Polish J. of Environ. Stud. Vol. 20, No. 3 (2011), 725-731

Original Research

Analysis of Airborne Contamination with Bacteria and Moulds in Poultry Farming: a Case Study

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Received: 12 October 2010
Accepted: 4 January 2011

Abstract

Our paper presents microbiological air pollution inside and outside two different poultry farms in Lower Silesia, Poland. The smaller poultry house (A) with 18,000 broiler chickens was surrounded by a protective green tree belt; the larger poultry house (45,000) was surrounded by a field without trees. Air samples were taken at ten sampling sites using a MAS-100 air sampler (Merck) in the summer, autumn, and winter of 2009 and in spring 2010. Seasonal variations were observed in all locations with regard to the numerous microorganisms. According to the Kruskal-Wallis test, the difference between the total number of bacteria in the indoor air of the poultry houses and surrounding area was statistically significant. It has been stated that the total number of heterotrophic bacteria (as CFU/m^3) in distant points (10 m, 50 m, 100 m) in summer at houses A and B was higher with regard to Polish Norms and ranged between between 6.0×10^3-2.6×10^4. Moreover, nearly all sampling points in both poultry houses were presumed to be heavily contaminated by staphylococci (0-1.4×10^4). In relation to Polish Norms, the air in points situated near the poultry farms can be referred to as highly-contaminated with fungal and bacterial microflora.

Keywords: airborne bacteria, airborne fungi, airborne contamination, bioaerosol in poultry farming

Introduction

Modern poultry production is usually polluted with large quantities of different microbial components, mainly aggregation of bacterial and fungal cells, their spores, and fragments of mycelium as well as metabolites like endotoxin (lipopolysaccharide, LPS) of Gram-negative bacteria and 1,3-beta-glucan of fungi [1, 2]. They are suspended as the indoor and outdoor bioaerosols that may be generated either as liquid droplets or as dry particles and transit in air individually or as clusters [3].

It is known that long-term or repeated exposure to high concentrations of airborne microorganisms can cause respiratory damage, allergenic, and immunotoxic effects [3]. A number of syndromes have been recognized in workers in the intensive animal industries. The more common clinical signs are exacerbate asthma, asthma-like syndrome, mucous membrane irritation, chronic bronchitis, acute inflammatory processes (called organic dust toxic syndrome), and chronic obstructive pulmonary diseases (COPD) [4, 5].

Most important for the possible transmission of a pathogen is its ability to survive in an airborne state over a longer period. The microorganisms are strongly influenced by environmental conditions such as temperature and humidity of the air and other factors, including radiation and sunlight [4].

Our paper presents the issue of seasonal microbiological pollution inside and outside two poultry houses located in different environmental areas in Lower Silesia, Poland.

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Materials and Methods

The studies were carried out in two poultry farms (A and B) located near Wrocław, Poland, Lower Silesia (Figs. 1, 2). Poultry farm A was smaller than B, with lower numbers of birds. The buildings held about 18,000 and 23,000 broiler chickens, respectively, and they were equipped with mechanical ventilation systems. Only poultry house A was surrounded by a protective green tree belt.

Air samples were taken using a MAS-100 air sampler (Merck KgaA, Darmstadt, Germany) based on the principle of the Andersen air sampler. The speed of air flow through the sampler was about 11 m/s, air volumes were 5-500 liters (depending on expected contamination levels) and the sampling rate was 100 l/min. Three parallel samples for each group of bacteria were taken at the central point of each building, 1.5 m from ground level. The emission level outside farming objects was determined similarly, i.e. 1.5 m with sampling points situated 10 m, 50 m, 100 m, and 200 m from the buildings (A and B). The following numbers were assigned various sampling sites: A_{10}, A_{50}, A_{100}, A_{200}, B_{10}, B_{50}, B_{100}, B_{200} – outside poultry house A and B at the distances of 10 m, 50 m, 100 m, 200 m; A and B – at the center of buildings A and B.

The temperature and air humidity of sampling sites were measured by a termohigrometer (La-bel, Poland). The studies were carried out in the summer, autumn, and winter of 2009, and in spring 2010.

Microbiological studies of air samples were used to determine the number of heterotrophic bacteria, *Enterobacteriaceae* representatives, mannitol+ staphylococci, *Salmonella* sp., and mould fungi. The heterotrophic bacteria were determined using TSA agar (BioMérieux, France). Incubation of those bacteria that had grown on the plate, conducted at 37ºC for 48 h. Representatives of the *Enterobacteriaceae* family were isolated using VRB medium (by Biomerieux) and incubated at 37ºC for 48 h. Estimation of staphylococci was done on Mannitol salt gar (BioMérieux, France) after 48 h incubation at 37ºC; typical *Staphylococcus aureus* colonies were recognized as yellow, with bright yellow zones. SS agar (BioMérieux, France) plates were inoculated for culturing and enumerating *Salmonella* sp. and incubated at 37ºC for 48 h; colonies are colorless to pale yellow with a black centre. Mould fungi were determined using Sabouraud (Merck) medium and cultured at 26ºC for 5 days.

Quantitative results were expressed in CFU/m^3 (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 [6].

Bacterial species were identified on the basis of gram staining, microscopic morphology, oxidase activity, catalase test results, and metabolic properties according to standard procedures [7]. The following commercial systems were used: API 20E for enteric gram-negative organisms, API 20 NE for fastidious and nonfermenting gram-negative organisms, and API Staph for gram-positive staphylococci. Biochemical identification systems, API Staph, API 20E, and API 20NE (BioMérieux, France) consisting of 20 microtubes containing dehydrated substrates were loaded with bacterial suspensions in a sterile physiological salt solution, and subsequently incubated for 18-24 hours at 35ºC (API 20E, API 20 Staph) and 24-48 hours at 30ºC (API 20NE).

Fungal colonies were identified on the basis of color, texture, topography of the surface of the culture, smell of the colony, color of the reverse of the colony, and the presence of a diffuse color pigment. Microscopic features of the fungal colonies (i.e. the presence of macroconidia and microconidia, their shape and appearance) were then identified. Fungi species of *Aspergillus* and *Penicilium* genera were identified using the keys by Raper and Fennell as well as Raper et al. [8, 9]. The other species were identified using the "Atlas of Clinical Fungi" [10].

The Kruskal-Wallis test was used to compare counts of bacterial and fungal colony-forming units. The degree of outdoor air contamination was evaluated in accordance with the Polish Norm [11, 12].

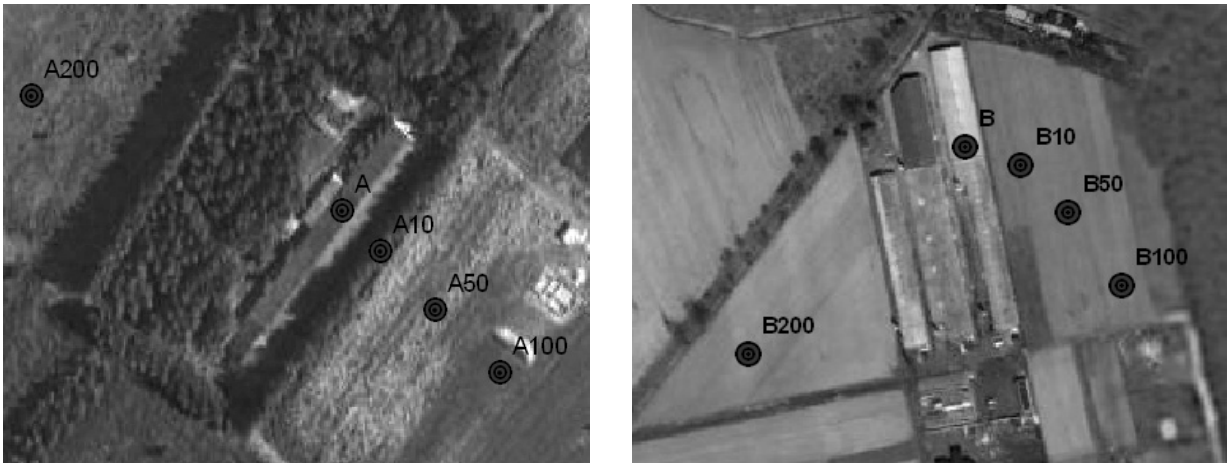
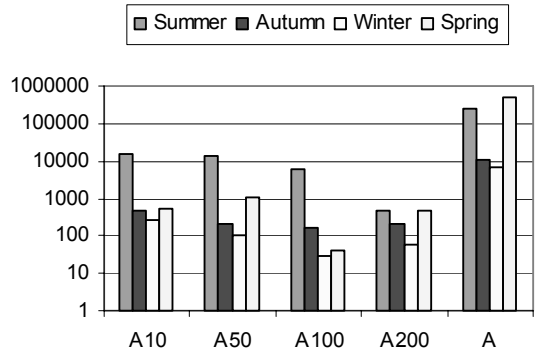
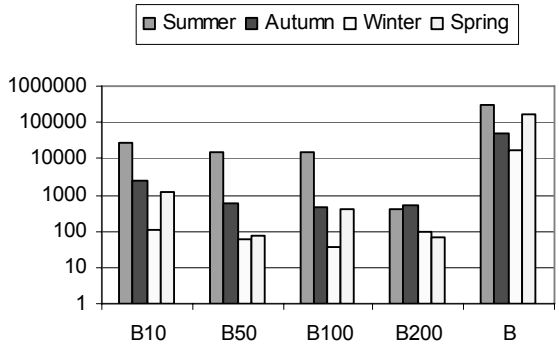


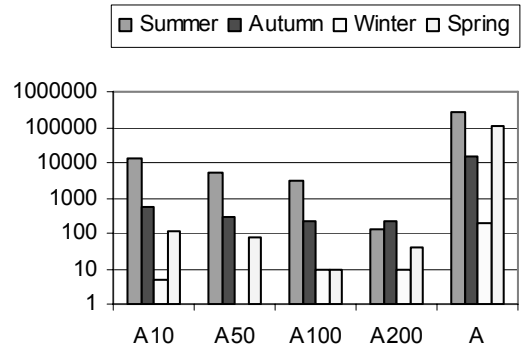
Fig. 1. Poultry houses A and B; the sampling sites outside the poultry house at distances of 10 m, 50 m, 100 m, and 200 m from the farming object, respectively, as well as at the center of building.



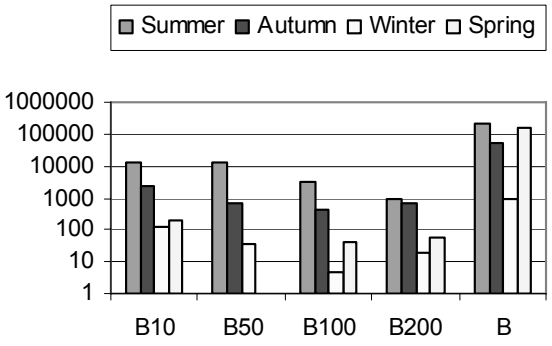
a) A heterotrophic bacteria



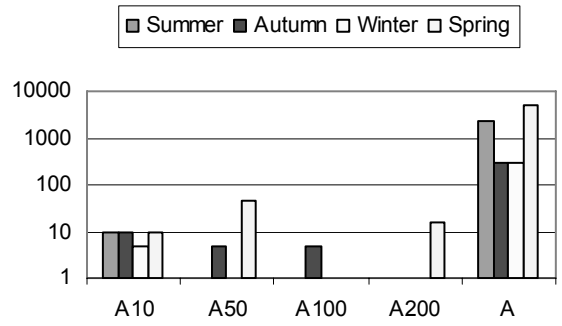
b) B heterotrophic bacteria



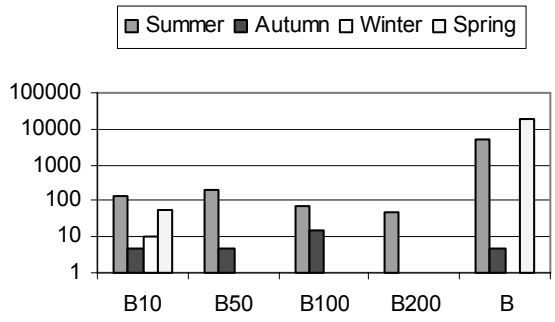
c) A staphylococci



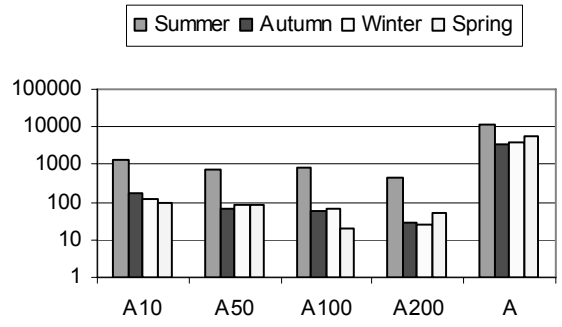
d) B staphylococci



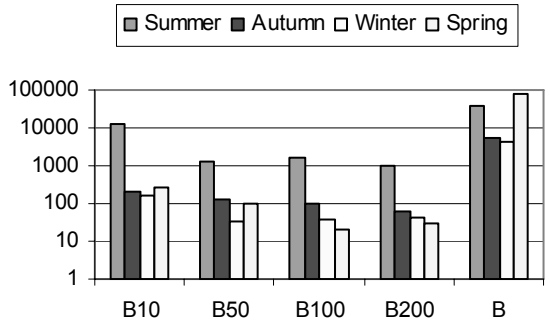
e) A *Enterobacteriaceae*



f) B *Enterobacteriaceae*



g) A moulds



h) B moulds

Fig. 2. Number of microorganisms in poultry houses A and B.

Results

The studies were carried out in the summer, autumn, and winter of 2009 and in spring 2010, when temperature of atmospheric air ranged between - 0.5ºC and + 25.5ºC; poultry house inside temperatures varied from 22ºC to 27ºC. Indoor relative air humidity was about 73-89%, outdoor ca. 55-85%. Seasonal variations was observed in all locations with regard to the numerous microorganisms (Fig. 2a-h). According to the Polish Norms, in summer the points A_{10}, A_{50}, A_{100}, B_{10}, B_{50}, B_{100} were presumed to be heavily contaminated by heterotrophic bacteria. Sampling sites A_{50} (in spring) and B_{10} (in autumn and spring), were considered average contaminated by this group of bacteria. Nearly all sampling places were presumed to be heavily contaminated by the staphylococci.

The highest numbers of heterotrophic bacteria, staphylococci, and moulds in the air of poultry A were recorded during summer and spring, and the lowest in winter. On the other hand, in poultry house B the highest numbers of these groups of microorganisms were during summer and autumn, and the lowest in winter. In the air (outside and inside) of poultry house A the lowest number of bacteria from the *Enterobacteriaceae* family was noted in winter and autumn (on average about 5.0×10^0 CFU/m^3), while the greatest number of these bacteria occurred in spring (A – 5.2×10^3 CFU/m^3). In the samples of air taken in house B, the highest number of this group of bacteria was found in spring (B – 1.9×10^4 CFU/m^3) (Fig. 2a-h).

In house A staphylococci were the most numerous organisms in all seasons and formed about 81% of the local microbial community. Less numerous heterotrophic bacteria and moulds constituted about 12% and 6%, respectively. The concentration of *Enterobacteriaceae* was fractional. However, atmospheric air of house B was characterized by a relatively small number of *Enterobacteriaceae*. On the other hand, heterotrophic bacteria dominated and formed about 54% of the local microbial community, whereas staphylococci and moulds constituted about 30% and 15 %, respectively. *Salmonella* sp. was not detected in either poultry house (Fig. 3a, b).

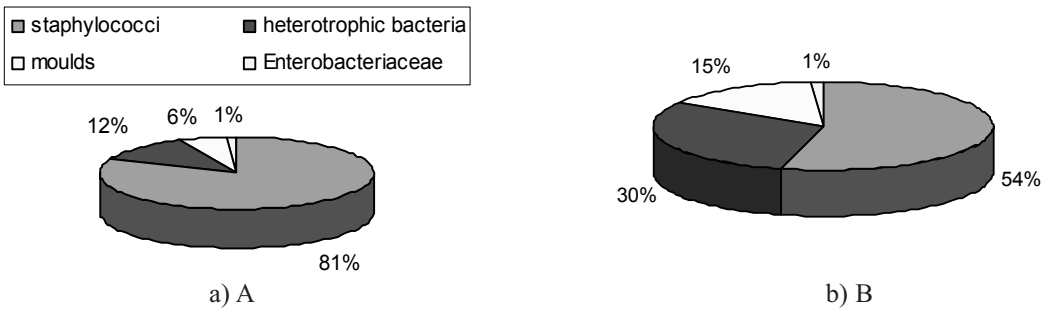
Bacterial concentrations were determined at 4 sites at distances of 10 m, 50 m, 100 m, and 200 m from poultry houses. The results of total bacteria and mould count measurements outside houses A and B at 10 m distance showed it to be several times lower than the total bacteria and mould counts in the poultry houses. The number of microorganisms increased at 5 m distance from the poultry houses and gradually decreased to reach the lowest value at a distance of 100 m.

According to the Kruskal-Wallis test, the difference between the total number of bacteria in the indoor air of the poultry houses and surrounding area was statistically significant ($p<0.05$). For both poultry houses the concentration levels of bacterial aerosols were higher indoors than outdoors in all sampling points (poultry house A $p<0.0037$ and poultry house B $p<0.0097$). In addition to the bacterial air contamination, the concentration of moulds indoor was also higher in the investigated poultry houses compared to the surrounding area. This difference in fungal contamination was statistically significant (house A $p<0.0094$, house B $p<0.0135$).

Both poultry houses and their surrounding habitats were characterized by predominating strains of bacteria as *Staphyloccus* (*lentus*, *epidermidis*, *aureus*, *sciuri*, *cohnii*, *urealitycum*, *warneri*), *Micrococcus* (*luteus*, *roseum*), *Pseudomonas* (*fluorescens*, *aeruginosa*, *chlororaphis*, *maltophila*), *Proteus* (*mirabilis*, *vulgaris*), *Enterobacter agglomerans*, *Shigella boydii*, *Bacillus mycoides*, *Klebsiella pneumoniae*, *Serratia* (*plymuthica*, *marcescens*), *Pasteurella multocida*, and *Acinetobacter* sp. Several moulds were isolated from inside and outside both poultry houses (Table 1).

Discussion

In Poland there are no standard regulations concerning the permitted number of bacteria and fungi in indoor air, including farming accommodations [13]. In the case of farm environments, Krzysztofik proposed the acceptable numbers of heterotrophic airborne bacteria (>10^5) and moulds (>2×10^3). In our studies in both poultry houses the proposal of allowed numbers of moulds was higher in every season, whereas the number of heterotrophic bacteria was higher in summer and spring. Moreover, according to the regulations of the Polish Norms [11, 12], air in surrounding areas can be classified as a highly contaminated at site B_{10} (in summer) with fungal microflora. At all other sampling sites the number of fungi in the air did not exceed 3.0×10^3 CFU/m^3. The total number of heterotrophic bacteria (as CFU/m^3) in points A_{10}, A_{50}, A_{100}, B_{10}, B_{50}, and B_{100} was



a) A b) B

Fig. 3. Groups of microorganisms in the air of poultry houses a) A and b) B in all seasons.

Table 1. Fungal genera isolated from poultry houses A and B and from the surrounding area during the study period (winter 2009-spring 2010).

Genus	Species	Sampling site									
		A_{10}	A_{50}	A_{100}	A_{200}	A	B_{10}	B_{50}	B_{100}	B_{200}	B
Moulds											
Penicilium	*chrysogeum*	+	+		+	+	+	+	+	+	+
	commune	+		+	+	+	+	+	+	+	+
	granulatum	+									
	steckii	+									
	citrinum	+				+					
	solitum	+									
	corylophilum						+	+	+		+
	solitum						+	+	+		+
Aspergillus	*fumigatus*					+	+				+
	clavatus	+	+			+	+				+
	flavus					+	+	+			+
	wentii						+				
	niger										+
	terreus										+
	fischerii						+	+			+
	glaucus										+
	versicolor						+				+
	proliferans						+			+	
	oryzae									+	
Alternaria	*alternata*	+	+		+	+	+	+	+	+	+
	tennuissimma					+					
Cladosporium	*cladosporoides*	+	+	+		+	+	+	+	+	+
Fusarium	*oxysporum*						+				+
	graminearum	+	+	+	+	+					
Mucor	*mucedo*				+						
Rhizopus	*nigricans*				+					+	
Scopulariopsis sp.						+					
Sporotrichum sp.										+	
Chrysosporium sp.		+	+			+					
Chaetomium sp.							+				+
Drechslera									+		

+ fungal growth was observed

higher with regard to Polish Norms and ranged between 6.0×10^3-2.6×10^4 CFU/m^3. A high number of mannitol+ staphylococci occurred in both poultry houses in nearly all sampling points. In some cases the number of staphylococci was higher than the number of heterotrophic bacteria on MPA agar.

A significant increase in the worldwide scientific database on air contamination in poultry houses has been noted in recent decades [14-24]. However, the literature data usually shows the air biopollutant concentration inside the poultry houses. Much less is known about the relationships between indoor and outdoor biological pollution as well as about the spreading of indoor bioaerosol in the surroundings of the farms. According to Radon et al. [21], Vučemilo et al. [19, 20], Agranovski et al. [18], and Lues et al. [24], the number of bacteria in poultry houses ranged from 10^3 to 10^{10} CFU/m^3, and the concentrations of fungi ranged from 2.5×10^1 to 4.9×10^6 CFU/m^3. In this investigation the num-

ber of microorganisms was comparable. The number of microorganisms inside poultry houses A and B ranged between 7.1×10^{3}-5.2×10^{5} for heterotrophic bacteria, 2.0×10^{2}-2.8×10^{5} for staphylococci, 5.0×10^{0}-1.9×10^{4} for coli group bacteria, and 4.1×10^{3}-3.6×10^{4} for moulds. On the other hand, the number of microorganisms (as CFU/m^3) outside of the poultry houses was smaller than indoor and ranged 3.0×10^{1}-2.6×10^{4} for heterotrophic bacteria, 0-1.4×10^{4} for staphylococci, 0-2.0×10^{2} for coli group bacteria, and 2.0×10^{1}-1.3×10^{4} for moulds. The comparison of microflora occurring in the atmospheric air taken 100 m and 200 m from farming objects and bacteria and fungi in the vicinity and inside animal houses revealed that the source of microorganisms is probably farm objects. For both poultry houses, the indoor concentration of bacteria and moulds were always higher compared with the outdoor concentrations for the four different sites.

Moulds can live practically anywhere and have particularly favorable conditions inside poultry houses [20]. In this work we detected 31 species representing 13 fungal genera. The most frequent species in poultry house B was *Cladosporium cladosporoides,* which comprised 58.4% of all the identified species. Eight species of the genus *Penicilium* were isolated and identified, among them *Penicilium chrysogeum* prevailed and comprised 44.5% of all identifies species in poultry house A. Fungi of the genus *Aspergillus* were isolated in both poultry houses and represented 11 species, with a predominance of *A. fumigatus*, *A. flavus*, and *A. clavatus*. The majority of these species are known to be potential respiratory allergens and exposure to their spores may provoke immune responses in susceptible individuals. As a result, diseases such as allergic rhinitis, bronchial asthma or extrinsic allergic alveolitis may develop in certain individuals [2]. Agranowski et al. isolated and identified many fungal strains, including the genera: *Cladosporium*, *Aspergillus*, *Penicillium*, *Scopulariopsis*, *Fusarium*, *Epicoccum*, *Mucor*, *Trichophyton*, *Alternaria*, *Ulocladium*, *Basidiospores*, *Acremonium*, *Aureobasidium*, *Drechslera*, *Pithomyces*, *Chrysosporium*, *Geomyces*, and *Rhizomucor* from farming areas [18]. Romanowska-Słomka and Mirosławski described the occurrence of the moulds and yeast *Aspergillus* sp., *Penicillium* sp., *Candida* sp., and *Cryptococcus* sp. in poultry houses [22]. The presence of such fungi in farmhouses was proved by the results of this study.

The presence of high numbers of potentially pathogenic staphylococci was emphasized by Karwowska as a negative phenomenon [2]. They can probably serve as indicator species for bacterial pollution because they do not usually appear in relevant concentrations in normal outside air [23].

The highest total coliform counts were found in indoor air and outside (sampling point Nos. A_{50} in spring and B_{50} in summer). In this study *Escherichia coli*, *Proteus mirabilis*, *Shigella boydii*, *Citrobacter farmerii*, *Enterobacter agglomerans*, *Klebsiella pneumoniae*, *Serratia plymuthica*, and *S. marcescens* were identified. Similar results were observed by Vučemilo et al., who found four dominating species of the *Enterobacteriaceae* family: *E. coli*, *Pantoea* sp., *Serratia plymuthica*, and *Serratia marcescens* [20]. According to Lues et al., *E. coli* and the other members of the coliform bacteria family could be good indicators of air contamination [24].

Conclusions

Microorganisms were more numerous in the air in house B than in A. This result was strongly influenced by the total number of broiler chickens and by locations. The trees and shrubs that surround poultry farm A may contribute to lower levels of bacteriological and mould contamination by functioning as a buffer zone that hampers the distribution of pathogenic bioaerosol. On the other hand, the higher number of microorganisms in poultry house B than in farming house A may be the result of the lack of a protective green tree belt and by the higher number of chickens on the farm.

The high levels of microorganism air pollution should be assessed in two aspects: a possibility of causing infections from aerosols and the allergic effect of microorganisms. These results are alarming, because poultry houses have been built usually close to residential areas. Moreover, the outcome of this study proves that indoor air standards should also be created for poultry farm settings to guarantee proper hygienic and epidemiological conditions of farming settings and to prevent the emission of bioaerosols into atmospheric air.

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