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ORIGINAL ARTICLE

Poultry house as point source of intense bioaerosol emission

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Abstract

Introduction and Objective. Intensive poultry farming is usually associated with massive exposure to organic dust, which is largely composed of microbiological origin particulates. The aim of the study is to assess occupational and environmental exposures to airborne bacteria, fungi, and Marek's disease virus emitted by a poultry house.

Materials and method. The concentrations of airborne microorganisms in a poultry house and its vicinity (250–500 m) at 3 different stages of the production cycle (i.e. empty poultry house, with 7-day-old and 42-day-old chickens) were stationary measured using Andersen and MAS impactors, as well as Coriolis and BioSampler impingers. The collected microbiota was taxonomically identified using molecular and biochemical techniques to characterize occupational exposure and its spatial dissemination.

Results. Although Marek's disease virus was not present in the tested air samples, the appearance of reared chickens in the poultry house resulted in an increase in airborne bacterial and fungal concentrations up to levels of 1.26×10^8 CFU/m^3 and 3.77×10^4 CFU/m^3, respectively. These pollutants spread around through the ventilation system, but their concentrations significantly decreased at a distance of 500 m from the chicken coop. A part of the identified microbiota was pathogens that were successfully isolated from the air by all 4 tested samplers.

Conclusions. The poultry house employees were exposed to high concentrations of airborne microorganisms, including pathogens that may lead to adverse health outcomes. To protect them, highly efficient hygienic and technical measures regarding the poultry house interior and its ventilation, respectively, should be introduced to prevent both unwanted pollution and subsequent emission of microbial contaminants during intensive chicken breeding.

Key words

bacteria, fungi, size distribution, bioaerosol, poultry house, Marek's disease virus, environmental dissemination

INTRODUCTION

Environmental protection is one of the most important aspects of the European Union (EU) sustainable development standards. According to Food and Agriculture Organization estimates, poultry production, which accounts for 35% of world livestock production, is responsible for only 7% of total waste emissions and water consumption [1]. Nevertheless, poultry farming, like any type of livestock production, produces waste and by-products that can have a negative impact on the environment. In the common perception, the most troublesome among them are solid waste (used litter, excrement) and liquid manure, as well as mixtures of organic and inorganic volatile chemical substances (odours). While poultry farming is usually associated with this type of pollution, much less attention is paid to the emission of microbiological contaminants, both within and outside the poultry farms.

Polish poultry processing plants are currently considered to be one of the most modern in the EU and their competitiveness in relation to other countries is systematically growing. According to the AVEC report from 2022 [2], Poland is a leader in the production of poultry meat in the EU (2.75 million tons in 2021, which is slightly above 20% of EU production) and is also the largest exporter in intra-EU trade (1.47 million tons in 2021). Such a dynamic development of the poultry industry forced the need to increase financial outlays for the modernization of poultry farms. On the other hand, to improve the efficiency of this livestock production, it was necessary to augment the number of birds kept per unit area. The consequence of these circumstances was the rapid change of breeding environment. These 'new' conditions, however, favour the development and spread of a wide range of microorganisms at every stage of poultry production [1, 2].

Poultry rearing usually takes place on specialized chicken farms with an intensive fattening system, or on small farms with more traditional forms of breeding. Irrespective of the poultry farming method used on the farms, from the employees' point of view, being in a chicken coop is associated with a long-term stay in a highly polluted environment. Commercial poultry farming is usually linked with the massive formation, as well as subsequent generation and environmental spread of organic dust, which is predominantly composed of microbiological origin components. This type of dust contains, in addition to minerals of soil origin, settled dust particles (consisting of feed, litter, excrements, fragments of feathers, or exfoliated epidermis) originating

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from the livestock housing, as well as microorganisms developing in it and being transported by the air (bacteria, fungi, viruses), noxious gases (NH_3, CO_2, H_2S), and chemicals (e.g. from fertilizers, pesticides or disinfectants) [3]. Long-term exposure to harmful microbiological agents contained in organic dust may result in many respiratory disorders and diseases. These adverse effects usually include work-related respiratory symptoms, chronic pulmonary disease, decline in lung function, increased airway responsiveness, and allergic reactions [e.g. 3–8]. As poultry farm workers are primarily exposed to high concentrations of organic dust, the quantitative assessment and qualitative characteristics of airborne microbiological contaminants can help to ensure not only the appropriate conditions for rearing chickens (and thus the high quality of poultry meat products), but can also assist considerably in the effective protection of both the health of poultry farm employees and the quality of environment around it.

The aim of the study is to quantitatively and qualitatively assess occupational and environmental exposures to airborne bacteria, fungi, and Marek's disease virus within and around poultry house of intense chicken production. As intense poultry farming is usually associated with massive exposure to organic dust, which is largely composed of microorganisms, their airborne dissemination in the vicinity of poultry house is also characterized.

MATERIALS AND METHOD

Poultry farm characteristics. The studied poultry farm was located in north-eastern Poland. About 75% of the farm's surroundings are occupied by arable fields, the remaining 25% are residential premises and technical buildings of small companies not related to agricultural production, all located about 500 m from the farm. The investigated poultry house was a free-standing building 105 m long, 16 m wide, and 4.8 m high at the ridge. The building was equipped with an automatically regulated ventilation system with an exhaust chimney with a height of 5 m. The exhaust capacity of the ventilation system was 4,000 m^3 per hour. The operation of fans depended on the microclimate parameters inside the house set at a given stage of rearing. In the poultry house, broiler chickens were fattened in a bedding system. The poultry house also had automated watering and feeding systems. The production cycle of chickens lasted from the receipt of 1-day-old chickens to about 6 weeks of age, when their slaughter weight had reached about 2.5 kg. About 6 rearing cycles have been carried out on the farm annually. In house stocking rate (birds per usable area) was about 18 chickens per 1 m^2, which means that in 1 production cycle, about 30,000 chickens were bred in the studied poultry house. Manure (droppings with litter) was removed from the building after completing the full rearing cycle, and was not stored on the farm. For periodic disinfection of the henhouse between successive stockings with new chickens, the floor was washed with water, then sprayed (usually with ammonia water or sodium hypochlorite), and subsequently fogged with a bactericidal, virucidal, and fungicidal preparation. During the production cycle, about 5 employees took care of the chicken flock. Workers, although equipped with personal protective equipment, usually wore goggles and gloves, avoiding the use of respiratory protection.

Sampling strategy. The concentrations of airborne microorganisms were measured at workplaces in the poultry house, in its immediate vicinity to established background (outdoor) concentrations, and at a distance of 250–500 m from the henhouse to check the ability of microbial particles to be disseminated from such a source of their intense emission. In these first 2 cases (i.e. at workplaces and in background), altogether 6 sampling campaigns were carried out during 'winter' (3 campaigns in the period February – March, when the average outdoor air temperature was below 10 °C) and 'summer' (also 3 campaigns in the period June – September, when the average outdoor air temperature was above 20 °C) seasons. In each sampling season, the bioaerosol measurement campaigns covered 3 different stages of production cycle, i.e. in the clean and disinfected poultry house without chickens, poultry house with 7-day-old (i.e. 1 week after flock stocking), and 42-day-old chickens (i.e. about 1 day before their departure to the slaughterhouse). Each time at the workplaces in poultry house, the viable (understood here as culturable) bacterial and fungal aerosols were stationary measured using a 6-stage Andersen (model WES-710, Westech Instrument, Upper Stondon, UK) and single-stage MAS (model 100 NT, MBV AG, Stäfa, Switzerland) impactors, as well as Coriolis μ (Bertin Technologies, St-Quentin-en-Yvelines, France) and BioSampler (SKC Ltd., Eighty-four, USA) impingers. The flow rates and sampling times were 28.3 L/min and 3 min and 100 L/min and 1 min, as well as 300 L/min and 10 min and 12.5 L/min and 30 min, respectively. In addition, to determine a degree of background (outdoor) contamination, the atmospheric bacterial and fungal aerosols were simultaneously collected outside the poultry house using a 6-stage Andersen and single-stage MAS impactors. In both cases, the flow rates and sampling times remained the same (i.e. 28.3 L/min and 3 min and 100 L/min and 1 min, respectively).

In order to illustrate the environmental dissemination of microbial pollution, the measurements of bacterial and fungal aerosol concentrations were carried out using an MAS impactor (a battery-operated device), at a flow rate of 100 L/min with sampling time of 1 min, at 16 sampling points radially distributed at distances of 250 m (8 points) and 500 m (8 points) around the tested poultry house. During all indoor and outdoor measurements, all sampling instruments (Andersen, MAS, Coriolis, and BioSampler) were placed at a height of 1–1.5 m above the floor or ground level to simulate aspiration from the human breathing zone, and at a distance of 1 m from each other to avoid possible interferences between them. At each sampling point and for all samplers, all performed bioaerosol measurements were duplicated.

In the case of Andersen and MAS impactors, at the beginning of each measurement cycle inside and outside of the poultry house, an aerobic bacterial aerosol was collected on blood trypticase soy agar (TSA 43001, bioMérieux, Marcy l'Etoile, France), followed by sampling of anaerobic airborne bacteria on Schaedler agar with 5% additive of sheep blood (SCS, Graso, Starogard Gdański, Poland) and, after final impactor reloading, fungi were aspirated on malt extract agar (MEA, Oxoid Ltd., Basingstoke, UK). In the intervals between these sampling sessions, the impactors were subjected to disinfection and cleaning with isopropyl alcohol and then dried by a stream of hot air. In turn, in the case of Coriolis and BioSampler impingers, the autoclaved

phosphate-buffered saline (PBS) was used as the liquid medium collecting bioaerosol samples. From each of the received Coriolis and BioSampler suspensions, the serial dilutions in deionized sterile water were prepared and a volume of 100 µL of each of these samples was cultivated (in triplicates) on an agar media suitable for specific bacteria (blood TSA and SCS) and fungi (MEA).

In the case of measurements performed to check the dissemination of microbial pollutants around the studied poultry house, at the beginning of each measurement cycle, the MAS impactor was first loaded with a Petri plate filled with blood TSA to sample bacterial aerosol, and then with plate filled with MEA to collect fungal aerosol.

Microbiological analyses of bacterial and fungal aerosol samples. The collected aerobic bioaerosol samples were incubated as follows: bacteria – 1 day at 37 °C, followed by 3 days at 22 °C and 3 days at 4 °C; fungi – 7 days at 25 °C. Prolonged incubation of the samples was intended to capture of both pathogenic and environmental species, considering in the case of bacteria psychrophilic strains growing in the lower temperature range [9]. In turn, the sampled anaerobic bacteria were incubated for 2 days at 37 °C, followed by 2 days at 30 °C, with the use of AnaeroGen™ system (Oxoid Ltd., Basingstoke, UK). After incubation, the microbial concentrations were calculated as colony-forming units (1 or more cells that grow to form a single visible colony) per cubic meter of sampled air (CFU/m^3) [10].

The bacterial biota collected in poultry house and around it was taxonomically characterized to the phylum, genus and/or species levels using molecular, biochemical, and culture-based techniques. Bacterial colonies from blood TSA and SCS media were used to isolate their DNA material to confirm the affiliation of individual strains to taxonomic phyla and genera employing molecular (NGS, PCR) techniques (see below). As viability of microorganisms in collected samples was of a great importance from the exposure assessment point of view, the molecular analyzes were supplemented by identification of isolated culturable bacteria to the genus and/or species level based on their morphology (after Gram staining; model Aerospray 7322, ELITechGroup Inc., South Logan, USA) [11], microscopic structure (model Eclipse E200, Nikon, Tokyo, Japan), and biochemical reactivity using analytical profile index (API) tests and APIweb database (bioMérieux). In turn, the isolated fungal colonies were directly identified under stereo (model SteREO Discovery V.12, Carl Zeiss, Gottingen, Germany) and light (Nikon) microscopes based on their macro- and micro-morphological characteristics. Filamentous fungi were taxonomically systematized using four identification keys: Fisher and Cook [12], Murray et al. [13], Samson et al. [14], and St-Germain and Summerbell [15]. The analysis of yeasts included Gram staining (ELITechGroup Inc.) and biochemical reactivity evaluation based on API 20C AUX test (bioMérieux).

DNA extraction and characterization of bacterial biota using next generation sequencing (NGS). The next generation sequencing (NGS) was applied to analyze bacterial communities in 5 bioaerosol samples collected using Andersen impactor. After sampling, all collected bacterial colonies were washed off the agar plates and pooled together using deionized water to a total liquid volume of 10 mL in a 50-mL corning tube. Then, 2 mL of the bacterial suspension for each sample was taken out for DNA extraction using the Bead-Beat Micro AX Gravity kit (A&A Biotechnology s.c., Gdańsk, Poland) according to the manufacturer's protocol. The extracted DNA samples were further resuspended into 50 µL deionized water. The quality and concentration of DNA was determined using a Nanodrop spectrophotometer (Thermo Scientific, Waltham, USA). One mock bacterial community standard (ZymoBIOMICS, #D6300) as a positive control was processed alongside experimental samples.

The bacterial biota was characterized on the basis of the V3 and V4 hypervariable region of the 16S rRNA gene using the next generation sequencing method [16]. NGS was performed by Genomed S.A. laboratory (Warsaw, Poland) using paired-end Illumina MiSeq sequencing on MiSeq platform (Illumina Inc., San Diego, USA). The specific sequences of 341F (5′-*TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG*C CTACG GG NGGCWGCAG-3′) and 785R(5′-*TCTCGTGGG CTCGGAGATGTGTATAAGAGACAG*GACTACHVGGGTA TCTAATCC-3′) primers with adaptors were used to amplify the selected region and prepare the library. The primers contain Illumina adaptor sequence (in *italics*) and V3-V4 16S rRNA locus specific sequence [17]. All steps, including amplification, indexing, and library quantification were performed according to the 16S Metagenomic Sequencing Library Preparation' protocol [18]. Briefly, the PCR reaction was performed with Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs Inc., Ipswich, USA) under the following conditions: initial denaturation step (95 °C for 3 min); 25 cycles at 95 °C for 30 sec, 55 °C for 30 sec, 72 °C for 30 sec, with a final extension at 72 °C for 5 min. The resulting amplicons were then indexed with Nextera XT Index Kit [18]. Library size was evaluated on Bioanalyzer 2100 DNA High Sensitivity chip (Agilent Scientific Instruments, Santa Clara, USA). Sequencing was performed on the MiSeq 2×300 PE (paired-end) in order to obtain at least 50,000 read pairs per sample.

The classification of reads to the species level was performed with the QIIME 2 software package based on a database of reference sequences Silva 138 [19]. DADA2 package was also used to separate sequences of biological origin from those newly generated in the sequencing process, as well as to identify unique sequences called ASV (amplicon sequence variant). To create a phylogenetic tree, they were aligned with the MAFFT algorithm after which the FastTree method was used to draw a tree. Hierarchical cluster analysis was performed based on Bray-Curtis dissimilarity matrices of relative abundance of bacterial operational taxonomic units (OTUs) in the R package program (version 4.1.3) [20, 21]. Additional extended bioinformatics analyses were performed using the R programme and the phyloseq and vegan packages.

Additionally, for PCR analyzes of anaerobic strains from *Clostridium* genus, genomic DNA from SCS samples was extracted using Genomic Mini Kit (A&A Biotechnology) according to protocol recommended by the manufacturer. After sampling, all collected anaerobic bacterial colonies on SCS agar plates were flooded with 2 mL of PBS, and then transferred into suspension by scraping. The resulting suspension was poured into a sterile 2 mL tube. 200 µL of the suspended material was then transferred to a new tube and centrifuged for 3 min at 7,000 rpm. In the next step, the supernatant was removed and the remaining pellet re-suspended in 145 µL digestive tap. Then, 25 µL of lysozyme (in concentration of 10 mg/mL) and 10 µL of lysostaphin (in

concentration of 1 mg/mL) were added and the whole mix incubated for 40 min at 37 °C. Further DNA isolation steps were performed according to the manufacturer's protocol. The concentration of isolated DNA (in ng/mL), as well as its purity, was checked with a spectrophotometer (model DS-11, DeNovix Inc., Wilmington, USA) at wave lengths of 260 nm and 280 nm.

PCR analyzes of pathogenic strains. The isolated pathogens were additionally analyzed by molecular methods (polymerase chain reaction (PCR). In total, 19 aerobic bacterial and 2 fungal species were thus analyzed. DNA was isolated from pure bacterial/fungal cultures grown on TSA/MEA plates using QIAmp DNA (Qiagen, Hilden, Germany) or Fungi DNA (Syngen Biotech, Wrocław, Poland) Mini Kits. The isolated bacterial DNA was used as a template in PCR with BAK11w (5′-AGTTTGATCMTGGCTCAG-3′) and BAK2 (5′-GGACTACHAGGGTATCTAAT-3′) primer sets, which allow amplification of bacterial 16S rRNA gene fragment corresponding to *Escherichia coli* 16S rRNA gene positions from 10–806.

The isolated fungal DNA was used as a template in PCR with ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′ – as biased towards amplification of basidiomycetes) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′ – as analytically 'favouring' amplification of ascomycetes) primer sets [22], which allow amplification of the fungal genome fragment located between genes 18S and 28S rRNA, covering ITS1, 5.8S rRNA gene, and the ITS2 fragments. The amplified PCR products were purified, sequenced using DNA analyzer (model 3730; Applied Biosystems, Waltham, USA), and compared to GenBank database (National Center for Biotechnology Information, US National Library of Medicine, USA) using BLAST (Basic Local Alignment Search Tool) algorithm [23–25].

Molecular identification of *Clostridium* isolates. For *Clostridium* species, as bacteria with the highest percentage share among anaerobic microorganisms, isolated DNA was used as a template for PCR reaction with primer pair specific to *Clostridium* genus, i.e. Chis150f (5′-AAAGGRAGATTAATACCGCATAA-3′) and ClostIr (5′-TTCTTCCTAATCTCTACGCA-3′) [26], allowing amplification of the gene fragment encoding 16S rRNA. The reaction mixture (20 µL) contained 2 µL of 10 × reaction buffer with $MgCl_2$, 0.5 U of RUN-HS Taq Polymerase (A&A Biotechnology), 250 µM of each deoxynucleotide (dNTP), 50 pmol of each primer and 0.5 µL of template DNA. Amplification included 35 cycles preceded by initial denaturation (95 °C, 5 min). Each cycle included: denaturation (95 °C, 15 sec), annealing (58 °C, 60 sec), and elongation (72 °C, 60 sec) steps. The reaction ended with a final elongation (72 °C, 5 min). The size of PCR product and the specificity of primers were checked using electrophoretic analysis in 1.5% agarose gel (Certified™ Molecular Biology Agarose, BioRad, Hercules, USA, and the product size compared with the DNA fragment marker (GeneRuler 1kb DNA Ladder, Thermo Scientific).

Testing for the presence of Marek's disease virus. The air samples were collected with Coriolis μ impinger (Bertin Technologies) for 20 min at a flow rate of 200 L/min using sterile sampling cones filled with 15 mL of universal viral transport medium (Capricorn Scientific GmbH, Ebsdorfergrund, Germany). After sampling, all liquid media were concentrated by ultrafiltration using an Amicon Ultra-15 (molecular weight cut-off 30 kDa) centrifugal filter device (Merck Millipore Ltd., Livingston, UK) at 3,200 × g for 20 min at 4 °C. The centrifugal concentration step was repeated until the entire volume of the sample passed through the filter to obtain the volume of 200 µL. The extraction of viral DNA/RNA from concentrated samples was carried out with Kogene Power Prep Viral DNA/RNA Extraction Kit CE-IVD (Kogene Biotech, Seoul, South Korea) according to the manufacturer's instructions to produce a final volume of 45 µL. Obtained RNA/DNA samples were stored at -20 °C until further analysis.

Quantitative polymerase chain reaction (qPCR) was performed using CFX96 real-time PCR thermocycler (BioRad, USA). Detection and quantification of GaHV-2 was carried out using Gallid herpesvirus 2 Meq gene genesig Standard Kit (PrimerDesign, Ltd., Chandler's Ford, UK). According to the manufacturer, the kit was designed to have the broadest possible detection profile while remaining specific to the viral genomes. Based on a comprehensive bioinformatics analysis, the applied primers and probe sequences of these kits had 100% homology with a broad range of GaHV-2 sequences. Each reaction mixture (20 mL) contained: 10 mL oasig Precision PLUS 2X qPCR MasterMix (PrimerDesign, Ltd.), 1 mL primer/probe mix, 4 mL RNase/DNase-free water, and 5 mL of DNA sample. All studied mixtures were elaborated as follows: enzyme activation at 95 °C for 2 min, followed by 49 cycles of denaturation at 95 °C for 10 sec, and annealing at 60 °C for 60 sec. According to the manufacturer's procedure, the fluorogenic data were collected through the FAM channel. The samples with quantification cycles, Cq, ≤ 35 were considered as positive.

Control of microclimate parameters. The environmental conditions (temperature and relative air humidity) at workplaces in the poultry house and in atmospheric (outdoor) air were recorded during every sampling session with thermohygrometer (model Omniport 20, E+E Elektronik GmbH, Engerwitzdorf, Austria). All microclimate parameter measurements were performed in triplicate.

Statistical analyzes. After checking the normality of data distributions with the Shapiro-Wilk test, the collected data were statistically elaborated by analysis of variance (ANOVA), *t*-test, and Pearson correlation analysis using Statistica (data analysis software system) version 10. (StatSoft, Inc., Tulsa, USA). Probability values were treated as statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Bacterial and fungal contamination of indoor and outdoor air. The concentrations of culturable aerobic and anaerobic bacteria as well as fungi in the air samples collected using four bioaerosol samplers are presented in Figure 1. In the case of aerobic bacteria, the concentrations at workplaces in the empty poultry house (a), in the poultry house with 7-day-old (b) and 42-day-old (c) chickens, as well as in background (outdoor) air (d) obtained using Andersen and MAS impactors, and Coriolis and BioSampler impingers, ranged between:

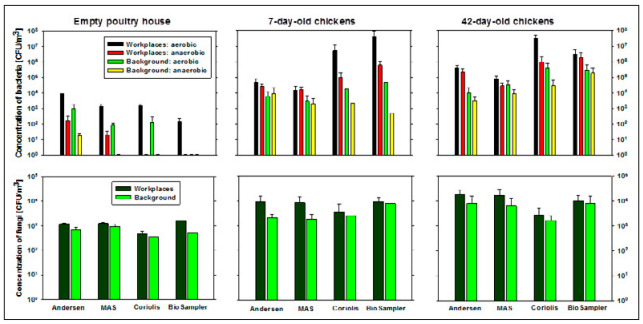

Figure 1. Bacterial and fungal concentrations in the poultry house air and background (outdoor) air measured using different bioaerosol samplers. The columns and whiskers represent mean concentrations and standard deviations, respectively

(a) 9.2–9.3×10³ CFU/m³ and 1.1–1.7×10³ CFU/m³ as well as 1.3–1.8×10³ CFU/m³ and 1.1–2.1×10² CFU/m³;
(b) 1.9×10⁴–1.1×10⁵ CFU/m³ and 1.3×10³–3.5×10⁴ CFU/m³ as well as 1.5×10⁵–1.9×10⁷ CFU/m³ and 2.1×10⁵–1.2×10⁸ CFU/m³;
(c) 2–6.6×10⁶ CFU/m³ and 2.4×10³–1.4×10⁴ CFU/m³ as well as 3.3×10⁶–7.6×10⁷ CFU/m³ and 5.8×10⁴–6.8×10⁶ CFU/m³, as well as
(d) 5.2×10²–2.4×10⁴ CFU/m³ and 7×10¹–6×10⁴ CFU/m³ as well as 1×10⁰–7.8×10⁵ CFU/m³ and 1×10⁰–5.9×10⁵ CFU/m³, respectively.

The respective anaerobic bacteria concentrations were as follows:
(a) 7.1×10¹–2.8×10² CFU/m³ and 1–3×10¹ CFU/m³ as well as 0–1×10⁰ CFU/m³ and 0–1×10⁰ CFU/m³;
(b) 1.4–4.4×10⁴ CFU/m³ and 9.9×10³–2.7×10⁴ CFU/m³ as well as 4.1×10³–2.2×10⁵ CFU/m³ and 6.4×10⁴–1.2×10⁶ CFU/m³;
(c) 4.7×10⁴–4.2×10⁵ CFU/m³ and 1.4–5.3×10⁴ CFU/m³ as well as 8.5×10³–3.8×10⁶ CFU/m³ and 3.6×10³–5.1×10⁶ CFU/m³, as well as
(d) 1.2×10¹–2.8×10⁴ CFU/m³ and 1×10⁰–1.9×10⁴ CFU/m³ as well as 1×10⁰–6.3×10⁴ CFU/m³ and 1×10⁰–3.7×10⁵ CFU/m³.

In turn, the respective values for fungi were as follows:
(a) 1.1–1.2×10³ CFU/m³ and 1.1–1.3×10³ CFU/m³ as well as 4–5.5×10² CFU/m³ and 1.6–1.7×10³ CFU/m³;
(b) 3.1×10³–2.3×10⁴ CFU/m³ and 2.4×10³–1.8×10⁴ CFU/m³ as well as 1.1×10²–8.7×10³ CFU/m³ and 3.2×10³–1.3×10⁴ CFU/m³;
(c) 3.7×10³–3.3×10⁴ CFU/m³ and 4.6×10³–3.8×10⁴ CFU/m³, as well as 9×10²–6.8×10³ CFU/m³ and 5.3×10²–1.7×10⁴ CFU/m³, as well as
(d) 3.5×10²–1.5×10⁴ CFU/m³ and 6×10²–1.3×10⁴ CFU/m³, as well as 3.5×10²–2.5×10³ CFU/m³ and 5.3×10²–1.5×10⁴ CFU/m³.

The concentrations of (aerobic) bacterial and fungal aerosols measured in the examined poultry house against the background of scientific literature data showed that they were at the levels of those observed in henhouses in (respectively):
– Switzerland (5.7×10⁵–1.6×10⁹ CFU/m³ and 1.4×10⁴–1.1×10⁸ CFU/m³) [27];
– Croatia (2.9×10³–6.6×10⁷ CFU/m³ and 0.7×10³–3.6×10⁷ CFU/m³) [28, 29];
– Romania (2.3×10⁵–2.2×10⁶ CFU/m³ and 1.7×10⁴–8.1×10⁴ CFU/m³) [30];
– France (6.6×10⁴–1.3×10⁷ CFU/m³ and 6.4×10¹–3.3×10⁴ CFU/m³) [31, 32];
– UK (2.7×10²–2×10⁸ CFU/m³ and 9.3×10¹–6×10⁵ CFU/m³) [33, 34];
– Egypt (4×10³–5.3×10⁷ CFU/m³ and 9.7×10²–2.7×10⁴) [35, 36];
– China (0.2×10⁻¹–4.5×10⁴ CFU/m³ and 0.2×10⁻¹–4.5×10³ CFU/m³) [37–39];
– South Korea (1.2×10³–1.8×10⁴ CFU/m³ and 2.5×10²–8.8×10³ CFU/m³) [40];
– Taiwan (5×10⁴–1.7×10⁷ CFU/m³ and 2.8×10²–4.7×10⁴ CFU/m³) [41];
– Australia (1.1×10⁵–6.4×10⁶ CFU/m³ and 4.4×10³–6.2×10⁵ CFU/m³) [42];
– USA (2.5×10³–3×10⁶ CFU/m³ and <1×10³–2.2×10³ CFU/m³) [43–45];
– in earlier Polish studies (2.5×10²–5.6×10⁶ CFU/m³ and 1.7×10²–3.2×10⁶ CFU/m³) [46–50].

Interpretation of the results of quantitative bioaerosol measurements performed in both indoor and outdoor environments is difficult due to the lack of widely-acceptable hygienic standards and/or threshold limit values (TLVs) for microbiological agents. Determination of a degree of such pollution expressed by the number of colony forming units (CFU) in 1 m³ of the air to-date is the best known and most frequently used measure of exposure to these contaminants [51]. In the hygienic assessment of poultry house workplaces and outdoor air surrounding the studied poultry farm, the elaborated TLVs, based on volumetric sampling methods proposed by the Expert Group on Biological Agents at the Polish Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment, was used (Tab. 1) [52, 53]. Comparison of bioaerosol concentrations with these hygienic standards showed that bacterial (including mesophilic and Gram-negative species) and fungal levels in the air measured at certain points in the production cycle (e.g. feeding or hay bedding heating in warmer periods of chicken growth) in the poultry house with 7-day-old and 42-day-old chickens, as well as outdoors in the vicinity of studied poultry house exceeded the TLVs established for this type of workplaces in occupational environment, as well as for ambient air.

Usability of impactors and impingers in quantitative assessment of poultry house bioaerosol. The analysis of

Table 1. The proposals of threshold limit values for microorganisms in the air at workplaces polluted with organic dust and in outdoor (atmospheric) air [52, 53]

Microbial agent	Workplaces polluted with organic dust	Outdoor (atmospheric) air	
		Acceptable level	Unacceptable level
Mesophilic bacteria	1×10⁵ CFU/m³	≤5×10³ CFU/m³	>5×10³ CFU/m³
Gram-negative bacteria	2×10⁴ CFU/m³	≤2×10² CFU/m³	>2×10² CFU/m³
Thermophilic actinomycetes	2×10⁴ CFU/m³	≤2×10² CFU/m³	>2×10² CFU/m³
Fungi	5×10⁴ CFU/m³	≤5×10³ CFU/m³	>5×10³ CFU/m³
Agents from risk groups 3 and 4	0 CFU/m³	0 CFU/m³	0 CFU/m³

culturable bioaerosol particle concentrations, irrespective of whether they were obtained using Andersen and MAS impactors or were measured with Coriolis and BioSampler impingers, did not show statistically significant differences between these 4 samplers (in all cases – ANOVA: $P > 0.05$). As can be seen, however, when chickens were introduced into the poultry house, the highest bacterial concentrations were noted, with impingers reaching in extreme cases the values of 1.26×10^8 CFU/m^3 and 5.07×10^6 CFU/m^3 for aerobic and anaerobic species, respectively. The opposite picture was visible for concentrations of airborne fungi. In this case, the highest levels of these pollutants were measured using impactors reaching 2.33×10^4 CFU/m^3 and 3.77×10^4 CFU/m^3 for the poultry house with 7-day-old and 42-day-old chickens, respectively.

These regularities may be due to the character of the medium used for the collection of microbial particles, their aerodynamic diameters conditioning the time period, in which they are suspended in the air, as well as the shape of the sampler inlet. In impingers, all microorganisms are 'gently' collected into the liquid, which minimize injury to the aspirated particles. This phenomenon is of particular importance, especially in the case of relatively fragile vegetative bacterial cells. Moreover, the bacterial cells are usually aerodynamically smaller (between 0.6–1.3 μm) than fungal conidia (between 1.7–5.6 μm) [54, 55]. Hence, their 'half-life' times (i.e. the time period, in which they remain airborne and potentially 'available' for sampling) are much longer (from several to several dozen hours) than the expected 'half-life' times for fungal conidia (from several to tens of minutes) [56, 57]. Furthermore, in impingers like the BioSampler or Coriolis, the sampler inlet is usually designed as a 'curved neck' (that simulates the human throat) to reduce cell injury, but at the same time, that inlet shape restricts larger bioaerosol particles from entering the sampler collection area. In the curved inlet tube, larger particles can already be deposited on the tube wall by inertial force and, by that, may not reach the liquid that was supposed to capture them or reach the sampling liquid, but in much smaller numbers [56, 58, 59].

On the other hand, in impactors, the physical sampling steps, such as aspiration through the jets in each impactor stage and subsequent deposition of particles onto the collection agar medium, could lead to particle losses and affect the representativeness of collected sample.

The following processes may play a crucial role in this context and affect the accuracy of sampling:

- too strong an impact of the aspirated particles with the air stream on the metal surface of the impactor stage or the dried collection (agar) surface, their friction against internal walls of the impactor inlet nozzles and drying of particles already deposited on the capture surface (desiccation stress) resulting in the loss of viability of microorganisms;
- a too low inertia force acting on particles in the impactor (related to the aerodynamic diameters of particles and the so-called cutoff size of the given impactor stage), allowing it to stay in the air stream passing through the sampler without the possibility of being deposited on the collection surface;
- the bounce of particles from the collection surface, resulting in their reaerosolization;
- the possibility of depositing more than one particle on the collection surface by the same inlet nozzle of the impactor stage;
- the possibility of clogging (e.g. with other aerosol particles, fibres or other debris) of the impactor nozzle during aspiration of the bioaerosol stream, and thus preventing the deposition of any microorganism on the collection surface [60–63].

Moreover, the biological features of the collected bacterial and fungal cells could also be an important source of the observed quantitative differences. The higher numbers of fungi (of which the vast majority consisted of filamentous fungi) collected using impactors may result from the different bacteria shapes and cellular structures influencing their survival abilities (i.e. from the most resistant round or globose, thick-walled conidia of e.g. *A. flavus*, *P. commune* or *P. brevicompactum*, to the most sensitive rod-shaped, thin-walled vegetative cells of e.g. *Pseudomonas* species) [12–14, 64].

As shown above, the concentrations of bacterial (both aerobic and anaerobic) and fungal aerosols at workplaces and in background air when the poultry house was empty, were much lower than these noted at workplaces and in background air when the poultry house was occupied by 7-day-old and 42-day-old chickens; however, statistical analyses confirmed significant differences between the empty poultry house and the poultry ouse with 42-day-old chickens only (at workplaces – in all 3 cases, i.e. for aerobic and anaerobic bacteria as well as fungi, Scheffé test: $P < 0.05$; in background air – also in all these 3 cases, Scheffé test: $P < 0.05$). Compared to the situations when chicken were already introduced indoors, the empty poultry house was characterized by relatively stable and low ambient concentrations of microbial propagules in the practically undisturbed atmosphere. Despite this, in all 3 tested variants, i.e. when poultry house was empty or housed 7-day-old and 42-day-old chickens, indoor bioaerosol concentrations of aerobic and anaerobic bacteria, as well as fungi, always exceeded those recorded in the atmospheric air, regardless of the sampler by which they were measured. Statistical analyses (*t*-test) confirmed these differences between workplace and background concentrations as significant in the case of:

Andersen impactor – in the empty poultry house for aerobic bacteria ($P < 0.01$);

- in henhouse with 7-day-old chickens for aerobic ($P < 0.01$) and anaerobic ($P < 0.05$) bacteria;
- in poultry house with 42-day-old chickens for aerobic ($P < 0.001$) and anaerobic ($P < 0.05$) bacteria, as well as for fungi ($P < 0.05$);

MAS impactor – in empty poultry house for fungi ($P < 0.05$);

- in henhouse with 7-day-old chickens for aerobic ($P < 0.05$) and anaerobic ($P < 0.01$) bacteria, as well as for fungi ($P < 0.05$);
- in poultry house with 42-day-old chickens for aerobic ($P < 0.05$) and anaerobic ($P < 0.05$) bacteria;

Coriolis impinger – in empty poultry house and in henhouse with 42-day-old chickens for aerobic bacteria (in both cases: $P < 0.05$);

BioSampler impinger – in empty poultry house for fungi ($P < 0.001$).

Environmental dissemination of microbiological aerosols. Such a distribution of pollutants, confirmed by statistically

significant relationships, in the absence of significant sources of microbiological emission in the vicinity of the chicken farm, suggests that the tested poultry house itself was a significant point source of bioaerosol emission to the atmosphere. In order to confirm this thesis, and thus to illustrate the environmental dissemination of microbial pollution, the measurements of bacterial and fungal aerosol concentrations were carried out using the MAS impactor (as battery operated device) within the area around the tested poultry house.

Pursuant to the Ordinance of the Minister of the Environment of 26 January 2010 on reference values for certain substances in the air (specifying, among others, reference methodologies for modeling their levels), the impact zone around the poultry house as point emitter was defined as 50 times the height of this emission source [65, 66]. Taking into account the height of the emitter, i.e. the height of the exhaust chimney of the poultry house ventilation system – 5 m (calculated from the ground level), the area around the tested poultry house where the immission is expected to be the highest, was 250 m. Moreover, since the nearest residential buildings were located at a distance of about 500 m from the tested poultry house, this maximum distance was adopted to determine the grid of bioaerosol measurement points. Furthermore, taking into account that the highest species richness and diversity of studied microbiota in the poultry house was noted for 42-day-old chickens (see NGS results below), dissemination measurements had to be carried out over a fairly large area in stable weather conditions, i.e. primarily in the absence of precipitation, it was decided to carry out these tests in the 'summer' season. Figure 2 shows the location of 17 measurement points of the grid along with bioaerosol concentrations measured therein. The average concentrations of bacterial (Fig. 2a) and fungal (Fig. 2b) aerosols in the source, i.e. inside the studied poultry house, near the ventilation system chimney inlet (marked in red), were 3,240 CFU/m^3 and 31,470 CFU/m^3, respectively. For bacterial aerosol, its average concentration drops at distances of 250 m and 500 m from the centre of the poultry house, and were 2.47-fold and 1.70-fold, respectively; however, these reductions – although clearly visible – were not statistically significant ($P > 0.05$).

In the case of fungal aerosol, its average concentration decreased at the same distances of 250 m and 500 m from the studied poultry house and were much more pronounced (8.09-fold and 13.68-fold, respectively) and statistically significant ($P < 0.05$). The pollutant plume leaving the emitter is subject to complex processes during its movement, which are fundamentally influenced by topographic (including: topography, type of land cover and ways of its use, collectively defined as aerodynamic area roughness coefficient) and meteorological (wind speed and direction, atmospheric diffusion and turbulence, temperature gradient, mixing layer thickness, precipitation and chemical changes in the air) factors [66–68]. Since culturable microorganisms were considered in this study, their numbers in the air were certainly influenced by factors causing their inactivation leading to their death or elimination due to certain environmental or meteorological conditions, such as high temperatures, ultraviolet radiation, and humidity changes [68].

As dissemination measurements were performed in the warmer season (i.e. in 'summer'), some of the above factors without a doubt played a crucial role in influencing the results presented above; however, from among those determinants only the influence of air temperature and humidity were controlled in more detail (see below). Comparison of directional concentration distributions with the wind rose at the location of the studied poultry house (Fig. 3), and revealed that they did not coincide with the main directions of atmospheric air flows around the poultry house. This may indicate that local fluctuations in the direction of the stream inflow may be of great importance, and incidentally – quite naturally in this case – the conducted concentration measurements may only give a picture of the temporary distribution of microbial pollutants. However, on days where the wind strength was not very important (so-called days with no wind and/or with light wind when turbulences are suppressed), the basic properties of aerosol particles determined the possibility of their environmental dissemination [56, 69, 70]. In this context, the observed trends can be explained, in part at least, by the physical form in which these particles exist being suspended in the air, characterized by real (i.e. measured) size distribution

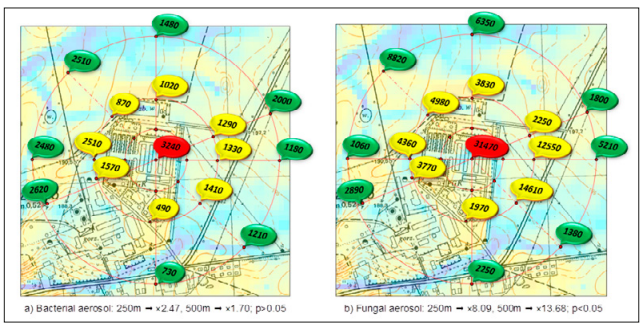

Figure 2. Environmental dissemination of bacterial (a) and fungal (b) aerosols around the poultry house. The average bioaerosol concentrations inside the poultry house and at the distance of 250 m and 500 m from the studied facility, are marked in red, yellow and green, respectively

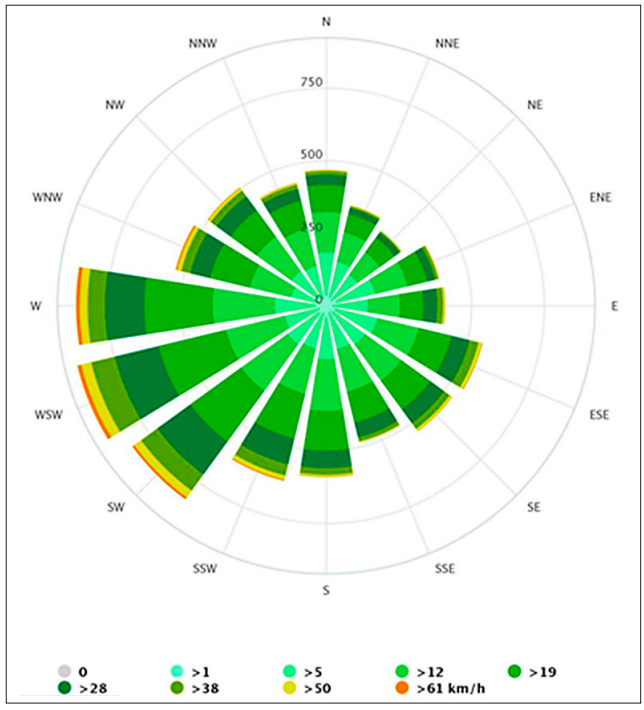

Figure 3. Wind rise for the poultry house area. The respective colours indicate the number of hours in a year when the wind blews at a certain speed in a specific direction.
Source: meteoblue.com

of disseminated microbiological particles (see below) and 'half-life' times closely related to their airborne diameters. Bacterial aerosol particles were environmentally spread mainly as small bacterial and/or bacterial-dust aggregates, whereas fungal aerosol particles as single conidia and coarse fungal and/or fungal dust aggregates (Figure 7, green and black step plots). As such, bacterial propagules were able to maintain their airborne presence somewhat longer that the fungal propagules, and with that their concentration dropped at the distances of 250 m and 500 m from the emission source, and were less pronounced than that observed for fungal aerosol.

Airborne microbial contaminants generated by the animal farms may affect the quality of neighbourhood atmosphere and the health of both animals and farm employees [8, 37, 38]. Studies on the environmental spread of microbial particles revealed that generally the concentrations of microbial pollutants decreased with a distance from the poultry house. Hartung and Schulz [71] measuring the concentrations of species from the *Staphylococcaceae* family (i.e. according to the authors, the typical bacteria in broiler house that can serve as indicator for bacterial pollution of the air) noted ~10-fold and ~20-fold decreases between the source of emission (i.e. poultry house) and locations 250 m and 500 m away from the source, respectively. In turn, Gladding et al. [34], studying airborne transport of microorganisms from broiler farm, observed ~7.8-fold and ~16-fold drops in bacterial and fungal concentrations, respectively, at points located 250 m from a chicken broilers farm. A much smaller (1.4-fold) decrease in bacterial concentrations in the air was noted by Plewa-Tutaj et al. [72]; however, the measurement point was placed only 100 m from the chicken farm. On the other hand, Baykov and Stoyanov [73], controlling microbial contamination of the atmospheric air in the vicinity of an industrial broiler farm, noted a 1,335-fold decrease between the production buildings (16,020±320 CFU/m^3) and measuring stations located 500 m from the poultry buildings (12±4 CFU/m^3).

The distance from the emission source in the form of an intensive chicken production farm influences the adverse health outcomes observed among both workers and residents living in a vicinity of the farm. van Dijk et al. [74] found a higher prevalence of respiratory symptoms among residents living within 500 m from a poultry farm housing more than 14,000 birds. They also confirmed chronic obstructive pulmonary disease if the residents lived within a 500 m radius from the poultry farm with chicken densities up to 12,499 birds [75]. Moreover, Borlée et al. [76] found a positive association between the distance of poultry farm (500 m) and atopic asthma among individuals living in its vicinity. Radon et al. [77] also observed that individuals living with more than 12 animal houses within 500 m of their home had a higher odds ratio for wheezing (without cold), decreased FEV_1, and lower FEV_1/FVC ratio.

Qualitative analyzes of airborne microbiota. To qualitatively characterize the exposure at workplaces in the poultry house and in the background air around it, the collected airborne microbiota was taxonomically identified using molecular (NGS and PCR) and biochemical (API tests) techniques. In comparison with conventional culture methods, 16S sequence analyses have a higher sensitivity for the detection of specific bacteria, and, as such, can be used for accurate bacterial species identification [78, 79]. As 16S metagenomic sequencing also provides a comprehensive tool for identification of both human and animal pathogens [80, 81], including those that are non-culturable or cannot be identified based on phenotypes, the qualitative characteristics of isolated bacterial biota started from this analysis. A total of 6 samples were assessed in this way, i.e. 5 randomly selected bacterial aerosol samples collected using an Andersen impactor at workplaces in the poultry house representing the environmental conditions as follows: 7-day-old chickens during 'winter' (K1) and 'summer' (K3) seasons, 42-day-old chickens during 'winter' (K2) and 'summer' (K4) seasons, empty poultry house (K5), and control (ZYMO) sample (K6). The samples from the Andersen impactor were selected for such analysis as this 6-stage sampler has been recommended and used as a reference device [56, 82–89], and impaction is a widely approved method of bioaerosol collection [60, 90]. Although Andersen impactor utilizes Petri type plates filled with appropriate agar as a medium suitable for sampling and subsequent growth of collected microorganisms, successful attempts have been made to scrape off the deposited microbial propagules with water, and analyze them using molecular methods [91].

The NGS analysis allowed to obtain 436,913 raw sequence reads from all tested bioaerosol and control samples. The average number of reads per sample was 72,819 (SD=7543.7). This study revealed the presence of 356 amplicon sequence variants (ASVs), among which all belonged to bacteria. Archaea were not found in the studied microbial communities. All ASVs were assigned into 4 phyla, 7 classes, 27 orders, 49 families, 94 genera, and 115 species. A phylogenetic summary of the results is presented in Table 2. Among the tested samples, the bacterial biota in poultry houses with 42-day-old chickens had the highest species richness and diversity (Tab. 3). The highest values of alpha diversity (evaluation from within sample [20]) were obtained for these two (K2 and K4) samples, including the richness (based on the observed OTU values and Chao index) and diversity (based on Shannon and Simpson indexes). The bacterial aerosol samples collected at workplaces in the poultry house with 7-day-old chickens (K1 and K3) and in the empty poultry house (K5) were less rich and taxonomically diverse.

Table 2. Percentage of reads assigned to appropriate taxonomic levels for analyzed bacterial aerosol samples

Sample	Kingdom	Phylum	Class	Order	Family	Genus	Species
K1	100	100	100	100	100	99.89	92.12
K2	100	100	100	100	99.99	99.05	60.90
K3	100	100	100	100	100	100	55
K4	100	100	100	100	100	99.97	63.82
K5	100	100	100	100	100	99.52	28.47
K6 (control)	100	100	100	100	100	100	79.33

Table 3. Alpha diversity indexes for analyzed bacterial aerosol samples

Sample	Observed TUs	Chao1	Shannon	Simpson
K1	53	53.33	2.45	0.88
K2	151	220.00	3.40	0.94
K3	81	82.00	2.88	0.91
K4	117	144.50	3.18	0.92
K5	59	66.00	2.22	0.84
K6 (control)	18	18.00	2.31	0.89

The beta diversity, indicating sequence variant composition differences among microbial communities between bioaerosol samples [20], was observed for samples collected at different stages of chicken breeding (Figs. 4 and 5). The principal coordinate analysis (PCoA) showed a high similarity in the structure of bacterial biota between K2 and K4 samples collected at workplaces in the poultry house with 42-day-old chickens in 'winter' and 'summer' seasons, as well as between K1 and K2 samples (i.e. with 7-day-old and 42-day-old chickens, respectively) taken therein during 'winter' season. These results visualized using PCoA are shown in Figure 4.

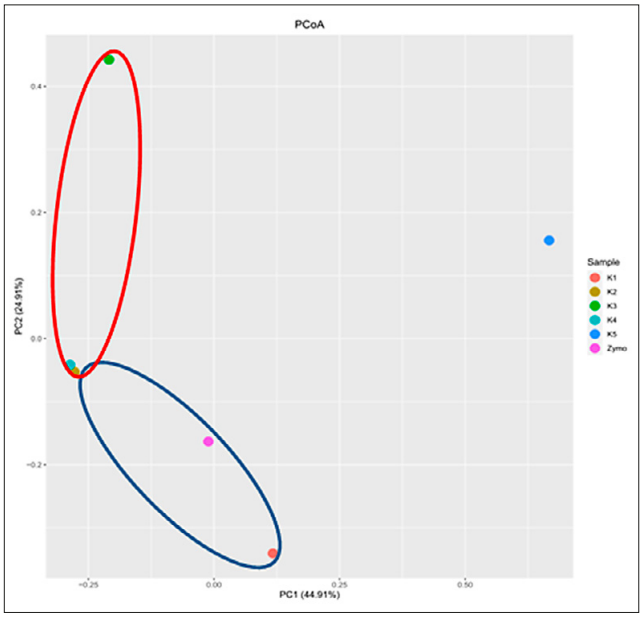

Figure 4. Bray-Curtis distance metric showing overall variation in bacterial communities during different stages of chicken breeding, visualized using principal coordinate analysis (PCoA). Blue and red ellipses represent the winter and summer seasons, respectively

In turn, the patterns of bacterial community beta diversity based on taxonomic assignments to genus level are shown as a heat map in Figure 5. Figure 7 shows the observed alpha diversity analysis and the beta diversity evaluation, which also identified some patterns in bacterial biota structure corresponding to different stages of chicken breeding among site-specific microbiomes, especially between samples from the poultry house with 42-day-old chickens. The notable different community composition across the collected samples was found in the sample from empty the poultry house (K5). In order to highlight the differences observed in both alpha and beta diversity analyses, the resulting data are additionally presented in the form of heat trees showing the differences in abundance of each taxon in the analyzed bacterial aerosol samples (Fig. 6) [92–94].

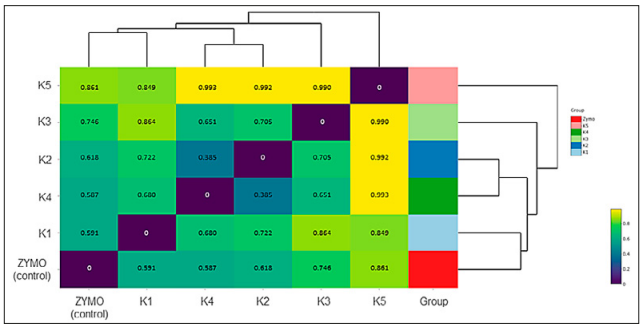

Figure 5. Heat map illustrating Bray-Curtis similarities of bacterial aerosol samples based on taxonomic assignments to genus level. The colour intensity scale reflects the composition similarity

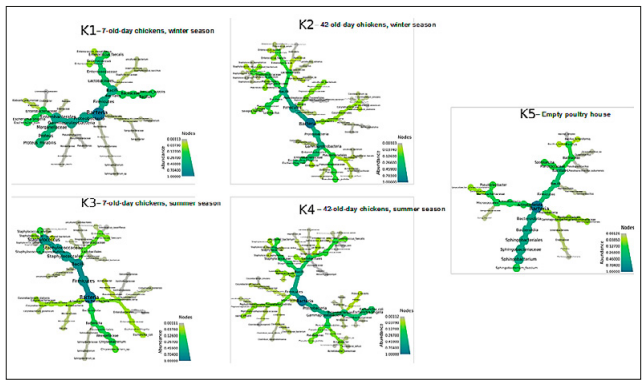

Figure 6. Heat tree visualizations of taxonomic distribution of bacterial population in bioaerosol samples from workplaces in the poultry house. The size and colour of nodes and branches are correlated with the abundance of microorganism sequences in each studied community

The 16S amplicon sequencing enabled qualitative profiling and estimation of relative abundance of bacterial strains at different taxonomic levels. The bacterial community of tested aerosol samples consisted of representatives of 4 phyla: *Actinobacteriota* (*Actinomycetota*), *Bacteroidota* (*Bacteroidetes*), *Firmicutes* (*Bacillota*), and *Proteobacteria* (*Pseudomonadota*). Their percentage contributions to the total microbiota varied depending on the production cycle stage and season. In the empty poultry house (K5), the air was dominated by bacteria belonging to the following phyla: *Bacteroidota* (48.5%), *Firmicutes* (28.5%), *Actinobacteriota* (17.5%), and *Proteobacteria* (5.5%) (Fig. 7a). This picture

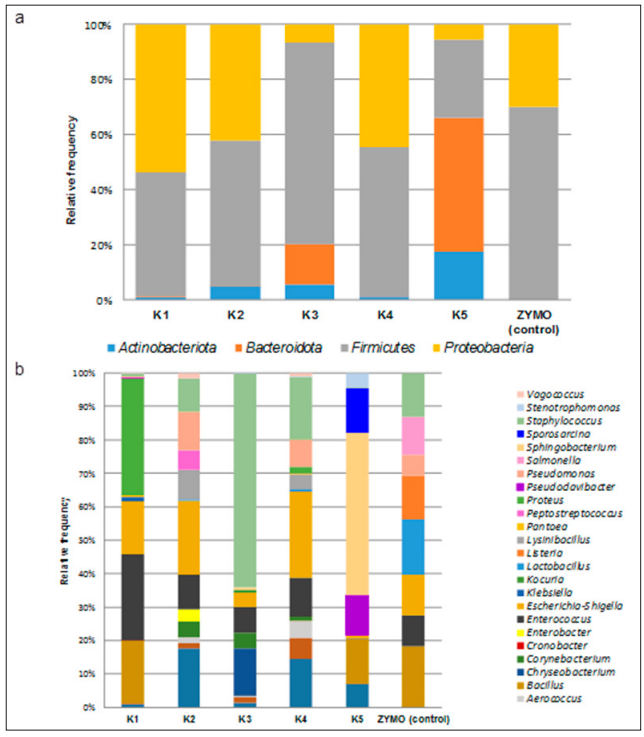

Figure 7. Bacterial community characteristics at phylum (a) and genus (b) levels

changed profoundly after introduction of a new young chicken flock to the poultry house. When 7-day-old chickens entered the poultry house, the *Firmicutes* and *Proteobacteria* phyla began to dominate in the air. This situation stabilized after 5 weeks and the structure of bacterial community in the poultry house with 42-day-old chickens in both 'winter' and 'summer' seasons was already very similar (K2 vs. K4). The comparison of these results with other bioaerosol studies revealed that similar regularities were observed in poultry houses in China [16, 39, 95, 96] and Australia [97]; however, O'Brien et al. [98] studying American broiler production facilities noted the dominance of *Proteobacteria* over *Firmicutes* and *Actinobacteriota*. Analyzing the bacterial community at genus level, in samples collected indoors with 7-day-old chickens, the most common taxa were *Staphylococcus* (32.4%), *Proteus* (17.5%), *Enterococcus* (16.7%), *Escherichia-Shigella* (10%), and *Bacillus* (9.3%), while with 42-day-old chickens there prevailed *Escherichia-Shigella* (24%), *Staphylococcus* (14.3%), *Enterococcus* (11%), and *Pseudomonas* (9.8%) (Fig. 7b). A very similar structure was described by researchers from e.g. Austria [99], Germany [71], Croatia [28], UK [34], and USA [43, 98] (see below).

In turn, with regard to the qualitative composition of poultry house mycobiota, the phyla with the highest contents in all analyzed bioaerosol samples, were *Ascomycota*, *Basidiomycota*, and *Mucormycota*, which is in good agreement with the results of studies by e.g. Chen et al. [37], Nieguitsila et al. [32], Radon et al. [27], Viegas et al. [100], and Yang et al. [39] studies. The analysis of airborne mycobiota at the genus and species levels in the studied poultry house revealed the presence of taxa characteristics for chicken farms and already identified on different continents by e.g. Egyptian [35], Chinese [39, 95], American [98], Australian [97], Austrian [99], French [32], and other Polish [47] authors (Tab. 4).

From the view point of the health status of both employees and reared chickens, an important element of the qualitative analysis was the characteristics of viable air microbiota of the studied poultry house, taking into account the infectious potential of human and animal pathogens. Moreover, in order to evaluate the usefulness of 6-stage Andersen and single-stage MAS impactors, as well as Coriolis and BioSampler impingers, for the assessment of microbiological air quality at workplaces in poultry house and in the vicinity of the source of such intense bioaerosol emission, all the air samples collected using these 4 sampling instruments were subjected to a detailed species analysis. It is known that Coriolis and BioSampler impingers are the collectors that have unidirectional inlets and as such are sensitive to both direction of the air stream and its velocity [83, 101]. To be effective, they should operate within the plume of incoming particles from which they collect them. As the conditions outside the henhouse were highly variable and turbulences created by the air stream around the building made impossible to clearly determine the direction of stream inflow, only 2 impactors offering omnidirectional sampling and high physical and biological collection efficiencies were used in the tests of the outdoor air [56, 82–88].

Percentage contribution of bacterial and fungal groups to the total viable microbiota and its species composition. The results of percentage contribution of microbial groups to the total viable bacterial and fungal biota isolated from the air at workplaces of the poultry house and in the background (outdoor) environment using different bioaerosol samplers together with qualitative characteristics of the air samples to the species level are presented in Figures 8 and 9, and Table 4. In total, 126 (including 108 aerobic and 18 anaerobic) bacterial species belonging to 52 (42 and 10, respectively) genera and 63 fungal species (48 filamentous fungi and 15 yeasts) belonging to 23 genera (17 and 6, respectively) were identified (Tab. 4). Analysis of percentage contribution showed that the dominant groups of culturable microorganisms in the air at workplaces and in outdoor environment were Gram-positive cocci, endospore forming Gram-positive rods, and filamentous fungi (Fig. 8). The results obtained at workplaces using 4 different bioaerosol samplers revealed a fairly uniform trend in this respect (for both bacteria and fungi – ANOVA ($P > 0.05$).

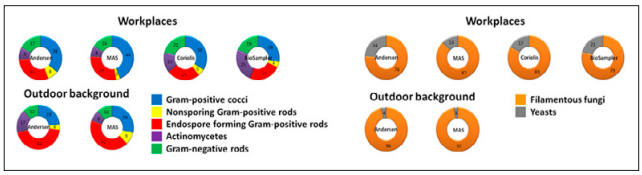

Figure 8. Percentage contribution of microbial groups to the culturable bacterial and fungal biota isolated from the air of the poultry house, and background (outdoor) air using different bioaerosol samplers

The percentage distributions of culturable microbiota determined with Andersen and MAS impactors did not show significant differences in the qualitative composition of bioaerosol between the workplaces and outdoor background (in the case of bacteria and fungi, for both impactors in all *t*-tests ($P > 0.05$); however, attention should be paid to the clearly visible prevalence of endospore-forming Gram-positive rods in the atmospheric (outdoor) air (compared to the studied workplaces), reaching almost half of the culturable bacterial species isolated. Moreover, despite the lack of statistically significant differences in the percentage contribution, the use of 4 different bioaerosol aspirators in this study showed dissimilarity in the numbers of isolated microbial species between them. Of the 4 samplers used, in the case of aerobic and anaerobic bacteria as well as fungi, the Andersen impactor (compared to MAS impactor as well as Coriolis and BioSampler impingers) always showed higher numbers of culturable species in the air samples (i.e. 60/11/55 compared to 26/11/37, 45/11/13, and 29/6/10, respectively). However, statistical analysis confirmed this regularity for differences in the numbers of isolated fungal species between Andersen impactor and both impingers (in both cases – Tukey tests ($P < 0.05$) (Fig. 9).

In the poultry house, the taxonomic structure of bacterial aerosol wa usually variable and depended on the quality

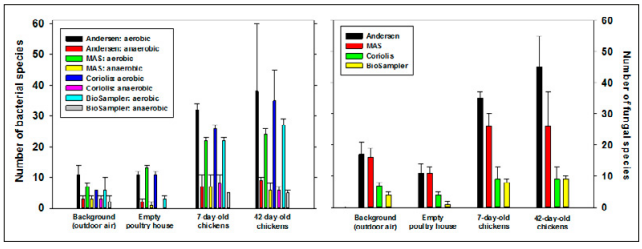

Figure 9. Numbers of microbiological species identified by different bioaerosol samplers. Columns and whiskers represent mean concentrations and standard deviations, respectively.

Table 4. Viable microorganisms isolated from the air at workplaces in poultry house and in background (outdoor) environment. Species affiliation to specific phylum is indicated as follows: A – *Actinobacteriota*, B – *Bacteroidota*, P – *Proteobacteria*, F – *Firmicutes*, Am – *Ascomycota*, Bm – *Basidiomycota*, and M – *Mucoromycota*. The species having potential to compose more than 50% of isolates in individual sample (in specific environment at specific stage of chicken breeding) are given in squares

Microorganisms			Phyla	Background (outdoor air)	Empty poultry house	Poultry house with 7-day-old chickens	Poultry house with 42-day-old chickens
Aerobic bacteria	Gram-positive cocci	*Aerococcus* spp.	F				✓
		Enterococcus avium	F		✓		✓
		Enterococcus durans	F			✓	✓
		Enterococcus faecalis	F	✓		✓	✓
		Enterococcus faecium	F	✓		✓	✓
		Enterococcus gallinarum	F			✓	
		Enterococcus hirae	F				✓
		Enterococcus malodoratus	F			✓	✓
		Enterococcus spp. *	F	✓		✓	✓
		Gemella morbillorum	F				✓
		Kocuria kristinae	A				✓
		Kocuria rosea	A			✓	
		Kocuria varians	A				✓
		Kocuria spp.	A				✓
		Micrococcus spp.	A	✓		✓	✓
		Staphylococcus aureus *	F	✓		✓	✓
		Staphylococcus auricularis	F	✓		✓	✓
		Staphylococcus capitis ssp. *capitis*	F			✓	✓
		Staphylococcus capitis ssp. *urealyticus*	F			✓	
		Staphylococcus caprae	F				✓
		Staphylococcus chromogenes	F	✓			✓
		Staphylococcus epidermidis	F	✓		✓	✓
		Staphylococcus gallinarum	F				✓
		Staphylococcus haemolyticus	F				✓
		Staphylococcus hyicus	F	✓		✓	
		Staphylococcus intermedius	F				✓
		Staphylococcus kloosii	F				✓
		Staphylococcus lentus	F	✓		✓	✓
		Staphylococcus saprophyticus	F	✓		✓	✓
		Staphylococcus sciuri	F				✓
		Staphylococcus simulans	F				✓
		Staphylococcus warneri	F	✓	✓	✓	✓
		Staphylocuccus xylosus	F	✓	✓	✓	✓
		Staphylocuccus spp.	F	✓	✓	✓	✓
		Streptococcus mutans	F			✓	
		Streptococcus suis *	F	✓		✓	
		Streptococcus spp. *	F			✓	✓
		Vagococcus fluvialis	F				✓
	Nonsporing Gram-positive rods (NG+r)	*Arthrobacter* spp.	A			✓	
		Brevibacterium spp.	A			✓	✓
		Cellulomonas spp.	A	✓		✓	
		Corynebacterium amycolatum	A			✓	
		Corynebacterium propinquum	A			✓	
		Corynebacterium striatum	A				✓
		Corynebacterium spp. *	A			✓	
		Listeria grayi	F				✓
		Listeria innocua	F			✓	✓
		Listeria monocytogenes *	F			✓	✓

Microorganisms		Phyla	Background (outdoor air)	Empty poultry house	Poultry house with 7-day-old chickens	Poultry house with 42-day-old chickens
	Listeria welshimeri	F				✓
	Listeria spp.	F			✓	✓
	Microbacterium spp.	A	✓	✓	✓	✓
	Oerskovia spp.	A	✓	✓		
	Rothia spp.	A			✓	✓
Endospore forming Gram-positive rods	*Bacillus cereus*	F	✓	✓		✓
	Bacillus firmus	F			✓	✓
	Bacillus licheniformis	F	✓	✓	✓	✓
	Bacillus megaterium	F	✓	✓	✓	
	Bacillus spp.	F	✓	[✓]	✓	✓
	Brevibacillus spp.	F			✓	
	Lactobacillus johnsonii	F				✓
	Paenibacillus spp.	F		✓		
Actinomycetes (Act.)	*Actinomyces* spp. *	A	✓		✓	
	Rhodococcus equi	A			✓	✓
	Rhodococcus spp.	A			✓	✓
	Streptomyces albus *	A			✓	
	Streptomyces spp. *	A	✓	✓		✓
Gram-negative rods	*Acinetobacter* spp.	P				✓
	Aeromonas hydrophila	P				✓
	Alcaligenes facealis	P			✓	
	Burkholderia cepacia *	P	✓			✓
	Citrobacter freundii	P		✓		✓
	Citrobacter koseri	P				✓
	Citrobacter spp.	P				✓
	Enterobacter asburiae	P				✓
	Escherichia coli	P	[✓]		[✓]	✓
	Hafnia alvei	P			✓	✓
	Klebsiella oxytoca *	P	✓		✓	✓
	Klebsiella pneumoniae *	P			✓	✓
	Klebsiella spp. *	P				✓
	Ochrobactrum anthropi	P	✓		✓	
	Ochrobactrum spp.	P	✓			✓
	Pantoea agglomerans *	P			✓	
	Pantoea spp.	P				✓
	Pasteurella multocida *	P				✓
	Proteus mirabilis *	P	✓		✓	✓
	Proteus spp.	P				✓
	Pseudomonas aeruginosa *	P	✓			✓
	Pseudomonas caricapapayae	P			✓	
	Pseudomonas fluorescens	P			✓	
	Pseudomonas fulva	P				✓
	Pseudomonas graminis	P	✓		✓	
	Pseudomonas libanensis	P			✓	
	Pseudomonas luteola	P	✓			✓
	Pseudomonas oleovorans	P			✓	
	Pseudomonas oryzihabitans	P	✓	✓	✓	✓
	Pseudomonas putida	P	✓	✓	✓	✓
	Pseudomonas savastanoi	P	✓		✓	
	Pseudomonas spp.	P	✓	✓	[✓]	✓
	Ralstonia pickettii	P				✓

Microorganisms			Phyla	Background (outdoor air)	Empty poultry house	Poultry house with 7-day-old chickens	Poultry house with 42-day-old chickens
		Salmonella spp. *	P			✓	✓
		Serratia fonticola	P	✓			✓
		Serratia liquefaciens	P				✓
		Serratia odorifera	P				✓
		Serratia spp.	P				✓
		Shigella sonnei *	P			✓	✓
		Shigella spp.	P			✓	✓
		Sphingobacterium faecium	B				✓
		Sphingobacterium spp.	B				✓
		Sphingomonas paucimobilis	P				✓
		Stenotrophomonas maltophilia	P				✓
Anaerobic bacteria	Gram-positive cocci	*Peptostreptococcus anaerobius* *	F	✓			✓
		Peptostreptococcus spp.	F	[✓]			✓
		Staphylococcus saccharolyticus	F	[✓]		[✓]	✓
		Streptococcus intermedius	F	✓		[✓]	
	NG+r	*Bifidobacterium* spp.	A			✓	
		Propionibacterium granulosum	A	✓		✓	
	Endospore forming Gram-positive rods	*Clostridium beijerinckii/butyricum*	F			✓	
		Clostridium difficile *	F	✓		✓	✓
		Clostridium perfringens *	F	✓			✓
		Clostridium ramosum	F			[✓]	✓
		Clostridium tertium	F	✓		[✓]	
		Clostridium spp. *	F	[✓]	[✓]	[✓]	[✓]
	Act.	*Actinomyces* spp. *	A	[✓]	✓	[✓]	[✓]
	Gram-negative rods	*Bacteroides caccae*	B	✓			✓
		Bacteroides spp. *	B	✓		✓	
		Fusobacterium spp.	F			✓	
		Prevotella intermedia	B			✓	
		Prevotella spp. *	B			✓	✓
Fungi	Filamentous fungi	*Absidia* spp.	M	✓		✓	✓
		Acremonium spp.	Am	✓			✓
		Alternaria alternata	Am	✓	✓	✓	✓
		Alternaria strictum	Am	✓			✓
		Alternaria spp.	Am	✓		✓	✓
		Aspergillus clavatus	Am	✓	✓	✓	✓
		Aspergillus flavus *	Am	✓		✓	✓
		Aspergillus fumigatus *	Am			✓	✓
		Aspergillus glaucus	Am	✓		✓	✓
		Aspergillus nidulans	Am	✓		✓	✓
		Aspergillus niger	Am	✓	✓	✓	✓
		Aspergillus ochraceus	Am	✓		✓	✓
		Aspergillus sydowii	Am	✓		✓	✓
		Aspergillus terreus	Am	✓		✓	✓
		Aspergillus versicolor	Am	✓		✓	✓
		Aspergillus wentii	Am				✓
		Chrysosporium spp.	Am	✓		✓	✓
		Cladosporium cladosporioides	Am	✓			
		Cladosporium herbarum	Am	✓	✓	✓	✓
		Cladosporium spp.	Am	✓		✓	✓
		Curvularia pallescens	Am	✓		✓	✓
		Epicoccum nigrum	Am	✓		✓	✓

Microorganisms		Phyla	Background (outdoor air)	Empty poultry house	Poultry house with 7-day-old chickens	Poultry house with 42-day-old chickens
	Fusarium culmorum	Am	✓		✓	✓
	Fusarium oxysporum	Am	✓	[✓]	✓	✓
	Fusarium solani	Am	✓		✓	✓
	Fusarium spp.	Am	✓		✓	✓
	Geomyces spp.	Am			✓	✓
	Mucor circinelloides	M			✓	✓
	Mucor plumbeus	M	✓		✓	✓
	Mucor spp.	M	✓		✓	✓
	Penicillium brevicompactum	Am	[✓]	✓	[✓]	✓
	Penicillium chrysogenum	Am	✓	✓	✓	✓
	Penicillium citreonigrum	Am	✓		✓	✓
	Penicillium citrinum	Am	✓		✓	✓
	Penicillium commune	Am	[✓]	[✓]	✓	✓
	Penicillium digitatum	Am	✓		✓	✓
	Penicillium expansum	Am	✓		✓	✓
	Penicillium fellutanum	Am			✓	
	Penicillium glabrum	Am			✓	✓
	Penicillium italicum	Am	✓		✓	✓
	Penicillium verrucosum	Am			✓	
	Rhizopus spp.	M	✓	✓	✓	✓
	Sarocladium strictum	Am				✓
	Scopulariopsis brevicaulis	Am			✓	✓
	Scopulariopsis fusca	Am				✓
	Scopulariopsis spp.	Am			✓	✓
	Talaromyces rugulosus	Am			✓	✓
	Ulocladium spp.	Am				✓
Yeasts	*Candida ciferrii*	Am	✓	✓		✓
	Candida colliculosa	Am			✓	✓
	Candida famata	Am			✓	✓
	Candida krusei	Am				✓
	Candida lambica	Am				✓
	Candida lusitaniae	Am				✓
	Candida magnoliae	Am				✓
	Candida rugosa	Am			✓	✓
	Candida thermophila	Am			✓	✓
	Cryptococcus albidus	Bm			✓	✓
	Geotrichum candidum	Am	✓		✓	✓
	Rhodotorula minuta	Bm	✓		✓	✓
	Rhodotorula mucilaginosa	Bm	✓		✓	✓
	Saccharomyces cerevisiae	Am				✓
	Trichosporon asahii	Bm				✓

* pathogens from risk group 2 according to Commission Directives (UE) 2019/1833 [118] and Ordinance of Polish Minister of Health [119], identified using both biochemical (API) and molecular (PCR) methods

of the litter (contaminated with faeces), feed, purity of water, frequency of feeding (all of which were responsible for additional emission of particulate pollutants when disturbed by air flow and/or animal activities), the birds themselves (exhaled air, faecal or skin microbiota), and microclimatic conditions accompanying the chickens' stocking in particular seasons. Poultry litter is primarily a mixture of bedding materials (e.g. chopped straw, sawdust, wood shavings, and rice hulls), bird excreta, dander, and feed [102]. During the growth a chicken, a constant influx of nutrients and faeces together with permanent availability of water, results in a complex litter microbiota [103]. Poultry litter (especially in fresh bedding) is usually dominated by *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria* phyla [e.g. 97, 103, 104]. Most bacteria in the poultry litter are Gram-positive, including both aerobic

(species of *Staphylococcus*, *Enterococcus*, *Streptococcus*, *Bacillus*, *Lactobacillus*, *Aerococcus*, *Vagococcus*, *Arthrobacter*, *Brevibacterium*, *Cellulomonas*, *Corynebacterium*, and *Listeria* genera) and anaerobic (mainly species of *Clostridium* genus). Gram-negative bacteria (species of *Escherichia*, *Salmonella*, *Pseudomonas*, *Stenotrophomonas*, *Campylobacter*, *Bordetella*, *Alcaligenes*, *Xanthmonas*, *Acinetobacter*, *Sphingobacterium*, and *Shigella* genera) account for a small fraction of the total microbiota, but due to their high concentrations, their numbers can still be very high [97, 103–108]. The majority of bacteria from these genera were also found in the air of the poultry house examined in this study (Tab. 4).

Litter is also a major contributive factor to fungal contamination in poultry farms. Litter spreading results in the high exposure of poultry workers to fungi and their metabolites, including mycotoxins [100, 109]. Among the fungi, the most prevalent representatives isolated from poultry litter are species of *Aspergillus*, *Fusarium*, *Mucor*, and *Penicillium* genera [102, 110]. Viegas et al. [100] and Gerber et al. [111], analyzing mycobiota from fresh and aged poultry litter samples, isolated fungal species most frequently representing the *Penicillium*, *Scopulariopsis*, *Alternaria*, *Trichosporon*, *Cladosporium*, *Aspergillus*, and *Fusarium* genera. A similar prevalence of these species was observed in the air of the poultry house investigated in this study (Tab. 4).

Organic materials such as feed may serve as carriers for microorganisms. They originate from a variety of ecological niches, such as soil and water, and their dissemination depends on how the feed is given (i.e. dry vs. wet, pellet vs. powder etc.) [102, 110]. All these variabilities translate into a diverse microbial population of both aerobic and anaerobic strains. Among bacterial contaminants of feed are species of *Alcaligenes*, *Agrobacterium*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Pseudomonas*, *Achromobacter*, *Acinetobacter*, *Actinomycetes*, *Azotobacter*, *Moraxella*, *Micrococcus*, *Mycobacterium*, *Streptomycetes*, *Streptococcus*, *Enterobacter*, and *Rhizobium* genera [110]. In turn, among the fungi are those of field (mainly *Absidia*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Diplodia*, *Phaeoramularia*, *Rhizopus*, *Drechslera*, and *Fusarium*) and storage (*Aspergillus*, *Penicillium*, *Absidia*, *Mucor*, *Candida*, and *Hensenula*) origin [14, 110]. Many of the above listed microbial species were present in the air of studied poultry house (Tab. 4).

Birds themselves shed microorganisms mainly by means of faecal excretion, dander, and to lesser extent exhaled air [102]. Overall, *Firmicutes* is the most dominant phylum in the chicken intestine, with *Lactobacillus* the primary bacterial taxa found in the gizzard, duodenum and ileum. *Clostridium*, *Ruminococcus*, *Streptococcus*, *Candidatus*, and *Arthromitus* (phylum *Firmicutes*), as well as *Escherichia* and *Enterococcus* (phylum *Proteobacteria*), have also been reported in the ileum. The most abundant phyla found in the caecum are *Firmicutes*, followed by *Bacteroidetes*, while *Proteobacteria* and *Archaea* are present in lesser amounts. The dominant bacterial taxa in the caecum are *Lactobacillus*, *Ruminococcus*, *Clostridium*, *Faecalibacterium*, *Bacteroidaceae*, *Sporobacter*, *Acetanaerobacterium*, *Pseudobutyrivibrio*, *Oscillospira*, *Subdoligranulum,* whereas *Lactobacillus*, *Clostridium*, *Bacillus*, *Ruminococcus*, *Faecalibacterium*, and *Eubacterium* are predominant in the cloaca and excreta [97, 112–116].

According to available data, microorganisms from the respiratory tract of animals might not readily become suspended in the air or expelled from the body. Hence, even if this process is visible, it is not a major source of airborne microbiota, compared to, e.g., faeces [102].

Airborne microorganisms are exposed to numerous environmental stressors such as oxidation, radiation, and microclimate changes. As for the latter, both temperature and relative humidity play a crucial role at different chicken breeding stages, shaping, among others, the survival abilities of airborne microbes in poultry houses. Generally, regardless of the season, when the chickens are young, a higher temperature is needed to keep them warm. The temperature is reduced when the chickens become bigger. In the winter season, a more airtight poultry house allows avoiding heat losses at high temperatures, together with sufficient moisture content indoors, and through that creat conditions supporting the growth of specific bacterial groups (e.g. Gram-negative rods from *Proteobacteria* phylum) [117]. In the studied henhouse such situation was noted at the beginning of the 'winter' breeding period (K1 in Fig. 7). For 'summer' breeding of 7-day-old chickens, heat losses within the building were probably not that significant, and the structure of indoor microbiota shifted towards the more environmentally resistant *Firmicutes* (K3 in Fig. 7).

Isolated pathogens and potential health threats they may cause. Among the isolated culturable microbiota, 21 aerobic and 7 anaerobic bacterial pathogens as well as 2 fungal pathogens important from human health point of view were found (Tab. 4). All isolated human pathogens belonged to group 2 according to their level of risk of infection [118, 119]. The presence of microbial particles in the air, especially in high concentrations and with high percentage of pathogenic strains, may represent significant immunological and toxic challenges to human health. Among the identified pathogens were those directly responsible for adverse health effects, from decline in lung functions, through acute and chronic work-related symptoms (including cough, phlegm, eye and throat irritation, dyspnea, chest tightness, nasal congestion, wheezing, sneezing, fever), to allergic disease such as asthma and allergic alveolitis (e.g. farmer's lung disease) [e.g. 6, 16, 33, 37, 120–124].

Among isolated culturable bacterial species, nearly 20% of airborne microbiota were Gram-negative bacteria, present as both aerobic and anaerobic strains. They are a source of immunologically-active endotoxins which pose a serious threat to the health of exposed individuals. The pathogenic effects of these bacteria are manifested mainly in the form of fever with chills and/or inflammatory reactions in the respiratory system. Such adverse effects can be caused by even picogram amounts of these highly reactive particles [125].

In turn, among the identified filamentous fungi, numerous strains are able to produce mycotoxins. Of these, attention should be paid to species producing: *A. alternata* – alternariol; *Aspergillus clavatus* – patulin and cytochalasin E; *A. flavus* – aflatoxins B and G; *A. fumigatus* – fumigaclavine and fumitremorgins; *A. nidulans* – sterigmatocystin and nidulin; *A. ochraceus* and *P. commune* – ochratoxin A and penicillic acid; *A. terreus* – patulin and citrinin; *A. versicolor* – sterigmatocystin; *A. wentii* – kojic acid; *Cladosporium herbarum* – epicladosporic acid; *Epicoccum nigrum* – flavipin; *Fusarium culmorum* – zearalenone, deoxynivalenol, and diacetoxyscipernol; *F. oxysporum* and *F. solani* – zearalenone;

Penicillium brevicompactum – mycophenolic acid; *P. chrysogenum* – roquefortine C, meleagrine, penicillin, ochratoxin A; *P. citrinum* and *P. fellutanum* – citrinin; *P. commune* – cyclopiazonic acid and rugulovasine A and B; *P. digitatum* – tryptoquivalins; *P. expansum* – roquefortine C, patulin, citrinin, communesin A and B, and chaetoglobosin C; *P. glabrum* – citromycetin; *P. rugulosum* – rugulosin; *P. verrucosum* – ochratoxin A and citrinin [14, 126–129]. Filamentous fungi are able to produce a high number of these secondary metabolites, which they probably need for survival in their natural habitats as a response to the influence and/or interaction of other bacteria and moulds [130, 131]. They are known to interfere with RNA synthesis and may cause DNA damage [132]. Mycotoxins have been suggested as one of the major possible cause of the health problems indoors [133, 134]. They are responsible for numerous adverse health outcomes ranging from acute poisoning to long-term effects, such as immune deficiency and tumours as they exhibit carcinogenic, mutagenic, teratogenic, nephrotoxic, hepatotoxic, tremorgenic, immunotoxic, and immunomodulatory effects [88, 135–139]. Some studies have already shown that mycotoxins can have a profound toxic effect on alveolar macrophages [140], suggesting that they are more toxic when exposure occurs by inhalation rather than ingestion [141, 142]. This means that the mycotoxin dose required to cause particular adverse effects is typically one order of magnitude less when administered by the respiratory route, than by the alimentary tract [137]. As the vast majority of mycotoxins are not volatile, an exposure by inhalation is most likely to occur via inhalation of fungal conidia [88, 140]. Hence, their quantitative and qualitative assessment in the air is of a great importance from the occupational exposure point of view. Regarding other fungal group, the yeast species, *Candida albicans*, secrets an acid protease, which has been reported to induce atopic asthma being by that an important allergen of itself [141].

Bioaerosol size distribution and its adverse effects in humans and animals. The use of a 6-stage Andersen impactor allowed obtaining data on particle size distribution of microbial aerosols in all 4 studied environments (Fig. 10). Regarding the aerodynamic diameters (D_{ae}) of the dominant species (i.e. with the highest percentage contributions to the total microbiota – see above), i.e. from bacteria – staphylococci and enterococci with D_{ae} from ~0.75 µm to ~1 µm, aerobic bacilli (*Bacillus* genus) and Gram-negative rods with D_{ae} about ~0.8 µm, anaerobic bacilli (*Clostridium* genus) with D_{ae} between 1.4–1.9 µm, actinomycetal species with D_{ae} from 0.6 µm to 1.1 µm, as well as from fungi – *Penicillium* species with D_{ae} from ~2.3 µm to ~3 µm, *Aspergillus* species, and from ~2.1 µm to ~3.6 µm, *Cladosporium* species with D_{ae} about ~1.8 µm, *Fusarium* species with D_{ae} between 4.35–6.8 µm [143–147], the size distribution analysis revealed that in case of airborne bacteria:

a) the concentrations of bioaerosol particles in poultry house with 42-day-old chickens in all studied aerodynamic fractions, i.e. from 0.65 µm to above 7 µm, significantly augmented the concentrations recorded indoors with 7-day-old chickens, and when the poultry house was empty as well as those noted in outdoor (atmospheric) air (Scheffé tests: $P < 0.01–0.001$);
b) when both 42- day-old and 7-day-old chickens were present indoors, the size distribution step plots had similar courses showing that bacteria in the air appeared mainly in the form of fine aggregates, composed solely of vegetative cells or spores and/or their aggregates with dust particles of D_{ae} between 2.1–4.7 µm;
c) when the poultry house was empty (i.e. without chickens and with the floor not covered with litter), the air was dominated by fine aggregates of D_{ae} between 2.1–3.3 µm as the lack of bedding and food significantly limited the possibility of forming aggregates with dust particles;
d) in background (outdoor) air, these microbial particles forming coarse bacterial and or bacterial-dust aggregates with D_{ae} above 7 µm clearly prevailed. In turn, the size distribution of fungi revealed that:
e) in the case of 7-day-old chickens, their introduction into the poultry house resulted in the appearance in the air of single conidia with D_{ae} of 1.1–3.3 µm, together with fine fungal and/or fungal-dust aggregates with D_{ae} of 3.3–4.7 µm;
f) the size distribution from indoor workplaces with 7-day-old chickens mirrored that observed in the empty poultry house, with the proviso that the noted concentrations were 10 – 60 times higher in particular ranges of aerodynamic diameters after the introduction of chickens into the building;
g) when the chickens were almost at slaughter age (42-day-old), the size distribution changed with a clear predominance of fine (3.3–4.7 µm) and coarse (above 7 µm) fungal and/or fungal-dust aggregates;
h) all indoor concentrations of fungal aerosol within the aerodynamic diameters of prevailing conidia (i.e. above 1.1 µm) were higher than that noted in background (outdoor) air. This situation in terms of size distribution, in relation to fungi and bacteria discussed above, confirms

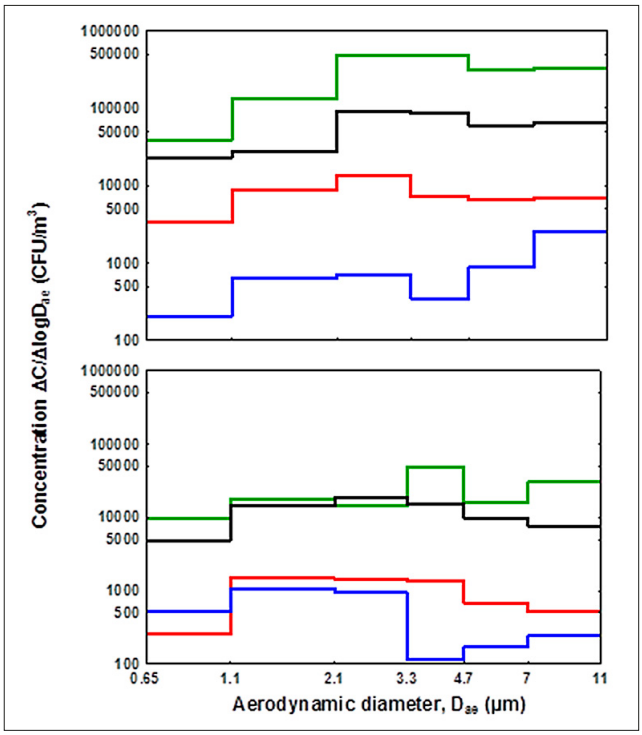

Figure 10. Size distribution of bacterial (top) and fungal (bottom) aerosols at workplaces in the poultry house and in background (outdoor) environment. Colours of step plots indicate: green – workplaces with 42-day-old chickens, black – workplaces with 7-day-old chickens, red – empty poultry house, and blue – background (outdoor) air

that the intense emission of particles from the poultry house is the main source of microbial contamination in and around the farm under study.

The dissemination of microorganisms in the work environment in the form of bioaerosols is the most common way in which they cause occupational health risks. Bioaerosols are diverse in terms of size, composition and biological properties, and are an important transmission route for infectious and sensitizing agents [62, 70]. As respiratory symptoms and diseases are the most common health effects associated with bioaerosols, inhalation exposure is therefore of the greatest importance [148].

With regard to workplaces in the studied poultry house, microbial particles being inhaled may reach and be deposited in the case of bacteria within the trachea, primary and secondary bronchi, and in the case of fungi, within the nasal and oral cavities, trachea, primary, secondary, and terminal bronchi. As interactions between airborne particles and respiratory system cells greatly depend on the site of their deposition, airborne bacteria may be responsible for the occurrence of adverse health effects in exposed workers in the form of asthmatic reactions and allergic inflammations. Research shows that bacterial particles (especially those smaller than 2.5 μm) have the ability to bypass numerous defence systems in the respiratory tract (e.g. epithelial cilia, mucus, saliva etc.), and can deliver a significant amount of potentially dangerous substances relatively deeply in the body, often causing damage at the cellular level [149]. In turn, airborne fungi in the empty poultry house and in the premises by 7-day-old chickens may be responsible for the appearance of asthmatic reactions and allergic inflammations. When 42-day-old chickens are reared in the poultry house, an inhalation of airborne mycobiota may most often provoke mucous membrane irritations and asthmatic reactions. All these adverse effects were already observed in other studies at poultry farms, as shown above.

Airborne pathogens are dangerous not only for humans, but also for the health of poultry. They can interact directly with poultry by inhalation, or indirectly through elements of the environment that can pollute through their presence (e.g. litter, feed, and water). As aptly expressed by Chen et al. [37], the respiratory system of chickens is an 'open' structure from nose to lung, abdominal organs, bones, and related tissues. Therefore, infections of the respiratory tract tend to occur and spread to the abdominal organs, and even to the entire body. On the other hand, numerous bacteria and fungi already present in litter, feed, water, and feathers, may provoke a wide range of adverse effects. Among the bacteria isolated in the examined poultry house, the following pathogens had a significant negative impact on the health of the chickens, and were responsible for: *Clostridium* (incl. *C. perfringens*) species – necrotic enteritis; *Escherichia coli* – septicemia, cellulitis, swollen head syndrome, and airsaculitis; *Pseudomonas aeruginosa* – infections leading to septicaemia and death; *Salmonella* spp. – enteritis, diarrhea, dehydration, septicaemia and death in young chickens; *Shigella* spp. – diarrhea, dysentery, blood stool, and even death; *Staphylococcus aureus* – bumble foot; *Enterococcus* (incl. *E. avium*, *E. faecalis*, *E. faecium*, *E. hirae*, *E. durans*, *E. gallinarum*) species – sepsis, endocarditis, arthritis, arthropathies; *Listeria monocytogenes* – encephalitis and septicaemia [96, 107, 110, 111, 150].

Although the period of chicken rearing is relatively short, broilers are very likely to be also infected with fungi, mainly due to the high concentrations of fungal conidia in the aerosol, litter, feed, and water. Among the filamentous fungi and yeasts, the following pathogens are responsible for adverse health outcomes in chickens: *Aspergillus* (incl. *A. fumigatus*) species for avian aspergillosis (acute in young birds resulting in high morbidity and mortality, chronic in older birds causing lesser mortality); *Cladosporium* and *Alternaria* species for respiratory diseases; *Candida albicans* for common mycosis of the digestive tract, manifested by dyspepsia, reduced feeding and feed conversion ratio, and even death [37, 110, 151, 152]. Poultry are also sensitive to *Aspergillus* and *Penicillium* mycotoxins (e.g. to aflatoxin B_1). Their inhalation may lead to impairment of neuromotor functions in the respiratory tract and tumours [110].

Marek's disease virus in air samples. The growing consumption of poultry meat and the related increase in the productivity of chicken farming translates today into shorter rearing periods, and cramped living conditions for the birds. The side-effect of these changes is the evolution of pathogens. A good example is Marek's disease virus (MDV), which has evolved from a relatively harmless paralytic syndrome into a highly virulent pathogen [153–155]. Marek's disease is caused by oncogenic *Gallid alphaherpesvirus* 2 (GaHV-2), commonly known as MDV type 1 (MDV-1) [154, 156, 157]. This disease is characteristic for young chickens, but older birds can also be affected. The virus is concentrated in the feather follicles and shed in the dander (sloughed skin and feather cells), and has a long survival time in dander. The viable virus can be isolated from poultry houses that have been depopulated for more than 12 months.

Marek's disease may produce a variety of clinical responses, all lymphoid in character. These are acute visceral, neural, ocular, skin or combinations of the responses that can be seen [158]. Infected birds show weight loss and signs of paralysis; mortality ranges from 5% – 50% in unvaccinated birds. In the classic form, the typical symptom is asymmetric paralysis of the limbs. Skin lesions associated with Marek's disease are the cause of many seizures in broilers on the slaughter line worldwide [156]. In the examined poultry house, all air samples collected at workplaces and in background (outdoor) environment were free from GaHV-2 strain. This may be the result of periodic disinfection with a virucidal preparation effectively used in the examined poultry house.

It is also worth mentioning that MDV is among the few herpes viruses that are potentially able to cross the species barrier and infect different hosts. As this virus can be transported in the environment on dust particles and skin scrapings, not only birds but also poultry farmers and veterinarians are prone to its inhalation. The exact impact of inhalation exposure to MDV is unknown. However, given the high degree of homology between MDV and Human herpesvirus-1, Epstein-Barr virus or Kaposi's Sarcoma-associated herpes virus, it is possible that MDV may also replicate in humans and pose a potential threat [159, 160]. On the other hand, Schat and Erb claim that there is no indication that avian oncogenic viruses are involved in human diseases (e.g. cancer or multiple sclerosis) or even able to infect and replicate in humans [161]. In the light of these various scientific considerations, for now, MDV is treated as a virus that is not infectious to humans [162].

Table 5. The mean values (and ranges) of air temperature and relative humidity (RH) at workplaces in poultry house and in background (outdoor) environment

Rearing period	Sampling season	Workplaces		Background	
		Temperature [°C]	RH [%]	Temperature [°C]	RH [%]
7-day-old chickens	'Winter'	33.7 (32.8–34.7)	28.8 (26.1–31.5)	–3.9 (–3.7–-4.1)	18.7 (18.5–18.9)
	'Summer'	25.5 (24.8–26.3)	48.4 (45.5–52.4)	28.2 (28.1–28.4)	36 (34.5–37.1)
42-day-old chickens	'Winter'	18.9 (16.6–23)	66.1 (60.5–71.8)	2.8 (2.6–3.1)	86.2 (85.3–87.1)
	'Summer'	22.1 (21.3–23.8)	69.4 (65.5–70.8)	16.5 (16–17.1)	73 (70.4–75.2)

Influence of microclimate parameters on microbial aerosol levels. The mean values (and ranges) of air temperature and relative humidity at workplaces in the poultry house and in background (outdoor) environment in 'summer' as well as in 'winter' seasons are presented in Table 5. The correlations between bacterial and fungal aerosol concentrations and values of air temperature and relative humidity revealed only 2 statistically significant relationships, both of which concerned concentrations of aerobic bacteria measured in the poultry house using a Coriolis impinger. In the case of temperature, the noted relationship was negative, whereas in the case of relative humidity – positive (Pearson correlation coefficients: $r = -0.96$ at $P < 0.05$ and $r = 0.96$ at $P < 0.05$, respectively). The remaining relationships between the concentrations of aerobic and anaerobic bacteria and fungi (measured with all 4 samplers), and the values of air temperature and relative humidity, were not statistically significant.

Limitations of the study. Nowadays, the next-generation sequencing (NGS) technique is widely used in microbiological studies. NGS has had a significant biotechnological impact on detecting bacteria and viruses, but is not equally applicable to fungi [163]. In the middle of the previous decade, only a few hundred approximate fungal genome sequences were available [164]. Compared to classic identification techniques, microbial identification using NGS is fast, accurate, and broad-spectrum; however, NGS-based strategies in fungal detection require bioinformatics skills, specialized software and equipment. The cost of analysis remains significant [165, 166]. Nilsson et al. [167] stated that, despite tremendous analytical progress, NGS workflows cannot replace the traditional culture and other analytical techniques, the major reason being the complexity of the bioinformatics analysis. From the isolated mycobiota, only 2 species of filamentous fungi of *Aspergillus* genus were recognized as human pathogens; the qualitative diagnostics of mycobiota in this study was carried out based on the molecular PCR technique (using ITS as the 'official fungal barcode') [168, 169] supplemented with classic (using identification keys) morphological diagnostics of fungal colonies on agar cultures.

The environmental dissemination of bacterial and fungal pollutants around the poultry farm building was determined for the 'summer' season only. Compared to the warmer part of the year, the 'winter' season has some important advantages as well a number of significant disadvantages. The study of airborne spread of microbiological contamination from a known point source of emission (the tested poultry house in this case) in 'winter' season at low air temperature and snow cover on the ground, would eliminate an important disturbing factor, which is the additional emission of microorganisms from the soil, large plant communities (forests or crops), and water reservoirs. On the other hand, due to the number and spatial location of sampling points around the poultry house, in this study it was necessary to use a battery operated sampler – in this case, a single-stage MAS impactor. Since the impactor uses Petri dishes filled with agar medium as the collection surface for microbiological particles, the possibility of using them in repeated measurements in winter at very low ambient temperature (often below 0 °C), if it did not prevent the measurement due to freezing of the microbiological medium, it at least significantly adversely affected the impactor collection efficiency (lack or limited possibility of particle deposition on the solidified agar, their bounce from the surface of the rigid agar, limited or even impossible growth of microorganisms deposited on the collection surface at a very low temperature etc.). Therefore, taking into account the unfavourable microclimatic conditions recorded in the 'winter' season, the measurements describing environmental dissemination of bacterial and fungal pollutants in the air were carried out in the 'summer' season only.

Moreover, analyzing the concentrations of microorganisms in the air of the poultry house, it can be seen that the average concentrations of bacterial aerosol (Fig. 2a) measured using MAS impactor in the source (i.e. inside studied poultry house near the ventilation system chimney inlet) were noticeably lower than their levels at workplaces located at a certain distance from the inlet to the chimney (Fig. 1, data with chickens in the poultry house) and the fungal aerosol concentrations near the chimney (Fig. 2b). This could be explained by 2 facts:

1) the ventilation system operating during chicken breeding 'sucked' bioaerosol from the entire hen house, which is a large-volume facility. The indoor transport of bacterial particles, which, as shown by size distribution analysis, consisted mainly of bacterial and/or bacterial-dust aggregates (i.e. particles with a relatively large mass), caused them to reach the central exhaust point of the ventilation system from various distant places in the poultry house. Most of these aggregates were deposited along the way on all other inanimate surfaces (floor, walls, litter, feeding and watering system elements etc.), and animate objects inside the poultry house. Therefore, only bacterial vegetative cells or spores and fungal conidia with small aerodynamic diameters, for which 'half-life' times were relatively long (amounting to hours or even days in turbulent air), reached near the ventilation system chimney inlet [70].
2) In order to determine bioaerosol concentrations for the purpose of describing environmental dissemination, the MAS impactor was used. This device is characterized by sampling efficiency described by the cuttoff size parameter, D_{50}, at the level of 1.7 μm [170]. The latter designates the particle diameter for 50% removal, which means that almost all particles larger than that size are collected with

high efficiency, but almost all particles smaller than that size may not be captured by this impactor [56]. As most of the identified airborne bacteria of dominant species had D_{ae} below 1.1 μm (see above), there was a possibility that not all of them were 'seen' by this sampler, so that their number may have been underestimated in relation to actual concentration in the air. As in the case of stationary measurements, all used bioaerosol samplers did not significantly differ from each other in terms of sampling efficiency of bacterial and fungal particles. The overlap of both the above-described reasons probably resulted in the observed lower concentration of bacterial aerosol near the ventilation system chimney inlet.

CONCLUSIONS

The introduction and subsequent rearing of chickens into the poultry house resulted in bacterial and fungal concentrations in the air reaching the levels of 1.26×10^8 CFU/m^3 and 3.77×10^4 CFU/m^3, respectively. A poultry house heavily loaded with bioaerosol particles was a significant point source of microorganism emission to the environment. These pollutants were spread around the poultry house through the ventilation system, but their concentrations significantly decreased at a distance of 500 m from the farm. The bacterial community of tested aerosol samples consisted of representatives of 4 phyla: *Actinobacteriota*, *Bacteroidota, Firmicutes*, and *Proteobacteria*, whereas fungal microbiota was composed of agents representing 3 phyla: *Ascomycota*, *Basidiomycota*, and *Mucoromycota*. Marek's disease virus was not present in any of the tested air samples.

The appearance of numerous bacterial and fungal species, including those pathogenic for humans and animals, was confirmed in the air samples collected with tested Andersen and MAS impactors, as well as Coriolis and BioSampler impingers; however, of these 4 samplers, a 6-stage Andersen impactor always isolated higher numbers of culturable bacterial and fungal species from the air samples than the other tested aspirators.

The presence of microbiological pathogens in the air of the poultry house and the possibility of their airborne migration poses real threats to exposed individuals. To protect them, highly efficient hygienic and technical measures regarding the poultry house interior and its ventilation, respectively, should be introduced to prevent both unwanted pollution and subsequent emission of microbial contaminants during intensive chicken breeding. This study also emphasizes the necessity for permanent monitoring of microbial air quality in poultry houses.

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