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MICROBIOLOGICAL AIR RATING IN A VARIETY OF OBJECTS DURING TREATMENT OF THE POST-SLAUGHTER POULTRY WASTES PART II. BACTERIA

MIKROBIOLOGICZNA OCENA POWIETRZA W RÓŻNYCH OBIEKTACH PRZETWARZANIA POUBOJOWYCH ODPADÓW DROBIOWYCH CZĘŚĆ II. BAKTERIE

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Streszczenie. W niniejszej pracy przeanalizowano skład powietrza pod względem bioaerozolu bakteryjnego na terenie zagospodarowania poubojowych odpadów drobiarskich. Próbki powietrza pobrano w 4 terminach z 5 różnych obiektów (z budynku przerobu wstępnego wraz z obróbką chemiczną, z basenu z odpadami ciekłymi, z obiektu składowania osadu z oczyszczalni biologicznej, z obiektu przygotowania odpadów do kompostowania oraz z kompostowni właściwej). Analizy wykonano zgodnie z procedurami mikrobiologii środowiskowej. Oznaczono liczebność bakterii ze szczególnym uwzględnieniem *Pseudomonas* sp. i *Clostridium* sp. oraz *Actinomycetes* sp. Na podstawie przeprowadzonych badań powietrza stwierdzono, że bioaerozol bakteryjny występował w różnych ilościach, w zależności od wybranych grup mikroorganizmów i różnił się pomiędzy terminami wykonywanych analiz i punktami badawczymi w zależności od rodzaju i przeznaczenia obiektu zagospodarowania tychże odpadów.

Key words: bacterial bioaerosols, air, composting, poultry waste.
Słowa kluczowe: bioaerozol bakteryjny, powietrze, kompostownie, odpady drobiowe.

INTRODUCTION

Poultry productions have become more common in many countries of the world. Such production that deals with large densities of animals in small areas is a significant source of microbial air contamination that may arise a risk to human health (Schulze et al. 2006). Exposure to high concentrations of airborne bacteria and particulate matter (PM) can harm the health of animals and workers (Andersen et al. 2004). Poultry production facilities are associated with high concentrations of airborne microorganisms compared to ambient environment (Nimmermark et al. 2009; Miao et al. 2010; Zhao et al. 2011).

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Air quality has become a source of major environmental trouble of the poultry industry. Dust, odors and bioaerosols (e.g. microbes, endotoxins and mycotoxins suspended in air) generated at production, manure storage facilities and during land spreading of poultry litter constitute the most frequent source of complaints against animal-based industries (Millner 2009).

Poultry by-products and waste may contain up to 100 different species of microorganisms, including pathogens in contaminated feathers, feet and intestinal contents (Arvanitoyannis and Ladas 2007). Recent reports on the microbial status of poultry litter have demonstrated the presence of genera known for their pathogenicity: *Staphylococcus, Clostridium perfringens, Salmonella, Campylobacter, Streptococcus, Enterococcus,* and *Pseudomonas* (Lu et al. 2003). Some microorganisms can be transmitted through the air and cause animal diseases (Otake et al. 2010; Zhao et al. 2011, 2013).

Clostridium perfringens is found in soil, water, air, food, and the intestinal tract of human and animals, and is the most rapidly growing foodborne pathogen. *C. perfringens* has the ability to grow over a temperature range of 15–50ºC, but it grows best at relatively high temperatures, typically 43–46ºC (Dahiya et al. 2006; Labbe and Juneja 2006; McClane 2007). Bacteria *Pseudomonas* are comprising the main spoilage microorganisms of meat (Jay et al. 2005). *Pseudomonas* sp. were the predominant bacteria in broiler house (Vucemilo et al. 2005). *Actinomycetes* are Gram positive bacteria having a heterogeneous group of filamentous bacteria resembling fungi (Ventura et al. 2007). They are a major component of bioaerosols emitted from composting facilities (Swan et al. 2003; Taha et al. 2006).

The aim of the study was to determine the bacterial bioaerosol in the air within facilities related to the processing of poultry waste with particular emphasis to bacteria of *Pseudomonas* genus, including *P. fluorescens*, *Clostridium perfringens* and *Actinomycetes*.

MATERIAL AND METHODS

Assays of bacterial bioaerosols composition were carried out at four dates, i.e. I – 19.03.2015, II – 21.05.2015, III – 25.06.2015, and IV – 14.01.2016 within the facilities of poultry waste management in West Pomeranian province.

The air for microbiological analyzes was taken at various points assigned to the individual stages of waste management (different locations), as described in detail in part I (Oraibi and Cybulska 2016). These were the following facilities marked as below:

1) reservoirs for liquid wastes,
2) preparation of wastes after processing for composting,
3) storage of sediments from biological treatment plant,
4) proper composting facility,
5) building for pre-treatment with chemical processing.

Analyses were made under different weather conditions, i.e. date I (6ºC, relative humidity 49%, wind force 1 m · s^{-1}); date II (13ºC, relative humidity 61%, wind force 1 m · s^{-1}); date III (17ºC, relative humidity 72%, wind force 1 m · s^{-1}); date IV (1ºC, relative humidity 92%, wind force 1 m · s^{-1}). Objects such as buildings, composting facility, as well as additional equipment for management and processing of poultry waste into the compost were secured with a uniform solid fence of height from 2 to 5 m.

The assays used sedimentation method and concentration of microorganisms was expressed as number of cells able to develop in 1 m^3 (cfu · m^{-3}) (PN-89/Z-04111/01; PN--89/Z-04111/02).

Microbial tests included count determination of:

- Total number of mesophilic bacteria on nutrient agar MPA (incubation at 30°C; 72 h);
- *Pseudomonas sp.* on King B substrate (incubation at 37°C; 2 to 5 days; colonies green or blue fluorescent in UV light at wavelength 366 nm were counted);
- *Clostridium sp.* – selective SPS substrate (incubation at 35°C; 24–36 h; characteristic black colonies were counted) – Downes and Ito (2001);
- actinomycetes – substrate according to Cyganov (incubation at 26°C for 6 days) – Cyganov and Žukov (1964).

The Petri dishes for air sampling were open for 15 minutes, in three replications. All colonies were assigned to analyzed groups and expressed in cfu per 1 m^3 of air and then results were subject to statistical processing in Statistica 12 software.

RESULTS AND DISCUSSION

The presence of bacteria was found at all the test points (Fig. 1) in amounts from nearly 580 cfu recorded in location No. 4 to almost 42.000 per 1 m^3 recorded in location No. 5. Many researchers have reported an increased number of airborne microorganisms in objects relating to both breeding and processing of poultry (Arvanitoyannis and Ladas 2007; Millner 2009; Nimmermark et al. 2009; Miao et al. 2010; Zhao et al. 2011). Statistical analysis of obtained results confirmed highly significant effect of the sampling point, date and interaction of these factors on the total bacteria count. The total number of airborne bacteria varied widely in different sampling times and measuring locations. However, the average values indicate that the largest air pollution took place at the location No. 5 (twice as high as the average level), while the smallest at location No. 3 (below half of the mean). Poorly marked tendency to reduce the level of air pollution on subsequent dates is shown at the first four locations; instead, the opposite trend is observed at the last location. Average values indicate a slightly lower air pollution on the second measurement date. The greatest dispersion of results was recorded for location No. 5, while the smallest for location No. 3; other locations are characterized by similar dispersion of results.

Reports on the microbiological state of poultry breeding environment have revealed the presence of different types of bacteria known for their virulence: *Staphylococcus, Clostridium perfringens*, *Salmonella*, *Campylobacter*, *Streptococcus*, *Enterococcus*, and *Pseudomonas* (Lu et al. 2003).

The study allowed to detect the presence of *Clostridium* in the air of all study points, however, both frequency of their occurrence on particular dates and observed count substantially varied (Fig. 2). Air contamination with *Clostridium* occurred in over a half (55%) of measurements. Most cases of air pollution and maximum values appeared on the first two sampling dates. Detected quantities reached from 0 (lack) up to 812 cfu · m^{-3}.

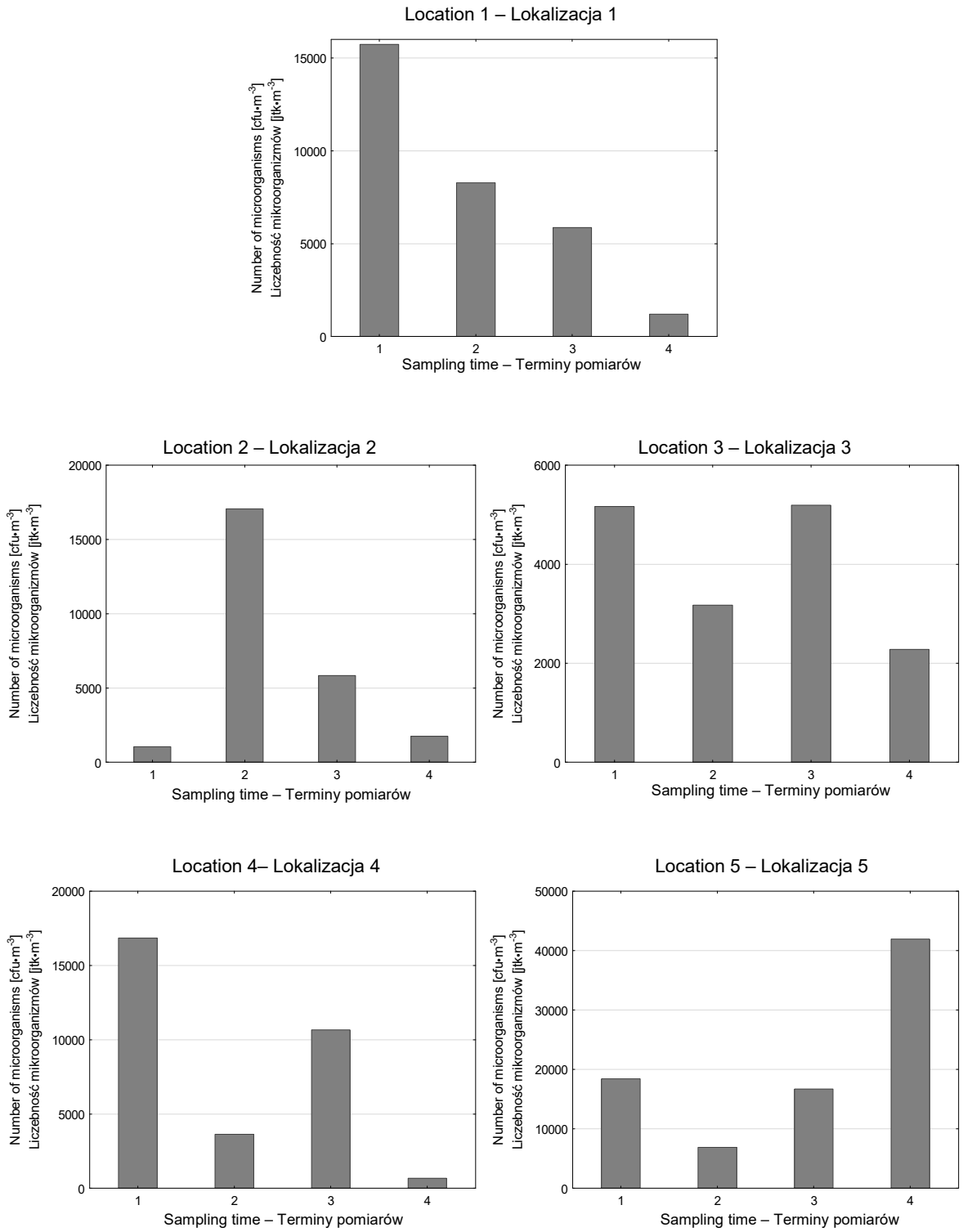

Fig. 1. Total airborne bacteria count in tested locations on particular measurement dates
Ryc. 1. Ogólna liczba bakterii w powietrzu w badanych obiektach w poszczególnych terminach pomiarowych

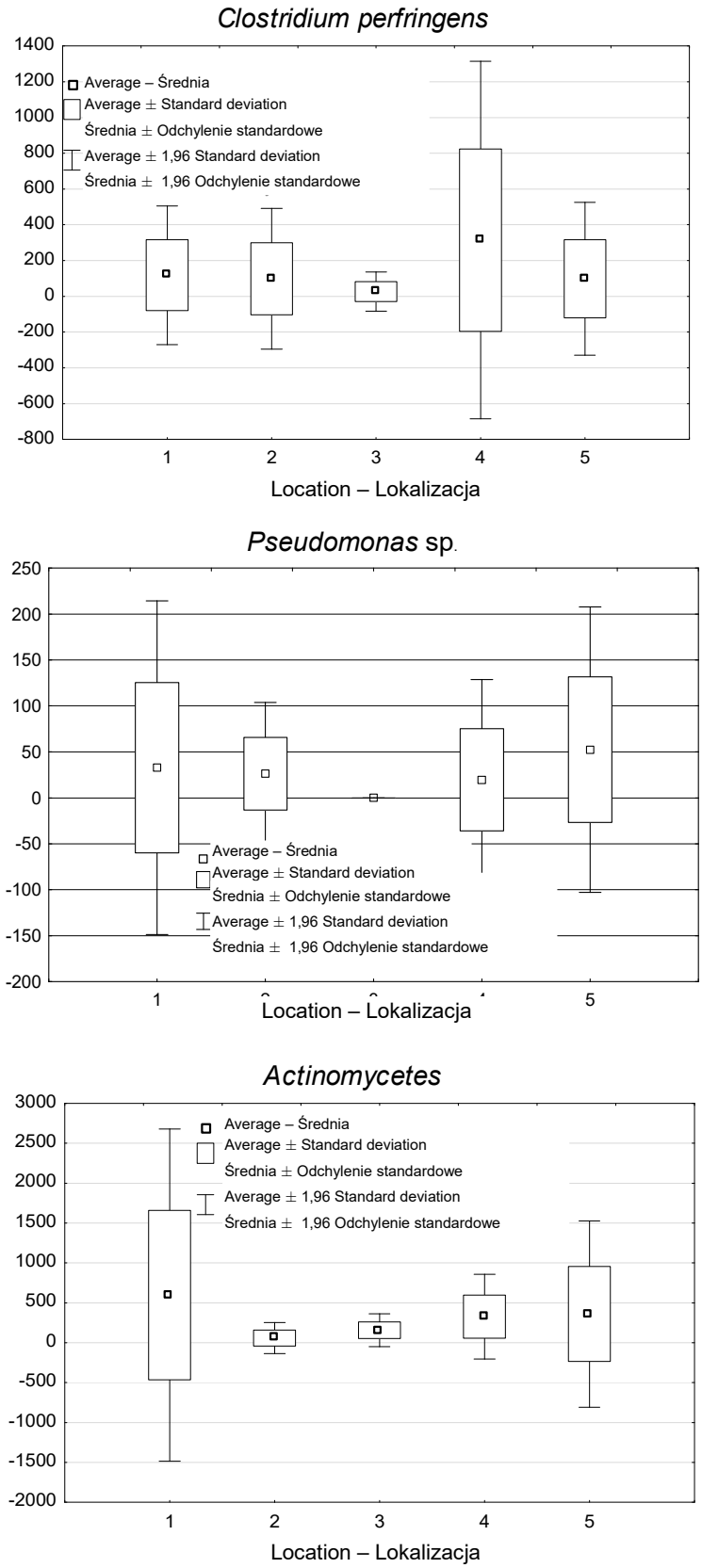

Fig. 2. Characteristics of recorded distributions of selected bacteria counts in the air of tested measurement points

Ryc. 2. Charakterystyka stwierdzonych rozkładów liczebności wybranych rodzajów bakterii w powietrzu w badanych punktach pomiarowych

Statistical analysis confirmed the presence of highly significant impact of sampling on the number of bacteria tested. Very small amounts of *Clostridium* bacteria occurred in the air near the landfill of centrifuge sludge from biological treatment plant (location No. 3), significantly higher in the air above the store of wastes prepared to composting, in a chemical pre-treatment facility, and at the pool for liquid wastes (locations No. 2, 5, 1). The largest amounts (314 cfu · m^{-3}, on average) were found in the air of the proper composting facility (location No. 4). This latter localization was also characterized by the greatest dispersion of results, whereas the least one was recorded at location No. 3 (landfill of wastes after centrifuging).

Quantities of airborne *Pseudomonas* sp. bacteria amounted from 0 to 157 cfu per 1 m^3 of air. Their presence was not detected only in the air of centrifuge waste landfill in biological treatment plant (location No. 3), while at other measurement points, they were found on one or two sampling dates. All cases of *Pseudomonas* sp. bacteria detection in the air occurred only on the first two dates of measurement.

Statistical analysis showed the presence of highly significant effect of the sampling point on the number of bacteria tested. The count of airborne *Pseudomonas* sp. bacteria revealed more spread in the air by the pool for liquid waste and chemical pre-treatment facility (locations No. 1 and 5), slightly less in the air above the landfill of materials prepared for composting and composting facility (locations No. 2 and 4). *Pseudomonas* family bacteria are a group of microorganisms occurring and well developing in sites containing meat products (Jay et al. 2005). *Pseudomonas sp.* were found particularly at broiler breeding (Vucemilo et al. 2005), thus their presence was also confirmed in the air of tested facilities.

The study allowed to detect the presence of actinomycetes in the air of all research points, however, both the frequency of their occurrence on a particular date and observed counts considerably varied (Fig. 2). The air pollution due to actinomycetes occurred in 75% of measurements. Statistical analysis confirmed the presence of highly significant effect of the sampling point, date, and interaction of both factors on the amount of tested bacteria. Only at locations No. 3 and 4 (landfill of sludge from centrifuge and composting facility) revealed the presence of airborne actinomycetes on all dates of measurement. At other measuring points, the occurrence of these organisms was found on two of the four dates.

The greatest dispersion of results was observed for location No. 1 (pool for liquid waste) and then in descending order, at locations No. 5, 4, 3, 2 (chemical pre-treatment, composting, centrifuge sludge landfill, sludge prepared for composting).

The airborne actinomycetes count amounted from 0 up to 2306 cfu · m^{-3}. In ascending sequence, localizations can be lined up as follows (mean values for all dates): landfill of waste prepared for composting (59 cfu · m^{-3}), centrifuge sludge landfill (157 cfu · m^{-3}), composting facility (327 cfu · m^{-3}, chemical pre-treatment (360 cfu · m^{-3}), and air over the pool for liquid wastes (596 cfu · m^{-3}). Their presence has been confirmed by many authors, particularly in various types of composting facilities and related installations (Swan et al. 2003; Taha et al. 2006).

The cluster analysis shows that among tested measurement points, location No. 5 (chemical pre-treatment) forms a separate group. The second one is composed of all the other locations, that are closely related to each other, with locations No. 1 and No. 2 (pool for liquid waste and landfill of materials prepared for composting) grouping the total bacteria and *Clostridium* genus; in the case of *Pseudomonas* genus bacteria, there were measuring points No. 2 and No. 4.

For airborne actinomycetes, the cluster analysis indicates that among tested measurement points, location No. 1 (air at the pool for liquid wastes) is a separate group. The other group contains all other locations closely related to one another, including locations No. 2 and No. 4 (landfill of materials prepared for composting and composting facility) forming a group with the closest proximity.

Analysis of the percentage of particular groups and bacterial genera reveals that different species and genera of bacteria make up over 90%, on average. *Escherichia coli* and actinomycetes were present in 3% each (Oraibi and Cybulska 2016), while 1% was shared by *Clostridium* genus bacteria; the lowest percentage was shown by *Pseudomonas* genus bacteria, including *P. fluorescens* species (Fig. 3).

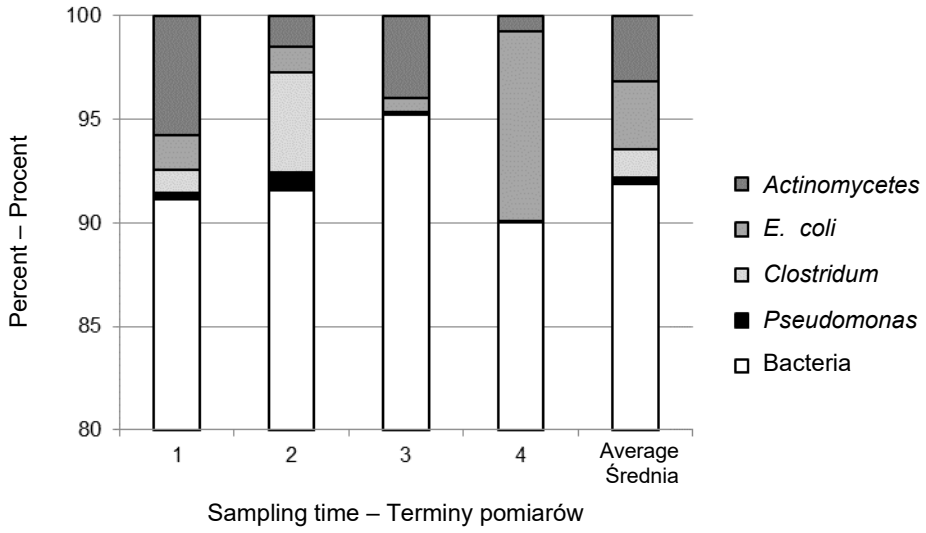

Fig. 3. Mean percentage of individual groups of bacteria depending on the measurement dates
Ryc. 3. Średni udział procentowy poszczególnych grup bakterii w zależności od terminów badawczych

CONCLUSIONS

Air of analyzed objects for poultry wastes processing unveiled the presence of bacteria, the count of which depended on type of the measurement point and testing date. The size of bacterial pollution amounted from 576 to 41942 cfu · m^{-3} air. Tested locations can be lined up in a following sequence in terms of ascending air contamination: the least at the centrifuge waste landfill (location No. 3), moderate at the pool for liquid wastes, at the store of wastes prepared for composting, and in composting facility (locations No. 1, 2, and 4), and the greatest in building of chemical pre-treatment (location No. 5).

The amount of contamination due to *Pseudomonas* gens bacteria amounted from 0 up to 157 cfu · m^{-3} air. Tested locations could be sequenced depending on the increasing pollution: centrifuge waste landfill (location No. 3), composting facility (location No. 4), landfill of wastes prepared for composting (location No. 2), pool for liquid wastes (location No. 1), chemical pre-treatment facility (location No. 5).

Bacteria of *Clostridium* genus occurred at the amount from 0 to 812 cfu · m^{-3} air. The localizations were detected, can be lined up in a following increasing contamination of air:

centrifuge waste landfill (location No. 3), landfill of wastes prepared for composting (location No. 2), chemical pre-treatment facility (location No. 5), and pool for liquid wastes (location No. 1), composting facility (location No. 4).

Number of actinomycetes oscillated from 0 up to 2306 cfu · m^{-3} air. Tested locations can be lined up in a following sequence in terms of ascending air contamination: landfill of wastes prepared for composting, centrifuge waste landfill, composting facility, chemical pre-treatment facility, air over the pool for liquid wastes (locations No. 2, 3, 4, 5, 1).

When analyzing the percentage of individual groups and types of bacteria, it can be concluded that, on average, more than 90% are the remaining species and types of bacteria. In terms of the incidence of other study groups (8% share, on average), these may be identified as follows: actinomycetes (75%), *Escherichia coli* (65%), *Clostridium* (55%), *Pseudomonas* (30%) respectivly.

Actinomycetes and *Clostridium* genus bacteria were detected at all measurement points and on all dates. Only *Pseudomonas* bacteria was not identified in the air of the centrifuge sediments landfill (location No. 3) on dates 1 and 2. The *Clostridium* genus bacteria were found at very small amounts on dates 3 and 4, and also in the air over the centrifuge sludge landfill (location No. 3). On some dates, actinomycetes were absent at measurement points No. 1, 2, and 5.

For every tested microorganisms, except from actinomycetes, the chemical pre-treatment facility (location No. 5) can be distinguished as a separate group differing in its pollution characteristics from other ones. For actinomycetes, that separate group was formed by the pool for liquid wastes (location No. 1).

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Abstract. In this paper, composition of the air in terms of bacterial bioaerosol in the management of post-slaughter poultry waste, was analyzed. The air samples were collected at 4 dates from 5 different locations – buildings (building for pre-processing with chemical treatment, pool for liquid waste, facility for storage of sludge from biological sewage treatment, object for preparation of waste subject to composting, and proper composting facility). Analyses were carried out in accordance with the procedures of environmental microbiology. Number of total bacteria was determined with particular emphasis put to *Pseudomonas* sp. and *Clostridium* sp. as well as *Actinomycetes* sp. Based on the air assays, it was found that the bacterial bioaerosol was present in varying degrees depending on the selected groups of microorganisms and differed between dates of analyzes and research points i.e. type and purpose of the object for these wastes management.