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Comparison of Indoor and Outdoor Bioaerosols in Poultry Farming

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1. Introduction

Intensive poultry production, implying large densities of animals in small areas, is a significant source of air pollution which may constitute a considerable health hazard to the birds, farmers and those living in the proximity of the farm (Lonc & Plewa, 2009). On the other hand, the spread of bioaerosols on the outside of animal housing may result in local or even more extensive environmental pollution (Bakutis et al., 2004).

Under commercial production the airborne particles will contain a mixture of biological material from a range of sources. The chickens produce large amounts of dust as a result of epithelial desquamation, as well as from feed, manure, faeces and litter (Matković et al., 2009). This dust consists of a variety of airborne particles of biological origin, i.e. bacteria, fungi, endotoxins (lipopolysaccharide, LPS) of Gram-negative bacteria, 1.3-beta-glucan of fungi, fungal spores and mycelium fragments. Hence, a more descriptive term for these airborne particles is bioaerosol in which the microorganisms can occur either as liquid droplets or as dry particles [Dutkiewicz, 1987; Matković et al., 2009**;** Nevalainen, 2007]. In specific conditions, bioaerosols may show pathogenic, toxic or allergy-causing effects. The particles in a bioaerosol are generally 0.3 to 100 μm in diameter; however, the respirable size fraction of 1 to 10 μm is of primary concern. Bioaerosols, ranging in size from 1.0 to 5.0 μm, generally remain in the air, whereas larger particles are deposited on surfaces (Srikanth et al., 2008).

Bioaerosol may contain representatives of Gram-positive bacteria: *Corynebacterium, Staphyloccocus, Streptococcus, Micrococcus, Pantoea* and *Sarcina* (Siemiński, 2001). Their presence in large numbers may present a significant immunological challenge to the human respiratory system. In dust are suspended also endotoxins (lipopolysaccharide complex - LPS) associated with the outer membrane of Gram-negative pathogens, such as *Escherichia coli, Salmonella, Shigella, Pseudomonas, Neisseria* and *Haemophilus influenzae.* LPS is composed of two major parts, the hydrophobic lipid A and the hydrophilic polysaccharide part (commonly called the "O" region). Most biological effects of LPS are due to the lipid A part, however O-region plays an important role in effective colonisation of host tissues. Inhalation of organic dust contaminated by endotoxins may cause chronic bronchitis and inflammatory reaction in the lungs (Bakutis et al., 2004, Schierl et al., 2007, Pomorska et al., 2009).

As with bacteria, the fungi present in the poultry dust bioaerosols may be derived from soil, dust feed and litter, but to a lesser extent from the birds themselves. Fungi are ubiquitous in all atmospheres. In general, both outdoor and indoor atmospheres are dominated by representatives of the genera *Cladosporium*, *Penicillium*, *Aspergillus, Alternaria* and by yeasts and Mycelia sterilia.*Cladosporium* is nearly always the dominant fungus in outdoor as well as indoor atmospheres. The abundance of the other fungi varies with the season and place. In relation to outdoor environments, indoor atmospheres typically display lower diversity (Araujo & Cabral, 2010). Long-term or repeated exposure to high concentrations of airborne fungal spores is recognised as contributing to the decline in lung function and allergic diseases such as asthma and allergic alveolitis known as farmer's lung disease (Crook et al., 2008).
Literature data usually pertain to the air biopollutant concentration inside the poultry houses. Much less is known about the relationships between the indoor and outdoor biological pollution, as well as about the spreading of indoor bioaerosols in the surroundings of the farms.
The aim of the study was to assess the influence of microbiological air contamination in the intensive poultry breeding, both inside and outside farms. The comparative quantity and quality analysis concerns bacteria as well as fungi isolated from the air samples taken during two seasons.

2. Materials and methods

Seasonal sampling was conducted in the summer of 2009 and spring of 2010 in two (I and II) poultry houses on family farms located near Wrocław in Lower Silesia, Poland (Fig. 1.)
Both farms were accommodated to 18 000 and 23 000 broilers, with the density of 16-17 chicken per square meter. The broilers were kept on the rye straw deep litter in buildings equipped with mechanical ventilation (inlet and outlet ventilators), heating with a central thermogen and artificial lighting with regularly distributed bulbs.
Air samples were taken using a MAS-100 air sampler (Merck KgaA, Darmstadt, Germany) which is representative of the new generation impactor samplers and is frequently used for indoor and outdoor sampling. These instruments are based on the principles described by Andersen and aspirate air through a perforated plate, which results in impaction of particles from an airstream onto the surface of agar medium. The speed of air flow through the sampler was about 11 m/s, air volumes were 5-200 litres (depending on the expected contamination level) and the sampling rate was 100 l/min. Indoor and outdoor samples were collected in the poultry biozone during the fattening period. The biopollutants were determined on the basis of airborne bacteria and fungi. The samples for each group of bacteria and fungi were taken at the central point of poultry houses 1.3 m from the ground level. The emission level outside the farming objects was determined similarly, i.e. 1.3 m with sampling points situated 10 m, 50 m and 100 m away from the farming buildings. At the same time both humidity and temperature were measured with a termohigrometer (Label).
Microbiological studies of the air samples were used to determine the number of mesophilic bacteria, *Enterobacteriaceae* representatives, mannitol+ staphylococci, *Salmonella sp.* and mould fungi. The mesophilic bacteria were isolated with the use of TSA agar (BioMérieux).
Enterobacteriaceae families were determined with the use of VRB medium (BioMérieux). The estimation of *Salmonella sp*. was done on SS agar (BioMérieux). Mannitol salt agar

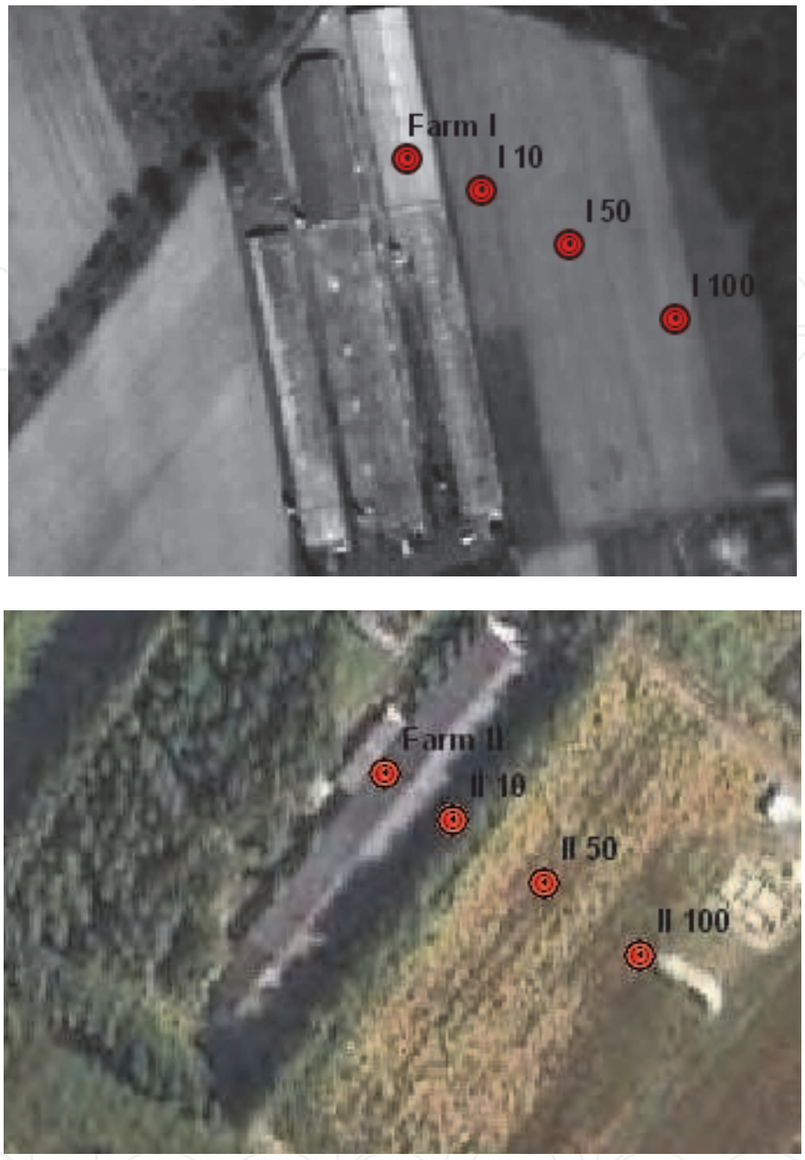

Fig. 1. Poultry houses I and II; the sampling sites outside the poultry house at the distance of 10 m, 50 m and 100 m from the farming object respectively, as well as at the center of building.

(BioMérieux) plates were inoculated for culturing and counting *staphylococci*. Mould fungi were determined with the use of Sabouraud (Merck) medium. Colonies were counted after 48 h of incubation at 37^0C for bacteria and after 5 days at 26^0C for moulds and subsequently the colony-forming units (CFU) were determined. Quantitative results were expressed in CFU/m3, i.e. colony forming units in 1 m^3 of the examined air and the total microbial count was corrected using the conversion formula devised by Feller:

$$Pr = N [1/N + 1/N-1 + 1/N-2+....+1/N-r+1]$$

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/moulds in tested air volume
Bacterial species were identified on the basis of gram staining, microscopic morphology, oxidase and coagulase activity, catalase test results and metabolic properties according to standard procedures described in Bergey's Manual of Determinative Bacteriology (2001). The following commercial systems were used: API 20E (BioMérieux, France) for enteric gram-negative organisms; API 20 NE (BioMérieux) for fastidious and nonfermenting gram-negative organisms; API Staph (BioMérieux) for gram-positive staphylococci, API 20C AUX (BioMérieux) for identification of yeasts, Slidex - Strepto Kit (BioMérieux) for the identification of Lancefield A,B,C,D,F et G group antigens of streptococci and Slidex Staph Plus (BioMérieux) to detect clumping factor, protein A and group-specific antigen bound to the *S. aureus*. Moulds colonies were identified on the basis of colour, texture, topography of the culture surface, smell of the colony, colour of the reverse of the colony and the presence of the diffuse pigment. Microscopic features of the fungal colonies, i.e. the presence of macroconidia and microconidia, their shape and appearance were identified later. Fungal species of the genera *Aspergillus* and *Penicilium* were identified with the use of the keys by Raper and Fennell as well as Raper et. al., while the *Fusarium* species were identified using the key by Kwaśna (1991). Other species were identified based on the "Atlas of Clinical Fungi"(2010).

3. Results. Quantitative and qualitative relationships between indoor and outdoor microflora examined in summer and spring

The studies were carried out in the summer of 2009 and in the spring of 2010, when the temperature of atmospheric air ranged between 15.2^0C and + 24.5^0C; the inside temperature in the poultry houses varied from 22^0C to 27^0C. Indoor relative air humidity was about 70-85 %, outdoor ca. 38-82%.
For both poultry houses, the indoor concentration of bacteria and moulds were always higher compared with the outdoor concentration at distance 10 m, 50 m and 100 m from the poultry houses. The number of microorganisms (as CFU/m^3) in the atmospheric air of both poultry houses ranged between 4×10^1– 7.2×10^3 for mesophilic bacteria, 0– 1.3×10^4 for staphylococci, 0 - 7×10^1 for coli group bacteria, and 2×10^1 – 1.3×10^4 for fungi (Fig. 2-5). *Salmonella sp*. were not found. On the other hand, the number of microorganisms inside both poultry houses was higher than in the surrounding area and ranged between 1.3×10^5– 5.2×10^5 for mesophilic bacteria, 1.4×10^5 – 2.6×10^5 for staphylococci, 2.0×10^2- 1.5×10^4 for coli group bacteria and 3.6×10^4 – $1{,}1 \times 10^5$ for fungi. *Salmonella sp*. similar like outdoor were not found.
Outside poultry house I mesophilic bacteria were the most numerous organisms in the spring and summer and formed about 55% of the local microbial community. Less numerous staphylococci and moulds constituted about 17% and 27.5%, respectively. The concentration of *Enterobacteriaceae* was fractional (0.5%). In contrast, outside poultry house II the most numerous group of bacteria were staphylococci (62%). Mesophilic bacteria and moulds constituted about 25% and 13% of the microbial community. However, air inside both poultry houses was characterized by a relatively small number of *Enterobacteriaceae*. On the other hand, mesophilic bacteria were dominant and formed about 44% (poultry house I) and 56.5% (poultry house II) of the local microbial community, whereas staphylococci constituted 40% and 27% and moulds 16% and 7% for poultry house I and II. *Salmonella sp*. was not detected in neither poultry houses.

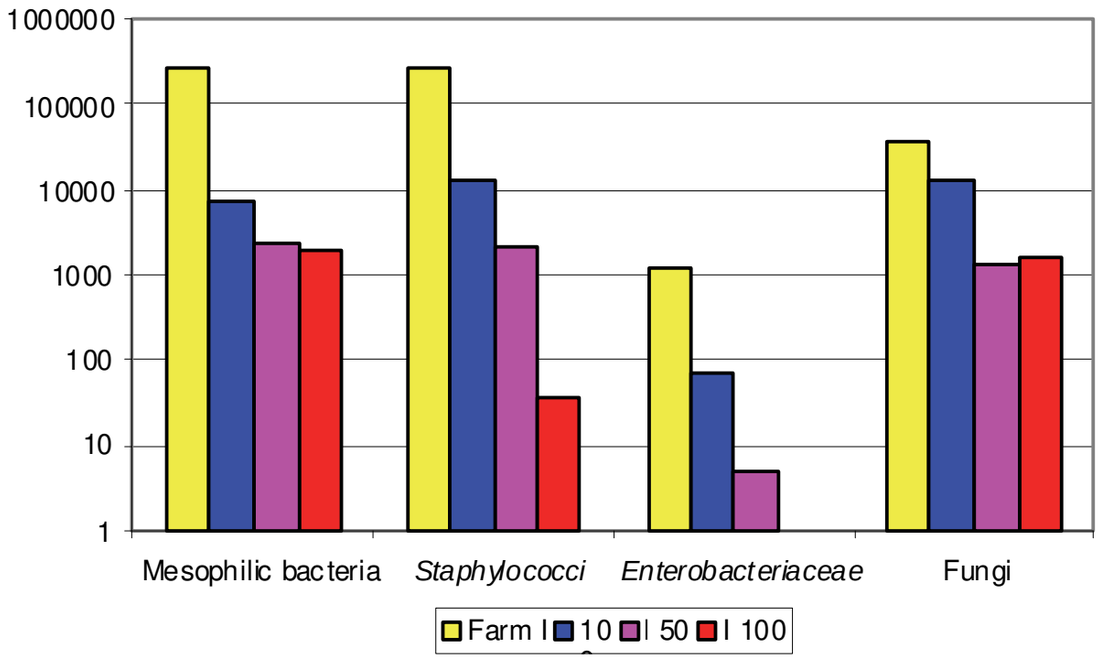

Fig. 2. Number of microorganisms in the poultry house I in summer.

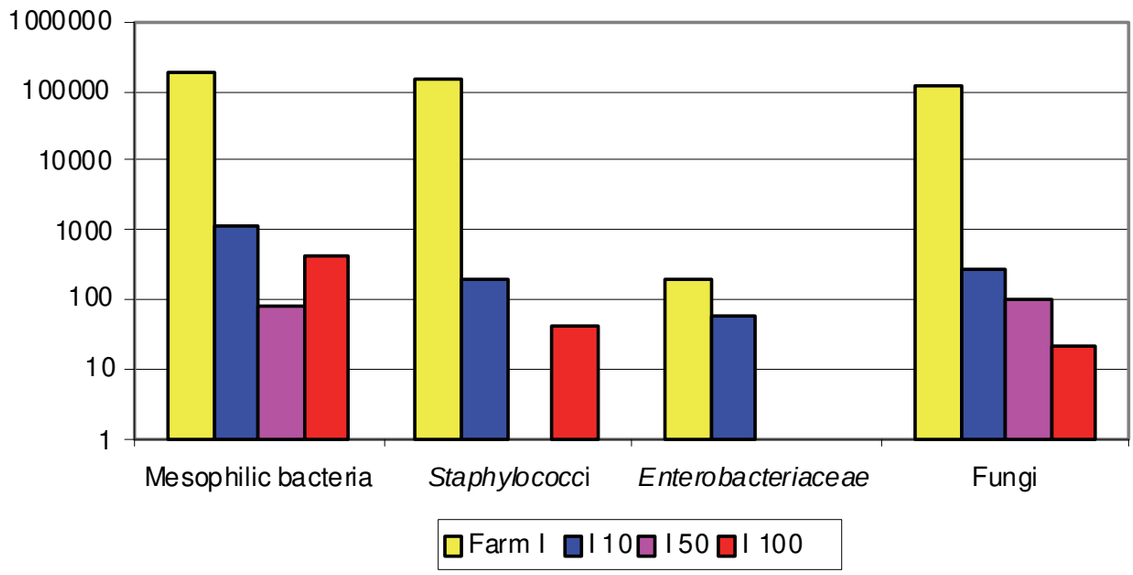

Fig. 3. Number of microorganisms in the poultry house I in spring.

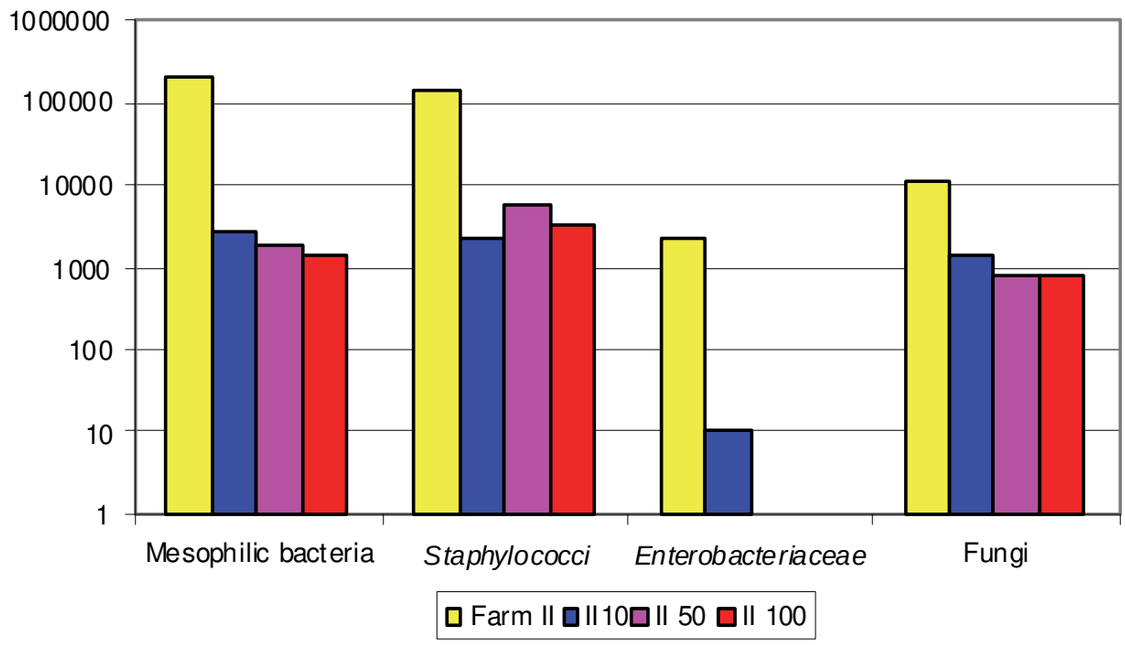

Fig. 4. Number of microorganisms in the poultry house II in summer.

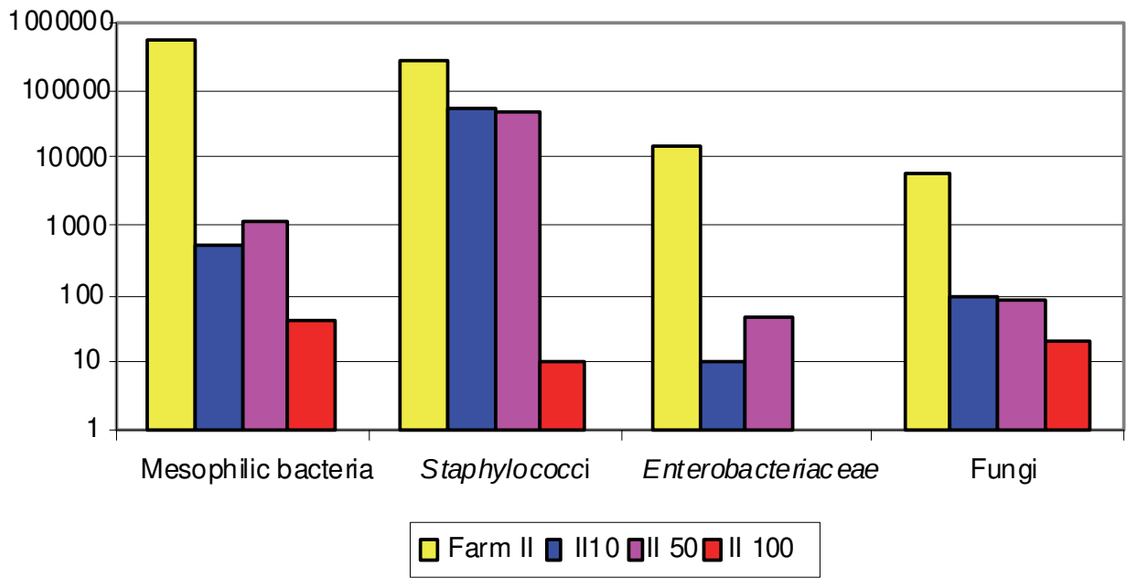

Fig. 5. Number of microorganisms in the poultry house II in spring.

The level of outdoor air contamination was evaluated in the accordance with the Polish Norm (Table 1). Contaminations by mesophilic bacteria and moulds in areas surrounding poultry house I in the summer was the highest in sampling point I 10. In relation to the Polish Norms this site was heavily polluted. On the other hand, the number of staphylococci at all sampling sites in both poultry houses (except sampling points I 50 and II 100 in the spring) indicated a high contamination. In contrast, the air in the surrounding areas could

be classified as medium-contaminated at all sites around the poultry houses II in the summer and in sampling point II 50 in the spring, with mesophilic bacteria. The evaluation based on the Polish Norm revealed that none of the researched measuring sites around the poultry house II was significantly contaminated by fungal microflora.

Polish Norm	Mesophilic bacteria	*Staphylococi*	Fungi
not pollution *	< $1x10^3$	0	$3x10^3$-$5x10^3$
medium pollution **	$1x10^3$- $3x10^3$	<25	$5x10^3$-$1x10^4$
heavily pollution ***	>$3x10^3$	>25	> $1x10^4$

Table 1. Polish Norm PN-89/Z-04111/02 and PN-89/Z-04111/03 (http://www.pkn.pl).

Fifteen species of bacteria detected in the indoor air represented 10 genera *Staphylococcus (S. aureus, S. xylosus, S. saprophitycus), Micrococcus (M. sedentarius, M. luteus), Enterococcus, Streptococcus (S. mitis), Corynebacterium (C. xerosis), Pseudomonas (P. aeruginosa, P. fluorescens), Citrobacter (C. farmerii), Escherichia (E. coli), Enterobacter (E. sakazakii, E. agglomerans)* and *Proteus (P. mirabilis)* – Table. 2. Quality outdoor set consisted of 19 species representing 10 bacteria genera: *Staphylococcus (S. sciuri, S. epidermidis, S. cohnii subsp., S. lentus,S. xylosus), Micrococcus (M. lylae, M. halobius, M. luteus), Streptococcus (S. mitis), Bacillus (B. mycoides, Bacillus sp.), Pseudomonas (P. aeruginosa, P. chlororaphis), Xantomonas (X. maltophila), Shigella (S. boydii), Providencia sp., Citrobacter (C. farmeri), Enterobacter (E. agglomerans)* and *Proteus (P. mirabilis)*. For the most part, the identified bacterial species were nonpathogenic or potentially pathogenic species, with the exception of *P. aeruginosa, S. aureus, Shigella sp., E. coli, P. mirabilis* which are regarded as primarily pathogenic.

In this work we detected 30 species (16 in poultry houses and 23 in surrounding areas) representing 16 fungal genera: *Aspergillus (A. flavus* – Fig. 6), *A. niger, A terreus, A. nidulans, A. parasiticus, A. glaucus, A. clavatus* - Fig. 7*), Penicillium* – Fig. 10 *(P. chrysogenum, P. solitum, P. sticticus), Cladosporium (C. cladosporoides* – Fig. 8*), Alternaria (A. alternata* – Fig. 9*, A. tennuissima), Scopulariopsis (S. brevicaulis, S. acremonium), Fusarium* – Fig. 11 *(F. oxysporum, F. graminearum), Mucor (M. mucedo) Drechslera (D. graminae, Drechslera sp.), Verticillium sp.,* Mycelia sterilia, *Ulocladium sp., Trichoderma (T. viridae), Scedosporium sp., Candida (C. albicans, C. inconspicua, C. lambica), Rhodotorula (R. rubrum), Cryptococcus (C. laurentii)* – Table 3. The majority of these species are known as the potential respiratory allergens. The most common airborne moulds, both indoors and outdoors, were *Penicilium sp., Aspergillus sp., Cladosporium sp., Alternaria sp.*, and *Fusarium sp*. The yeast were sometimes the dominant fungus in the indoor air comparison with the outdoor air, where this group of fungi occur very sporadically.

Among fungi identified from the farm I, there distinctly dominated the species belonged to genera *Candida spp*. which constituted on average, over 69% in summer and 82.5% in spring. In comparison with the predominated yeast inside poultry houses I, in outdoor air the most frequent species were moulds *A. flavus* (80% in sampling point I 50.36% in I 10 and 52.5% in I 100) and *F. oxysporum* (26.9%, 13.5%, 16.2% in sampling point I 10, I 50, I 100, respectively). On the other hand, in the air surroundings poultry house II dominated species were *A. clavatus* (about 16%) and *A. alternata* (38.4%) in spring and *C. cladosporoides* (in summer) which constituted 68.5%. Whereas, *A. clavatus* (54.5%) and *A. flavus* (27.3%) were dominated species inside poultry house II.

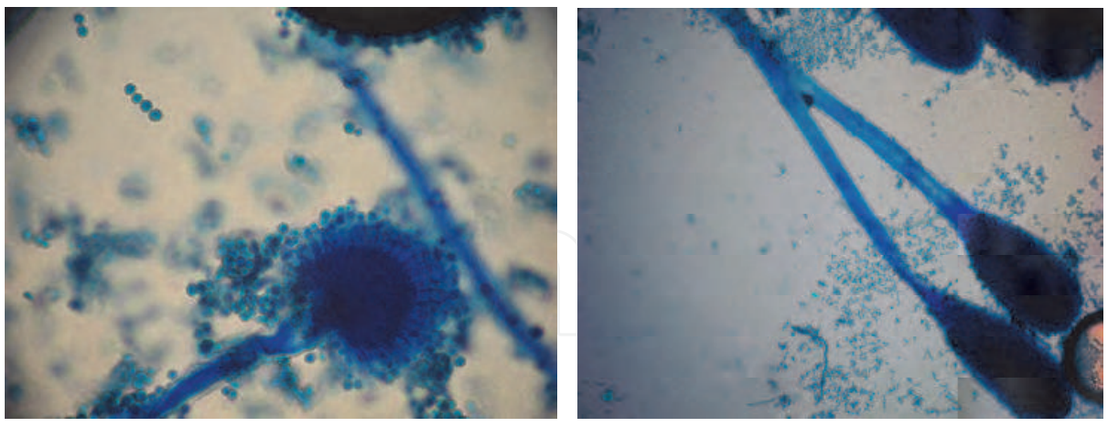

Fig. 6, 7. Conidiophores of *A. flavus* and *A. clavatus,* oryg.

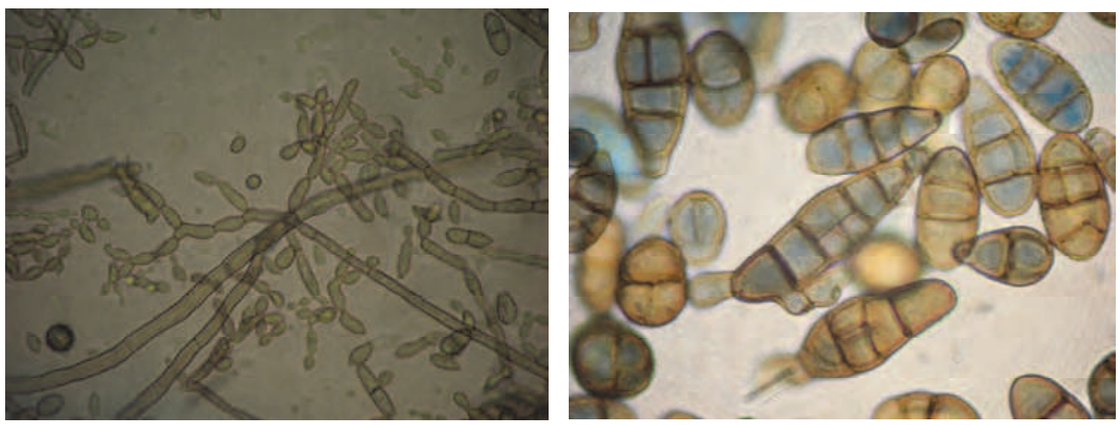

Fig. 8, 9. Conidiophores of *C. cladosporoides* and poroconidia of *A. alternata,* oryg.

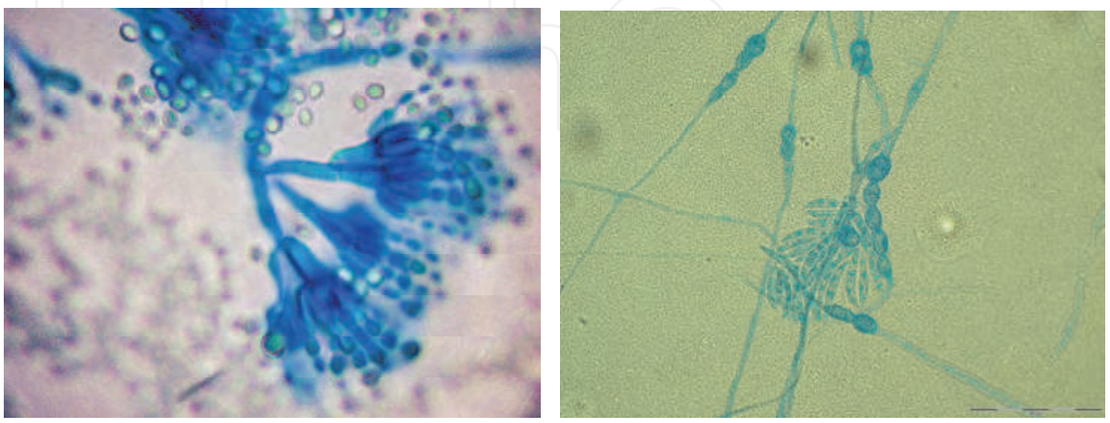

Fig. 10, 11. Conidiophores of *Penicilium sp.* and macroconidia of *Fusarium sp.*, oryg.

Genus	Species	Sampling site							
		Farm I	I 10	I 50	I 100	**Farm II**	II 10	II 50	II 100
Staphylococcus	*sciuri*		+				+		
	aureus					+	+	+	+
	epidermidis							+	
	cohnii subsp.2		+						
	lentus		+		+				
	xylosus	+	+						
	saprophitycus	+							
Micrococcus	*sedentarius*	+							
	lylae	+		+			+	+	
	halobius		+						
	luteus	+	+	+			+	+	
Enterococcus	*sp.*	+				+			
Streptococcus	*mitis*	+	+						
Bacillus	*mycoides*				+			+	
	sp.		+	+				+	
Corynebacterium	*xerosis*	+							
Psedomonas	*aeruginosa*	+	+		+				
	fluorescens	+							
	chlororaphis				+		+	+	
Xantomonas	*maltophila*				+				
Shigella	*boydii*		+						
Providencia	*sp.*		+	+					
Citrobacter	*farmerii*	+		+					
Escherichia	*coli*	+				+			
Enterobacter	*sakazakii*	+							
	agglomerans	+	+					+	
Proteus	*mirabilis*	+		+					

Table 2. Bacterial species isolated from the poultry houses I and II and from surroundings area during spring 2009 and summer 2010.

Genus	Species	Sampling site							
		Farm I	I 10	I 50	I 100	**Farm II**	II 10	II 50	II 100
Aspergillus	*flavus*	+	+	+	+	+			
	niger		+						
	terreus	+							
	glaucus		+						
	nidulans		+						
	parasiticus		+						
	clavatus					+	+	+	+
Penicilium	*solitum*	+							
	sticticus	+				+			
	chrysogenum		+		+				
Fusarium	*oxysporum*		+	+	+				
	graminearum		+	+	+				
Alternaria	*alternata*	+					+	+	+
	tennuissima					+		+	
Ulocladium	*sp.*				+				
Trichoderma	*viridae*	+	+						
Verticilium	*sp.*								+
Drechslera	*sp.*	+							
	gramineae				+				
Scopulariopsis	*brevicaulis*	+						+	+
	acremonium						+		
Cladosporium	*cladosporoides*					+	+	+	+
Mucor	*mucedo*	+	+			+	+	+	
Scedosporium	*sp.*					+	+	+	+
Mycelia	sterilia		+		+		+	+	
Rhodotorula	*rubrum*				+		+		
Candida	*albicans*	+				+			
	inconspicua	+							
	lambica							+	
Cryptococcus	*laurentii*	+				+			

Table 3. Fungal species isolated from the poultry houses I and II and from surroundings area during study period (spring 2009 and summer 2010).

4. Discussion

The literature data usually show the air biopollutant concentration inside the poultry houses. According to many studies (Agranovski et al., 2007, Radon et al., 2002, Vučemilo et al., 2006, 2007) the number of bacteria in poultry houses ranged from 10^3 to 10^{10} CFU/m^3, and the concentrations of fungi was from 2.5 x 10^1 to 4.9 x 10^6 CFU/m^3. Much less is known about the relationships between the indoor and the outdoor biological pollution. In comparison with the rate of microorganisms contamination in the poultry houses, the concentration of bacterial and fungi in the air in surrounding areas was considerable lower and did not exceed the values found inside farms. Airborne bacterial and fungi levels measured in poultry houses I and II were always higher than in adjacent areas. Therefore we can suggested that the source of microorganisms are probably the farm objects. Baykov & Stoyanov 1999 also reported higher bacterial levels inside broiler farms than in nearby areas and the average values were similar to our results, i.e. value of mean of 16020 CFU/m3 for farmhouses and range of2060 CFU/m3to 386 CFU/m3 for immediate areas (10 m and 100 m from farm object, respectively). Very high concentration of microorganisms may reflect on insufficient ventilation in relation to the number of animals kept in poultry houses.

The presence of bacteria and fungi in poultry air is a natural phenomenon. Their primary source are the animals themselves, feed and litter. Microorganisms are a constituent of solid and liquid bioaerosols. This mostly refers to saprophytes, however pathogenic microorganisms were also found in the poultry houses air. Aerial count of pathogenic bacteria and fungi depends on the health condition of animals kept in the poultry houses. In addition, microorganisms count inside poultry farms air and monitoring of its emission from this building to the adjacent environment are important parameters for the assessment of the influence of poultry houses on the environmental pollution (Matković et al., 2006). In the present study, microbiological air contamination were determined at three sites at a distance of 10 m, 50 m and 100 m from the poultry houses. The results of total microorganisms count measurements outside the both poultry houses showed it to be lower than the total bacterial and moulds count inside the poultry houses. Number of microorganisms increase at 10 m distance from the poultry houses and gradually decreased to reach the lowest value at a distance of 100 m.

Analyzing the results of the microbiological research about air pollution, it should be remembered that the results are temporary values, occurring at the moment the measurement. In connection with the physico-chemical properties of the air, the degree of contamination of the air can change diametrically within a few minutes (Donderski et al., 2005). Weather condition have a enormous influence on the count of microorganisms in the air. Temperature rise accompanied by rain scarcity can lead to a sudden increase in the concentration of microorganisms in the air. Consequently in summer with the weather conditions most friendly for the spread and development of numerous microorganisms, mesophilic bacteria, staphylococci, gram-negative bacteria and fungi were the more abundant in the air around the poultry houses than in early spring, where the temperature and humidity were lower.

Staphylococci seem to be a useful indicator bacteria (Schulz et al., 2004). Although this group of bacteria do not produce spores, they have the ability to survive in the air for a long time, which means spreading infections through the air. In the poultry houses I and II and outdoor air we observed very high numbers of potentially pathogenic staphylococci, what is really negative phenomenon. In our study the predominant species were coagulase

negative. *S. aureus* and *S. saprophitycus* are pathogens for humans and the other species isolated in our studies may act as opportunistic pathogens in humans and animals. Karwowska 2005 also described the very high number of staphylococci in the air farming.
Moulds and yeast can live practically anywhere and have particularly favorable conditions inside the poultry houses**.** Among fungi recovered from the farm I, the species belonged to genera *Candida spp*. were dominated. This is a large group of potentially pathogenic species. They are usually an etiological factor in mycoses and less frequently, in mycoallergies. As they can produce toxins (e.g. candotoxins), the species can cause mycotoxycoses and increase microorganism sensitivity to some bacterial infections (Ejdys et al., 2009). On the other hand, in outdoor air the most frequent species were moulds *Aspergillus flavus* and *Fusarium oxysporum*. Amongst the several secondary metabolites produced by *A. flavus* are aflatoxins, the most toxic and potent carcinogenic natural compounds ever characterized. Whereas, the *F. oxysporum* is potentially producers of zearalenone, scirpentriol, NT-2 toxin, nivalenol, acetoxyscirpenediol, acetyl T-2toxin and others dangerous toxins (http://www.esgtesting.com/Portal/Documents/Toxins%20and%20fungal%20origin.pdf).
In the air surroundings poultry house II dominated species were *Aspergillus clavatus*, *Alternaria alternata, Cladosporium cladosporoides*. Whereas, *A. clavatus* and *A. flavus* were the most frequent species inside poultry house II. We should emphasized that *A. alternata* is one of the most common fungi associated with asthma. Not only the presence of asthma but also persistence and severity asthma have been strongly associated with sensitization and exposure to *A. alternata* (Salo et al., 2006). On the other hand, the presence of opportunistic pathogens from the genus *Aspergillu*s poses a risk of invasive aspergillosis in farm workers and those living in the proximity of the farms. According to the data obtained by other authors (Soliman et al., 2009) fungi e.g. *Candida albicans, Aspergillus niger, A. nidulans, Penicilium sp.* and *Mucor sp.* were prevalent in broiler farms in Egypt. However, Romanowska-Słomka and Mirosławski 2009 described the occurrence of the moulds and yeast *Aspergillus sp., Penicillium sp., Candida sp. and Cryptococcus sp.* in poultry houses. Other investigators (Agranovski et al. 2007) isolated and identified many fungal strain, including genera: *Cladosporium, Aspergillus, Penicillium, Scopulariopsis, Fusarium, Epicoccum, Mucor, Trichophyton, Alternaria, Ulocladium, Basidiospores, Acremonium, Aureobasidium, Drechslera, Pithomyces, Chrysosporium, Geomyces and Rhizomucor* from farming areas. The presence of such fungi in farmhouses was proved by the results of this study.
The highest total *Enterobacteriacea* counts were found in indoor air and in areas nearby poultry houses I and II. In this present studies both in farms and surroundings areas *Escherichia coli, Proteus mirabilis, Shigella boydii, Citrobacter farmerii, Enterobacter agglomerans, E. sakazakii, Klebsiella pneumoniae, Providencia sp.* were identified. Different results was observed by Vučemilo et. al who found four dominating species of the *Enterobacteriaceae* family: *E. coli, Pantoea sp., Serratia plymuthica* and *Serratia marcescens*. According to Lues et. al 2007 *E. coli* and the other members of the coliforms bacteria could be a good indicators of air contamination.

5. Conclusions

The farming buildings are emitters of the considerable amounts of microbiological contaminants into the atmospheric air. This high emission of potentially pathogenic microorganisms via aerosols from animal housing facilities to the outdoor environment may constitute a considerable risk to human health and environmental pollution.

So far, in literature there are no reliable data about relationships between the indoor and the outdoor biological pollution. This study contributes to the understanding of the level concentration of bioaerosol and its composition with regard to the different distance from farms. The quantity and quality of microbial analysis shows both different bacteria genera and fungi in indoor and outdoor microbiological contamination. Comparing our own results with available literature data on the indoor and outdoor air biopollutant concentration in the poultry houses enables to understand the distribution process.

6. References

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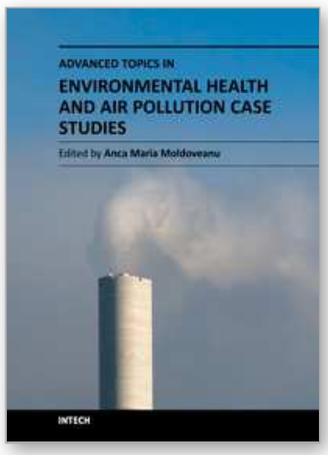

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