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# MICROBIAL POLLUTION OF MANURE, LITTER, AIR AND SOIL IN A POULTRY FARM

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## Abstract

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Hygienic evaluation of the microbial pollution of the fresh manure, litter, air and soil based on the number of cultivable microorganisms and the number of coliform bacteria at six points in a broiler farm (indoor points: Point 1 – building A and Point 2 – building B - fresh manure, litter and air; outdoor points: Point 3 - at 2.0 m, Point 4 - at 20.0 m, Point 5 - at 50.0 m and Point 6, control - at 500 m distances from the buildings – air and soil) with capacity of 43000 broiler chickens was performed in the present study. The number of cultivable microorganisms and number of coliform bacteria in all investigated parameters varied widely, with clear differences among the various elements of the chain "fresh manure–litter–soil at 2.0 m, at 20.0 m, at 50.0 m and at 500.0 m from poultry buildings" and in the indoor and outdoor air at the same distances as at the soil. In fresh manure number of cultivable microorganisms and number of coliform bacteria was much higher than litter and especially than indoor air, and later significantly decreased in outdoor air and soil at 2.0 m from poultry buildings, and to a lower extent in air and soil at 20.0 m, at 50 m and at 500.0 m from broiler buildings. The fresh broiler manure and litter are main sources of inside and outside air and soil pollution surround broiler buildings in a poultry farm with saprophytic microorganisms including coliform bacteria, subject to sanitary control. Increasing the distance from poultry buildings, led to reducing the number of cultivable microorganisms and number of coliform bacteria in the soil and in the air. When removed from the broiler houses manure and litter are not stored on the farm territory and no surface drainage process water from the facilities, the air from the production buildings is a major source of environmental contamination with microorganisms.

*Key words:* broiler farm, microbial pollution, fresh manure, litter, soil, air

*Abbreviations used:* BSS - Bulgarian state standards; CFU - Colony forming units; NCB - Number of coliform bacteria; NCM - Number of cultivable microorganisms; SG - State gazette

## Introduction

In intensive broiler production systems, stocking density in poultry houses is very high, thus making it difficult to maintain optimal microclimate and animal hygiene conditions. One of the prerequisites for the emergence of such difficulties is the fact that during the entire production cycle manure and litter remains inside. Thus, these wastes are becoming sources of pollution of environment by noxious gases, dust and microorganisms. In recent years, scientists have increased their interest to microbial pollution of the environment from livestock production systems. Surveys are in two main directions – microbial pollution of poultry litter and microbial pollution of indoor and outdoor air in poultry farms (Voermans, 1993; Nicholson, 1994).

Studies have been focused on microorganisms in poultry wastes (litter), having revealed the number of microorganisms – aerobic and anaerobic bacteria (Bratanov, 1979; Soupir et al., 2006), species composition of microorganisms in litter – saprophytes, enterococci,, coliforms, etc. (Bildirev, 1983; El-Jalil et al., 2008) and distribution of bacteria at different poultry litter depths (Barker et al., 2010).

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Many aspects of air pollution in poultry houses and surrounding area are also clarified. The air concentration of microorganisms in poultry houses and their surrounding reported in the literature greatly varies. Muller (1987) reported the concentration of airborne microorganisms in broiler houses up to 5860 CFU/dm$^3$ (6.77 log CFU/m$^3$ air), Baykov and Stoyanov (1999) up to 1.68×10$^5$ CFU/m$^{-3}$ air (8.22 log CFU/m$^3$), Karwowska (2005) up to 4.6x10$^4$ CFU/m$^3$ (4.46 log CFU/m$^3$), Vučemilo et al. (2007) up to 2.2x10$^5$ CFU/m$^3$ (5.34 log CFU/m$^3$). The number of microorganisms in the air around poultry houses (up to 500 m from poultry houses) ranged from 2.2x10$^3$ CFU/m$^3$ (3.34 log CFU/m$^3$) (Rusak and Rokicki, 1985) to 2060.0x10$^3$ CFU/m$^3$ (6.31 log CFU/m$^3$) (Petkov and Baykov, 1988). Microbiological contamination of the air in poultry farms, including species composition of microorganisms have been studied and analyzed in details from (Karwowska, 2005; Lonc, and Plewa, 2010; Plewa and Lonc, 2011). Herbut et al. (1982) and Petkov and Baykov **(**1988) found that the main source of microorganisms in poultry houses is birds, followed by feed, litter, and droppings, and that microbial counts are affected primary by the efficiency of ventilation systems and air dustiness.

The review of the literature on discussed issue revealed that no evidence of the content of microorganisms in fresh poultry manure, as well as their distribution in the chain "fresh manure-litter-soil in poultry farms". There is lack of date about the relationships between the content of microorganisms in fresh manure and litter, between fresh manure and litter in houses, from one side and the soil surround them, from the other, and between indoor and outdoor air. Therefore, the starting point of these studies should be the content of microorganisms, including sanitary indicative as coliform bacteria (*E. coli*) in fresh poultry manure, because in reality it is the main source of microorganisms in poultry houses. Manure plays an important role in the onset and distribution of diseases in both humans and poultry that is why that is must be a subject of a careful study and control by the veterinary and sanitary organs. The hygiene control of poultry manure is directed towards the establishment of an algorithm for evaluation of this animal waste from a hygienic and epizootically point of view in order to protect the air, the soil, the superficial and underground waters from pollution with intestinal faecal microflora, pathogenic microorganisms and parasites (Webb and Archer, 1994; Unc and Goss, 2004). From the other point of view, microbiological studies of the sanitary and hygienic condition of soil allow one to determine the organic or the microbial origin of pollution. The *E. coli* subject to sanitary control and the total number of microorganisms in a volume of soil (microbial number) are used as criteria for its microbial pollution (Danon-Moshe et al., 1985).

The aim of the present study was to perform a hygienic evaluation of the microbial pollution of the fresh manure, litter, air and soil based on the number of cultivable microorganisms and the number of coliform bacteria in a broiler chickens poultry farm.

## Materials and Methods

***Study area***

The study was conducted from September to December 2009 in a poultry farm with capacity of 43000 chicken-broilers, during two production cycles, each lasting 40 days. The farm is situated on leached cinnamon forest soils in the Central–South part of Bulgaria, Nova Zagora Municipality. The farm consists of 0.5 ha, and two production buildings (A – 850 m$^2$ with capacity of 23000 chicken-broilers and B – 750 m$^2$ with capacity of 20000 chicken-broilers), a warehouse for concentrated fodder and office. Both buildings were without windows and were equipped with automated systems for feeding and watering of broilers (Company Roxell, Belgium). Feeding grooved were placed in 2 rows in building A and in 3 rows in building B, and nipple drinkers were located in 3 (building A) and 4 rows (building B), respectively. Luminescent lamps mounted on the ceiling of the premises provided lighting of the both buildings.

Ventilation in the buildings was conducted through mechanical ventilation system – type negative pressure system. Under this system, exhaust fans (12 in building A and 9 in building B, each with a capacity 16.5 m$^3$/h), fitted to on one longitudinal wall, blow polluted air out of the buildings creating a slight negative pressure (vacuum) to draw air into the buildings through designed inlets, located on the opposite longitudinal wall. Microclimate in the buildings (heating, ventilation and cooling) was managed by an automated computer system of Fancom Company, The Netherlands.

***Study design***

During the study period, broiler hybrids – Cobb$^{45}$, Cobb$^{48}$, Cobb$^{49}$ and Ross$^8$ were reared on deep litter (chopped straw 0.10 m thick), with density 26 broilers/m$^2$ in building A and 25 broilers/m$^2$ in building B. All broilers were fed standard granular compound feed, consistent with the age of the broilers. The water supply for both drinking and technological purposes came from a central water supply system from the nearby town. All broilers had *ad libitum* access to feed and water. After the end of each production cycle (40d of broiler's age) manure and litter in the buildings were cleaned by mobile machinery. Removed wastes immediately were transported to landfill, located outside of the farm territory. Before loading new flocks of broilers the buildings were disinfected

with a solution of chlorine, NaOH and broad-spectrum insecticide - Nurele D. There were no cases of infectious diseases on the farm during the experiment. From a medical point of view, the broilers were clinically healthy.

***Monitoring points***

On the territory of the studied poultry farm 6 monitoring points for sampling of fresh manure, litter, air and soil were identified, as follows:

- Point 1 (building - A) (P-1) – fresh manure, litter, air;
- Point 2 (buildings B) (P-2) – fresh manure, litter, air;
- Point 3 (P-3) – at 2.0 m from the leeward side of the buildings – air and soil;
- Point 4 (P-4) – at 20.0 m from the leeward side of the buildings – air and soil;
- Point 5 (P-5) - at 50.0 m from the leeward side of the buildings – air and soil;
- Point 6 (P-5), Control - at 500.0 m from the leeward side of the buildings – air and soil;

***Sample collection and preparation***

***Fresh manure****:* Fresh manure samples were collected at 11:00–12:00 h from both broiler buildings (P-1 and P-2) during the first and last week of each production cycles. Within each building, than five random places about 0.2 kg of fresh manure sample was collected using sterile glass container (volume $5.10^{-4}$ $m^3$). The collected samples were transported to the laboratory in a cool bag (at 4–6°C), and processed for microbiological analysis up to 2 h after the collection (Alef, 1991; Jennifer et al., 2004). The total number of fresh manure samples collected was 4.

***Litter*****:** Litter samples were collected from both buildings (P-1 and P-2) using clear PVC pipe (0.05 m diameter and 0.5 m length) in the same time as fresh manure samples collected. Two pipes in each building were driven into the clay floor of the litter bed, so that each pipe contained layers of the litter bed. Ones the samples were collected, the bottom of each pipe was sealed using plastic bag and pieces of duct tape. Litter samples were transported up-right to the laboratory and starting for analysis in the same conditions as samples of fresh manure. The total number of litter samples collected was 8.

***Air*****:** Air samples - two for NCM and two for NCB, respectively were collected by the sedimentation method of Matusevich, 1975 (Petkov et al., 1999), using Petri dishes, at each monitoring point (inside: P-1 and P-2; outside: P-3, P-4, P-5 and P-6) in the first and in the last week during both production cycles (total 4 times) at a height 0.50 m from the floor/soil, in the same sequence from 9.00 to 12.00 h. According this method air sampling was performed in sterile cardboard cylinders of 1 $m^{-3}$. Then *in situ* an opening of cardboard cylinder was placed on sterile Petri dishes with Nutrient Agar (BBL™, USA) - for determination of NCM or with Endo Agar (BBL™, USA) - for determination of NCB, respectively, and the other side of the cylinder was covered with lid of the Petri dish. Cylinders remained in vertical position for 15 min, during which time the microorganisms from air sample sedimented on the agar medium. After that, cardboard cylinders were removed and Petri dishes were covered with their lids. The plated Petri dishes were transported to the laboratory in a cool bag (at 4–6°C) and processed for microbiological analysis immediately. The total number of air samples collected was 96 - 48 samples for NCM and 48 samples for NCB.

***Soil*****:** Soil samples (about 1.0 kg) were collected in clear plastic bags from three places on 0-0.10 m depth, around each outside point (P-3, P-4, P-5 and P-6) using metal probe (BSS EN ISO 10381). The collected soil samples were transported to the laboratory and analyzed at the same conditions as the fresh manure samples. The total number of soil samples collected was 16.

***Determination of air temperature, air relative humidity and air velocity***

Air temperature (°C), air relative humidity (%) and air velocity (m/s) were measured in all monitoring points, parallel with the air samples collection, as follow: temperature – by mercury thermometer with 0.1 °C resolution; relative humidity - by Aspirated-Psychrometer; air velocity – by Kata-thermometer (Petkov et al., 1999).

***Determination the cultivable microorganisms and coliform bacteria (E. coli)***

***Fresh manure, litter and soil*****:** The collected samples of fresh manure, litter and soil from different monitoring points were mixed and homogenized and an average sample of 0.01 kg of each one was prepared. The number of cultivable microorganisms (NCM – log CFU/$kg^{-3}$) in fresh manure, litter and soil was enumerated by performing serial dilutions (from 1:10 to 1:1.000.000) in Saline, Normal (Physiological) which was vortex agitated for 1 min prior to plating onto Nutrient Agar (BBL™, USA), plates and incubated for 24 h at 30±1°C under normal atmospheric conditions. From each serial dilution, $0.1L^{-3}$ was then spread plated onto two different Petri dishes. CFU in all plates were enumerated after 24 h (Danon-Moshe et al., 1985; Jeffery et al., 2004).

To determine the number of coliform bacteria (*E.coli*) (NCB – log CFU/$kg^{-3}$), samples were diluted as described above but plated onto MacConkey Agar (BBL™, USA) plates and incubated at 37°C under normal atmospheric conditions. CFU in all plates were enumerated after 24 h (Danon-Moshe et al., 1985; Jeffery et al., 2004).

***Air*:** The number of cultivable microorganisms in air (NCM – log CFU/m$^3$ air) was determinates after each plate was incubated at 37°C for 24 to 48 h, and then a count of the colonies on the plate surface was done, and found to yield colony-forming units. Obtained results were multiplied by 1000 to determine the number of cultivable microorganisms in 1 m$^3$ air (Danon-Moshe et al. 1985).

The number of coliform bacteria (*E. coli*) in air (NCB – log CFU/m$^3$ air) was determined by the same method as the number of NCM.

***Hygienic evaluation of microbiological pollution of the air/soil***

***Air*:** Indoor air (P-1 and P-2): The hygienic evaluation of microbiological pollution of air in broiler buildings (A and B) on the basis of the NCM (NCM - log CFU/m$^3$ air) was performed by comparison of the obtained results with reference values for maximum permissible limit of microbial content in the air of poultry houses – up to 250000 CFU/m$^3$ air (5.40 log CFU/m$^3$ air), (Regulation No 44/2006).

Outdoor air (at P-3, P-4, P-5 and P-6): In EU and Bulgarian legislation does not standard for permissible limit of bacterial concentration in atmospheric air. For that reason, the hygienic evaluation of microbiological pollution of the outside air was performed by comparison of the obtained results with suggested by us norm – up to 3000 CFU/m$^3$ air (3.47 log CFU/m$^3$ air), based on norms for clean atmospheric air adopted by Shaffer, 1975 (cited by Kochemasova et al., 1987) – up to 1500 CFU/m$^3$ air (3.17 log CFU/m$^3$ air) for the summer and up to 4500 CFU/m$^3$ air (3.65 log CFU/m$^3$ air) for the winter.

The hygienic evaluation on the base of number of coliform bacteria (*E. coli*) in air (NCB - log CFU/m$^3$ air) was not performed, because there is no standard for this parameter.

***Soil:*** The hygienic evaluation of microbiological pollution of the soil (at P-3, P-4, P-5 and P-6), on the basis of the NCM (log CFU/kg$^{-3}$/soil) was performed by comparison of the obtained results with reference values for microbial content in the soil adopted by Petkov and Baykov (1978): clean soil - up to 10 000 (4 log CFU/kg$^{-3}$); slightly contaminated soil – 10 000-100 000 (4-5 log CFU/kg$^{-3}$); contaminated soil – 100 000-1 000 000 (5-6 log CFU/kg$^{-3}$); heavily contaminated soil - over 1 000 000 (6 log CFU/kg$^{-3}$).

The hygienic evaluation of the soil on the NCB (log CFU/kg$^{-3}$/soil) was determined by Danon-Moshe et al. (1985) criteria: clean soil 0.0-1.0 (0 log CFU/kg$^{-3}$); slightly contaminated soil – 1.0-100.0 (0-2 log CFU/kg$^{-3}$); moderately contaminated soil – 100.0-1000.0 (2-3 log CFU/kg$^{-3}$); heavily contaminated soil – 1000.0 (3 log CFU/kg$^{-3}$) and over. We have used these norms for NCM and NCB for hygienic evaluation of microbiological pollution of the soil and in other similar our investigation (Petkov et al., 2006).

***Statistical analyses***

The statistical significance of the results was tested based on the standard deviation values calculated by the Student's t-test.

## Results

In the studied period - from September to December 2009, the controlled physical parameters of air ranged as follow: indoor air (broiler houses A and B): temperature - between + 20.4 and + 30.1°C; relative humidity - between 50.2 and 82.3%, air velocity - between 0.05 and 0.49 m/s; outdoor air: temperature - between + 3.0 and + 22.2°C; relative humidity - between 64.6 and 85.2 %, air velocity - between 0.23 and 1.85 m/s.

The results obtained for NCM and NCB in fresh broiler manure, litter, soil and air shows:

- Fresh manure (P-1 and P-2): The NCM (log CFU/kg$^{-3}$) varied between 7.77 (P-2) and 8.41(P-1). The average NCM in fresh manure from building B (P-2) was with 1.53 times (based on the real NCM values) greater than in building A (P-1), but this difference was not statistically significant. The coefficient of variation values demonstrated moderate levels of variability of NCM in fresh manure (Cv = 27.7 – 33.9%) (Table 1).

The NCB in fresh manure (log CFU/kg$^{-3}$) ranged between 3 (P-1) and 5 (P-1 and P-2). The average NCB in fresh manure from building B (P-2) was 1.83 times higher than in building A (P-1), but the difference was not statistically significant. The coefficient of variation values demonstrated large range of variation of that parameter – Cv = from 10.2 % at P-2 to 107.5% at P-1 (Table 1).

- Broiler litter (P-1 and P-2): The NCM in litter (log CFU/kg$^{-3}$) varied between 6.08 (P-1) and 6.92 (P-2). The average NCM in litter from building B (P-2) was 2.11 times more than in building A (P-1), but this difference was statistically insignificant. The values of coefficient of variation demonstrated moderate levels of variability of NCM in fresh manure (Cv = 37.3 – 41.7%) (Table 1).

The NCB in broiler litter (log CFU/kg$^{-3}$) ranged between 3 and 5 (P-2). The average NCB in litter from building B (P-2) was 5.05 times higher than in building A (P-1), but the difference was not statistically significant. The values of coefficient of variation showed as lack of variability of that parameter (Cv=0%, P-1), so and significant variability (Cv = 107.5%, P-2) (Table 1).

- Soil (P-3, P-4, P-5 and P-6): Maximum values for NCM (log CFU/kg$^{-3}$) have been found in the soil at P-3 (average for the investigated period – 4.89±0.31 log), which point was

**Table 1**
**Number of cultivable microorganisms (NCM) and Number of coliform bacteria (NCB) in manure, litter and soil in the studied poultry farm - mean (Cx), minimum (Cmin) and maximum (Cmax) values (log CFU/kg-3), standard deviation (SD) and coefficient of variation (Cv) values**

| Points | Samples | n | Cx±SD | $C_{min}$ | $C_{max}$ | Cv, % |
|---|---|---|---|---|---|---|
| Number of cultivable microorganisms (NCM) | | | | | | |
| Point 1 (building - A) | Fresh manure | 4 | 8,01±0,34[a] | 7.92 | 8.08 | 33.9 |
| Point 2 (building - B) | Fresh manure | 4 | 8,20±0,32[a] | 7.77 | 8.41 | 27.7 |
| Point 1 (building - A) | Litter | 4 | 6,46±0,26[b] | 6.08 | 6.65 | 37.3 |
| Point 2 (building - B) | Litter | 4 | 6,78±0,38[b] | 6.59 | 6.92 | 41.7 |
| Point 3, 2 m from the buildings | Soil | 4 | 4,89±0,31[c] | 4.65 | 5.1 | 44.1 |
| Point 4, 20 m from the buildings | Soil | 4 | 4,81±0,35[c] | 4.41 | 5.07 | 61.6 |
| Point 5, 50 m from the buildings | Soil | 4 | 4,65±0,27[cd] | 4.35 | 4.91 | 58.9 |
| Point 6, 500 m from the buildings, Control | Soil | 4 | 4,46±0,23[d] | 4.15 | 4.89 | 38.4 |
| Number of coliform bacteria (NCB) | | | | | | |
| Point 1 (building - A) | Fresh manure | 4 | 4,70±0,44[e] | 3 | 5 | 107.5 |
| Point 2 (building - B) | Fresh manure | 4 | 4,96±0,05[e] | 4.9 | 5 | 10.2 |
| Point 1 (building - A) | Litter | 4 | 4,00±0[e] | 4 | 4 | 0 |
| Point 2 (building - B) | Litter | 4 | 4,70±0,44[e] | 3 | 5 | 107.5 |
| Point 3, 2 m from the buildings | Soil | 4 | 2,84±0,33[f] | 2 | 3 | 85.7 |
| Point 4, 20 m from the buildings | Soil | 4 | 2,60±0,25[f] | 2 | 3 | 60.6 |
| Point 5, 50 m from the buildings | Soil | 4 | 2,60±0,25[f] | 2 | 3 | 60.6 |
| Point 6, 500 m from the buildings, Control | Soil | 4 | 2,0±0,50[f] | 1 | 3 | 70.4 |

*The differences among the points are statistically significant at $P < 0.05$, if symbols are not equal

**Table 2**
**Number of cultivable microorganisms (NCM) and Number of coliform bacteria (NCB) in air in the studied poultry farm - mean (Cx), minimum (Cmin) and maximum (Cmax) values (log CFU/m3), standard deviation (SD) and coefficient of variation (Cv) values**

| Points | n | Cx±SD | $C_{min}$ | $C_{max}$ | Cv, % |
|---|---|---|---|---|---|
| Number of cultivable microorganisms (NCM) | | | | | |
| Point 1 (building A) | 8 | 5,28±0,44[a] | 4.92 | 5.56 | 62.2 |
| Point 2 (building B) | 8 | 5,15±0,34[a] | 4.82 | 5.42 | 54 |
| Point 3, 2 m from the buildings | 8 | 4,87±0,30 | 4.72 | 4.97 | 29.2 |
| Point 4, 20 m from the buildings | 8 | 4,12±0,32[b] | 3.9 | 4.25 | 27.3 |
| Point 5, 50 m from the buildings | 8 | 3,97±0,40[b] | 3.54 | 4.24 | 74.3 |
| Point 6, 500 m from the buildings, Control | 8 | 3,92±0,33[b] | 3.39 | 4.23 | 69.9 |
| Number of coliform bacteria (NCB) | | | | | |
| Point 1 (building A) | 8 | 2,94±0,43 | 2.69 | 3.48 | 132.2 |
| Point 2 (building B) | 8 | 2,11±0,38 | NP** | 2.69 | 153.2 |
| Point 3, 2 m from the buildings | 8 | 3,37±0,44 | NP* | 3.95 | 161.3 |
| Point 4, 20 m from the buildings | 8 | 2,87±0,30 | NP* | 3.39 | 137.4 |
| Point 5, 50 m from the buildings | 8 | 2,39±0,35 | NP* | 2.99 | 143.2 |
| Point 6, 500 m from the buildings, Control | 8 | NP* | NP* | NP* | - |

*The differences among the points are statistically significant at $P < 0.05$, if symbols are not equal
** Not proved

nearest to the buildings (A and B), and minimum values at P-6, control (average for the period - 4.46±0.23 log), respectively, which point was outermost from the buildings (Table 1). The differences in NCM values between different points were statistically significant at $P<0.05$ only between P-3 and P-6, and between P-4 and P-6.

The NCB values of the soil (log CFU/kg$^{-3}$) from all monitoring points varied between 1 in P-6 and 3 in P-3, P-4 and P-5 (Table 1). However, the values of coefficient of variation (Cv=60.6-85.7%) revealed significant variability of this parameter, suggesting higher sensitivity to environmental factors. The differences of NCB values between different points were not statistically significant.

- Air (all points): The NCM (log CFU/m$^3$) in air varied widely between different monitoring points – from 3.39 in P-6 to 5.56 in P-1 (Table 2). However, there were points (P-1, P-2, P-5 and P-6) where the values of that indicator showed comparatively wide variation (Cv = 54.0-74.3%) and such points (P-4 and P-3) were variation was moderately (Cv = 27.3-29.2%). Maximum average values of NCM have been established in the air of both buildings (P-1 and P-2). In the same time the average values between the two buildings were differ materially. In P-1 NCM in the air was 1.36 times more than in P-2. In the first outside and nearest to the buildings point (P-3) average NCM in air sharply reduced. This trend was preserved for the remaining outside points. The differences in NCM between all monitoring points were statistically significant at $P<0.05$, except between P-1 and P-2, between P-4 and P-6, and between P-5 and P-6.

The NCB values (log CFU/m$^3$ air) varied more widely between different monitoring points than NCM – from points, where coliform bacteria were not proven in air (P-2, P-3, P-4, P-5 and P-6) to 3.95 in P-3 (Table 2). This high variability of the indicator's values was confirmed by the values of coefficient of variation (Cv =132.2 - 161.3%). Despite of the large differences in the values of the parameter between monitoring points, they were not statistically significant.

## Discussion

To properly manage built-up manure and litter, and prevent the outbreak of disease, on the one hand, and indoor manure, litter and air and outdoor air and soil of microbial pollution, of the other, it is necessary to understand interact with environment on the chain "fresh manure – litter – soil" and the role of air in these processes. The results obtained by the present study showed that NCM and NCB in fresh manure, litter, soil and air (log CFU/m$^{-3}$) were dynamic parameters, varying within broad ranges. It was evident they were influenced by numerous environmental factors.

There were lacks of data in the available literature about number of cultivable microorganisms and number of coliforms in fresh poultry manure and partly in the soil on the territory of the poultry farms, so we discussed our findings in a comparative aspect as with date for poultry, so with date for other animal species and farms. The date about NCM in fresh broiler manure (log CFU/kg$^{-3}$) – from 8.01±0.34 to 8.20±0.32 (Table 1) were lower than the results reported by El-Jalil et al. (2008) – $10^9$ CFU.g$^{-1}$ (10 log CFU kg-$^4$) for fresh poultry waste manure. Regarding the NCM in fresh manure by other animal species, our results were higher than those in fresh pig slurry - 5.86 – 6.41 log CFU/kg$^{-3}$ (Petkov et al., 2006), than the date for fresh cattle manure – $2x10^3$-$1x10^5$ CFU/g (3.30 - 5 log CFU kg$^{-3}$) and than the values for fresh sheep manure – $2x10^3$-$1x10^4$ CFU/g (3.30 – 4.0 log CFU/kg-$^3$) (Bildirev, 1983).

With respect to NCB in fresh manure (log CFU/kg$^{-3}$), our results 4.70±0.44 – 4.96±0.05, were higher to those of Soupir et al. (2006), who reported value of 3000 CFU/g (3.47 log CFU kg$^{-3}$) in turkey manure and our NCB values were in the range to those in the study of Petkov et al. (2006) in fresh pig slurry (2.97 – 5.12 log CFU kg$^{-3}$) and our results were lower than the values established by Bildirev (1983) in fresh cattle manure - $1x10^6$-$1x10^8$ CFU/g (6 - 8 log CFU kg$^{-3}$) and in fresh sheep manure - $1x10^5$-$1x10^6$ CFU/g (5 - 6 log CFU kg$^{-3}$). We assume that the main reasons for these differences in NCM and NCB in fresh manure from poultry and other animal species are associated with animal specie rearing in the farm as well as their feeding and the processes in the digestive tract.

In the next element of the chain, i.e. the broiler litter, the NCM (log CFU/kg$^{-3}$) 6.46±0.26 – 6.78±0.38 (Table 1) was lower than this found by Martin and McCann (1998) – 1.2-8 .$4x10^7$ CFU/g (7.07-7.92 log CFU/kg$^{-3}$) and by Lu et al. (2003) - $10^9$ CFU/g (9.0 log CFU/kg$^{-3}$) in broiler litter, and our results were close to the lower limit (anaerobic and aerobic bacteria) specified by Barker et al. (2010) – 6.38±0.09 – 7.59±0.13 log CFU/kg$^{-3}$ in broiler litter. As regards to NCB (log CFU/kg$^{-3}$), the established values 4.00±0 – 4.70±0.44 were much lower than the values reported by Barker et al. (2010) - 6.37±0.13 – 7.17±0.13 log CFU/kg$^{-3}$ for broiler litter. Obviously, this parameter also is affected by many factors of environment in each particular case, which determines the divergence of the results, obtained by the different authors.

Regarding the soil our date for NCM (log CFU/kg$^{-3}$) from different monitoring points 4.65±0.27 – 4.89±0.31 (Table 1) were in the range found by Stefanova (2012) in the soil of poultry farm for hens (11 250) and turkey (2 100) – $6.21x10^3$ – $375.0x10^3$ CFU/g (3.79 – 5.57 log CFU/kg$^{-3}$), by Petkov (2006) in the soil of pig farm with capacity for 600 swine – (3.73-5.31 log CFU/kg$^{-3}$), by Delev and Vitkov (1983) in soil of cattle

farms with capacity from 200 to 500 animals – 2600 – 62000 CFU/g (3.41-4.79 log CFU/kg$^{-3}$) and by Kostadinova (2003) in soil of dairy farms with capacity from 10 to 40 cows – 17.9x10$^3$ – 493.2x10$^3$ CFU/g (4.25-5.69 CFU/kg$^{-3}$). Obtained results for NCM in soil showed that the microbial contamination of the soil in the studied poultry farm is comparable to that of soils in other farms for poultry, cattle, pigs and sheep. Therefore, the microorganisms in manure and litter in the production buildings affect microbial soil pollution around them (A and B). In this case it must be considered, and many other environmental soil factors (type of soil, temperature, moisture, aeration, pH, organic matter and mineral salts, species composition of microorganisms) which impacts on the number of soil saprophytes. It is generally believed that the soil with a large number of microorganisms is biologically more active soil. However, the number of microorganisms in the soil is considered as an indication of its purity and quality.

The sanitary and hygienic evaluation of soil regarding to NCM, performed according to the criteria of Petkov and Baykov (1978) which revealed that, the soil at 2.0 m (P-3), at 20.0 m (P-4), at 50.0 m (P-5) and at 500 m from buildings (P-1 and P-2) could be referred as a slightly contaminated soil, because average, minimum and maximum values of NCM are between 4 and 5 log CFU/kg$^{-3}$. Only maximum values at P-3 and at P-4 define the soil from these points, such as contaminated soil (NCM is between 5 and 6 log CFU/kg$^{-3}$).

The date about NCB in soil (log CFU/kg$^{-3}$) 2.0±0.50 – 2.84±0.13 (Table 1) were close to the results reported by Stefanova (2012) for the soil in a poultry farm for hens and turkey 200-900 CFU/g (2.30 – 2.95 log CFU/kg$^{-3}$) and by Petkov (2006) for soil in a pig farm – up to 700 CFU/g (up to 2.84 log CFU/kg$^{-3}$), and our results were in the range of those found by Kostadinova (2003) in the soil of small dairy farms 40 – 70 000 CFU/g (1.60 – 4.84 log CFU/kg$^{-3}$). The presence of coliform bacteria reveals the potential danger of the soil contamination with pathogenic microorganisms. Their presence in the soil is a measure of potential risk in order to protect the health of humans and animals.

The sanitary hygienic evaluation of soil by the parameter NCB, regarding to the criteria of Danon-Moshe et al. (1985) characterized the soil at 2.0 m (P-3), at 20.0 m (P-4), at 50.0 m (P-5) and at 500 m from buildings (P-1 and P-2) as moderately contaminated soil (NCB is between 2 and 3 log CFU/kg$^{-3}$).

Results obtained for NCM in air of both buildings (P-1 and P-2) (log CFU/m$^3$) 5.15±0.34 - 5.28±0.44 (Table 2) were higher than the results found by Karwowska (2005) for number of staphylococci in poultry houses – from 1.5x10$^3$ to 4.6x10$^4$ CFU/m$^3$ (from 3.17 to 4.46 log CFU/m$^3$), and our results were lower than those reported by Rusak and Rokicki (1985) – from 1.9 to 4.6x10$^6$ CFU/m$^3$ (from 3.17 to 4.46 log CFU/m$^3$) in 5 broiler houses, each of them with capacity 16800 birds. Compared with the data of other authors, our results were with intermediate values. Baykov and Stoyanov (1999) found total number of microorganisms in air of broiler houses (20 birds/m$^2$) from 1.25×10$^3$ to 1.68×10$^5$ CFU/m$^3$ air (from 5.09 to 8.22 log CFU/m$^3$), Arganovski et al. (2007) – from 1.12x10$^5$ to 6.38x10$^6$ CFU/m$^3$ (from 5.04 to 6.80 log CFU/m$^3$), Bakutis et al. (2004) – from 109.2x10$^3$ to 714.7x10$^3$ CFU/m$^3$ (from 5.03 to 5.85 log CFU/m$^3$), Vučemilo et al. (2007) – from 1.7x10$^4$ to 2.2x10$^5$ CFU/m$^3$ (from 4.23 to 5.34 log CFU/m$^3$), 17 birds/m$^2$, and Plewa and Lonc (2011) - from 7.1x10$^3$ to 5.2x10$^5$ CFU/m$^3$ (from 4.85 to 5.71 log CFU/m$^3$) in broiler houses with capacity for 18000 – 23000 broiler chickens, respectively. The concentration of airborne microorganisms in poultry buildings in literature varies greatly, which could be explained as by different sampling methods used in different studies so and by different poultry species (broilers, hens and turkey), capacity of buildings, density of rearing of birds, age of birds, microclimate conditions, etc.

The animal hygienic evaluation of indoor air by the parameter NCM, regarding to the norm for poultry, according Regulation No44 (2006), determined the air quality in both broiler buildings (A and B) as air, that meets the requirements (NCM is up to 5.40 log CFU/m$^3$ air). The maximum value of NCM in both buildings only exceeded the maximum admissible concentration (NCM is over 5.40 log CFU/m$^3$ air) (Table 2).

The NCM in outdoor air (log CFU/m$^3$) - points P-3 at 2.0 m, P-4 at 20.0 m, P-5 at 50.0 m and P-5 at 500 m from the buildings (P-1 and P-2), 3.92±0.33 – 4.87±0.30 (Table 2) was close to those established by Rusak and Rokicki (1985) – 2.2x10$^3$-2.1x10$^4$ CFU/m$^3$ (3.34-4.32 log CFU/m$^3$) and by Petkov and Baykov (1988) – 12.0x10$^3$-2060.0x10$^3$ CFU/m$^3$ (4.07-6.31 log CFU/m$^3$) around similar of capacity broiler houses at distance up to 500 m from the buildings, and by Plewa and Lonc (2011) - 6.0x10$^3$-2.6x10$^4$ CFU/m$^3$ (3.77-4.41 log CFU/m$^3$) at distance between 10 and 100 m from the broiler houses.

The sanitary and hygienic evaluation of outside air regarding to NCM, performed according to accepted norm by Petkov and Baykov (1978), determined the air in all monitoring points (P-3, P-4, P-5 and P-6) as polluted air (NCM is over 3.47 log CFU/m$^3$ air). On the base of Polish norms (Plewa and Lonc, 2011) for outdoor air microbial contamination, the air in all points can be characterized as heavy polluted air, as the NCM (mesophilic bacteria) is over 3x10$^3$ CFU/m$^3$ - 3.47 log CFU/m$^3$ air. Besides this Polish standard include norm for clean air (when NCM < 1x10$^3$ CFU/m$^3$ - < 3.00 log CFU/m$^3$ air) and norm for medium polluted air (when NCM is from 1x10$^3$ to 3x10$^3$ CFU/m$^3$ – from 3.00 to 3.47 log CFU/m$^3$ air). Obviously, time has come for regulation of this parameter for atmospheric air quality in our country also.

The results for NCB in air of the buildings (A and B) – 2.11±0.38-2.94±0.43 log CFU/m$^3$ (Table 2) were in the range of those reported by Plewa and Lonc (2011) – from 1.69 to 4.27 log CFU/m$^3$, our results were closed to the date found by Karwowska (2005) – from 5.0x10$^0$ to 2.0x10$^2$ CFU/m$^3$ (from 1.69 to 2.30 log CFU/m$^3$) and they where much lower than the values established by Rusak and Rokicki (1985) – from 2.6 to 4.6x10$^4$ CFU/m$^3$ (from 4.41 to 4.66 log CFU/m$^3$). Although the NCM is small part of total number of microorganisms (from 0.09 to 0.45%) their presence in the air is indicative for sanitary conditions of the buildings.

With regard to the outside air results obtained for NCB at different points – P-3, P-4, P-5 and P-6 (from 0 to 2.94±0.43) (Table 2) were similar to the date received by Plewa and Lonc (2011) – from 0 to 2x10$^2$ CFU/m$^3$ (from 0 to 2.30 log CFU/m$^3$) at distance up to 100 m from the broiler buildings and our results were close to those established by Rusak and Rokicki (1985) – from 1.4x10$^2$ to1.6x13 CFU/m$^3$ (from 2.14 to 3.20 log CFU/m$^3$) at distance up to 500 m from the broiler houses. The NCB in outside air was up to 3.16% of NCM. The NCB can be used as indicator for assessing the quality and sanitary hygienic condition of air.

The analysis of data revealed a general tendency towards alteration in microbial counts along the chain "fresh manure – litter - soil at different points (P-3, P-4, P-5 and P-6)" and in the air at different points (P-1, P-2, P-3, P-4, P-5 and P-6). The NCM and NCB in fresh manure were greater compared to their values in litter and soil. The average NCM in fresh manure was from 26.1 (building B, P-2) to 35.8 (building A, P-1) times greater compared to litter in the same buildings and the differences in the average values were statistically significant at P<0.05. This tendency was preserved in respect of NCB, whose number was larger from 1.83 (P-2) to 5.05 times (P-1) in fresh manure vs. litter in both buildings. The main cause for the altered microflora in the litter compared to fresh manure is the change in the environmental conditions (especially the temperature, humidity and oxygen content of the substrate). After excretion, the temperature and humidity in the fresh manure sharply decreased and the oxygen content increased. These new conditions influence on the microbial survival and activities. A big part of intestinal microorganisms die, but new microbial species appears. Mixing of fresh manure with litter also affected these processes. The result of all this processes is the existence of considerably smaller number of microorganisms in the litter than in the fresh manure. Bratanov (1979), Bildirev (1983) and Ensminger (1992) pointed out similar explanations of this phenomenon in their studies for different animal species, including poultry.

An even more drastically reduces the number of microorganisms in the soil at 2 m distance from the buildings (P-3), compared to that in the litter (P-1 and P-2) – for NCM from 36.4 (P-3 vs. P-1) to 76.9 (P-3 vs. P-2) times and for NCB from 14.3 (P-3 vs. P-1) to 72.1 (P-3 vs. P-1) times. All differences in the average values of NCM and in the average values of NCB between inside points (P-1 and P-2) and outside points (P-3, P-4, P-5 and P-6) were statistically significant at P<0.05. The tendency was the same for NCM, although not so apparent, in the soil at the remaining points. Average NCM in soil at P-4 was 1.22 times lower than at P-3, at P-5 - 1.43 times lower than at P-4, and at P-6 - 1.54 times lower than at P-5. Only the differences between average values at points P-3 and P-4, from one side and point P-6, from the other, were statistically significant at P<0.05. Regarding the NCB in soil calculated ratios showed a different picture. Average NCM in the soil at P-4 was 1.75 times lower than at P-3, at P-5 it was equal to that at P-4 and, and at P-6 the NCB was 4.0 times lower than at P-5. Despite fluctuations in the NCB in soil from different monitoring points, for this parameter also there is a tendency to reduce their number with increasing the distance from the buildings.

A similar pronounced trend was found and for the NCM in the air. The NCM in air at 500.0 m (P-6) from buildings decreased 16.9-23.0 times over that air in the buildings (P-1 and P-2), 8.96 times over that air at 2.0 m distance from the buildings (P-3), 1.58 times over that air at 20.0 m distance from the buildings (P-4) and 1.11 times over that air at 50.0 m distance from the buildings (P-5), i.e. increasing the of the distance from the buildings led to reducing the NCM in air. The differences in NCM in air between P-1 and P-2, from one side and P-3, P-4, P-5 and P-6, from the other side, and between P-3, and P-4, P-5 and P-6 were statistically significant at P<0.05. Established dependence indicates that a major source of outside air microbial pollution in a poultry farm is a polluted inside air.

The average NCB in the air of both buildings (P-1 and P-2) was from 2.70 to 18.3 times lower than at outside air at P-3. The NCB in air at P-4 was 3.17 times lower compared to air at P-3, the NCB in air at P-5 was 3.00 times lower compared to air at P-4 and the NCB in air at P-6 was 250 times lower compared to air at P-5. The results characterized that parameter of air as more changeable. Nevertheless, and this parameter there is a tendency to reduce the values with increasing the distance from the production buildings.

Although the NCM and the NCB in the air of buildings (P-1 and P-2) was dramatically lower than in the manure and litter (average values for NCM – log CFU/kg$^{-3}$: fresh manure – 8.01-8.20; litter – 6.46-6.78, air /values were recalculated from NCM - log CFU/m$^3$ air to NCM - log CFU/kg$^{-3}$ air/ – 2.04-2.17 and for NCB - CFU/kg$^{-3}$: fresh manure – 4.70-4.96; litter – 4.00-4.70 and air – 2.11-2.94), there are reasonable

grounds to assume that the main sources for inside air microbial pollution are broiler manure and litter. This gives us reason to believe that through the air from buildings microorganisms dissipate in the atmosphere and then some of them sediment on the soil. Bearing in mind that litter removed from the premises is not stored on the farmyard and no surface runoff used for technological needs, the main source of environmental contamination by microorganisms is air from the production buildings. To support of this assertion are established similar dependencies to reduce the NCM and NCB, both in the air and in the soil with increasing the distance from the production buildings. Similar views stated and other authors (Bratanov, 1979; Mawdsley et. al., 1995; Doran and Michael, 2000; Soupir et al., 2006; Barker et al., 2010).

## Conclusion

NCM and NCB in fresh manure, in litter, in soil and in air of the investigated poultry farm varied widely, with clear differences among the various elements of the chain ''fresh manure–litter–soil at 2.0 m, at 20.0 m, at 50.0 m and at 500.0 m from poultry buildings" and in the indoor and outdoor air at the same distances as at the soil. In fresh manure NCM and NCB was much higher than litter and especially than air, and later significantly decreased in air and soil at 2.0 m from poultry buildings, and to a lower extent in air and soil at 20.0 m, at 50 m and at 500.0 m from broiler buildings.

The NCM in the soil at 500 m from poultry buildings decreased from 3515.3 to 5408.2 times over fresh manure, from 98.3 to 207.5 times over litter, 2.69 times over soil at 2 m, 2.21 times over soil at 20.0 m and 1.54 times over soil at 50.0 m from poultry buildings. For the NCB reduction in the number of coliforms in the soil at 500 m from the poultry buildings compared to fresh manure, litter and soil from other points are respectively – from 505.0 to 925.0 times (manure), from 100.0 to 505.0 times (litter), 7.0 times (soil at 2.0 m), 4.0 times (soil at 20.0 m) and 4.0 times (soil at 50.0 m).

The NCM in the air at 500 m from poultry buildings decreased from 16.9 to 23.0 times over air in poultry buildings, 9.0 times over air at 2.0 m, 1.58 times over air at 20.0 m and 1.11 times over air at 50.0 m from poultry buildings. For the NCB reduction in the number of coliforms in the air at 500 m from the poultry buildings compared to inside and outside air from different points are respectively – from 130.0 to 880.0 times (buildings), 2380 times (at 2.0 m), 750.0 times (at 20.0 m) and 250.0 times (at 50.0 m).

There are three major conclusions can be drawn: 1) the fresh broiler manure and litter are main sources of inside and outside air and soil pollution in poultry farm with saprophytic microorganisms including coliform bacteria, subject to sanitary control; 2) increasing the distance from poultry buildings, led to reducing the NCM and NCB in the soil and in the air; 3) when removed from the broiler buildings manure and litter are not stored on the farm and no surface drainage process water, the air from the production buildings is a major source of environmental contamination with microorganisms.

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