingly, there should be differences in contamination levels between old and new buildings, to the advantage of modern ones. However, statistical analysis of the results has revealed that there was no statistically significant diffrence between these two types of buildings.

In farming accomodations many fungal strains were isolated and identified. Moulds from genera *Alternaria*, *Cladosporium* and *Penicillium* were isolated by Krysińska-Traczyk [16] from farming objects. Barabasz and Jaśkowska [1] described the occurrence of the moulds *Alternaria sp.*, *Aspergillus sp.*, *Cladosporium sp.*, *Mucor sp.*, *Penicilliu sp.*, *Rhizopus sp.* and *Trichothecium sp.* in barn air. The presence of such fungi in agricultural settings was proved by the results of this study.

The number of microorganisms in objects connected with food production should be strongly limited. Because of that, the presence of significant amounts of mesophilic bacteria and potentially pathogenic staphylococci in the air of milk collection stores tested in this study, may be alarming.

Farm buildings were described as the emitters of microorganisms into the atmospheric air. Sawicka and Wieland [20] have revealed that the concentration of microorganisms in the vicinity of a pig farm was, respectively: $2.6 \cdot 10^3$ for bacteria, $1.4 \cdot 10^2$ for *Actinomycetes*, $3.2 \cdot 10^2$ for fungi. Contamination levels stated in this research work were comparable to literature data.

Comparison between microflora occurring in the atmospheric air taken far from farming objects (background sample) and bacteria and fungi in vicinity and inside animal houses revealed that the source of microorganisms are probably farm objects. In background air only two genera of moulds — Cladosporium and Penicillium were identified, compared with multiple strains of moulds from various genera occurring both in animal houses and in their vicinity.

It should be noticed that in case of this study measurements were accomplished in winter, so in warmer seasons the number of microorganisms detected in the air may be even higher.

Results of this research work prove that indoor air standards should also be created for farm work settings and animal accommodations in order to assure proper hygienic and epidemiological condition of farming environments and to prevent the emission of bioaerosols into atmospheric air.

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