

# Bacterial aerosols in an urban nursery school in Gliwice, Poland: a case study

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**Abstract** This work presents the results of the study of airborne bacteria in a kindergarten in Gliwice, Upper Silesia, Poland. In this study, the samples of bioaerosols were collected using six-stage Andersen cascade impactor (with aerodynamic cutoff diameters 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65  $\mu\text{m}$ ). The level of culturable bacterial aerosols indoors was about 3000 CFU  $\text{m}^{-3}$ —six to eight times higher than outdoors. In the classrooms, respirable bacterial particles,  $<4.7 \mu\text{m}$ , contributed up to 85 % of the total number of culturable bacteria, increasing the possible adverse health effects due to their inhalation. The identification of the bacterial species showing the dominance of gram-positive cocci in the indoor environment and non-sporing gram-positive rods in the outdoor air indicates that most of the bacteria present in the studied kindergarten are human origin. Using the obtained data, the nursery school exposure dose (*NSED*) of bioaerosols was estimated for the children and personnel of this kindergarten (nursery school). The highest value of *NSED* was obtained for younger

children (930 CFU  $\text{kg}^{-1}$ ) compared to older children (about 600 CFU  $\text{kg}^{-1}$ ) and to the kindergarten staff (about 300 CFU  $\text{kg}^{-1}$ ). This result suggests the elevated risk of adverse health effects in younger children exposed to the bioaerosols in the kindergarten, including infections.

**Keywords** Bioaerosols · Size distribution · Bacteria identification · Preschool

## 1 Introduction

People spend more than 90 % of the day in indoors environments (Ashmore and Dimitroulopoulou 2009; Lee and Chang 1986; Wichmann et al. 2010). In case of younger children, nursery school is the main indoor environment besides home. According to studies conducted in the last 20 years by the US Environmental Protection Agency, indoor air is sometimes 70–100 times more polluted than the outdoor air (Kotzias 2005). Consequently, early life exposure to bioaerosols found at nursery schools and their possible roles in airway diseases is a critical area of research. According to the Act on the education system of September 7, 1991, and the Regulation of the Minister for Education and Sport of December 31, 2002 on health and safety in schools and teaching—learning (DzU 1991, 2003) as well as the Framework Directive 89/391/EEG (EU 2000), the directors of these

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institutions are required to ensure safe and hygienic conditions of stay in these facilities. An appropriate microbiological concentration of air pollution is one of the elements of the management of health and safety in schools and education. The primary objective of microbial air quality is to determine the amount of existing bioaerosol particles and their identification. In this particular field, nursery schools could be a very interesting case study for two reasons: firstly, because children are more vulnerable to environmental pollutants compared to adults since they breathe more air relatively to their body weight and also have a lower ability to deal with the toxic chemicals due to their undeveloped airways (Branco et al. 2014; Santamouris et al. 2008; Selgrade et al. 2008) and secondly, because poor indoor air quality at classrooms was demonstrated to exert a negative impact on children's learning performance, with absenteeism and adverse health effects such as increased risk of asthma and other health-related symptoms (Aydogdu et al. 2010; Canha et al. 2013; Patelarou et al. 2015).

Studies on bioaerosols have been carried out in a variety of indoor environments: schools and pre-schools (Dumała and Dudzińska 2013; Górny et al. 2014; Kim et al. 2007; Salleh et al. 2011; Stryjawska-Sekulska et al. 2007), childcare centres (Aydogdu et al. 2010), hospitals and public buildings (Kim and Kim 2007; Pastuszka et al. 2005), and apartments (Karottki et al. 2015; Moon et al. 2014; Nasir and Colbeck 2010; Pastuszka et al. 2000). Most of these studies focused on the total concentration of bioaerosols and show a great variation in the total concentration of air pollutants. This information is indispensable for the assessment of population exposure, as well as for the identification of biological aerosols emission sources. However, not only the total concentration of bioaerosol particles is important. In particular, particle size is critical with regard to their fate in the air and their deposition in the human respiratory system (Latif et al. 2014). Bioaerosols vary considerably in size approximately from 0.02 to 100  $\mu\text{m}$ . The size distribution of bioaerosols depends upon the type of microorganism species, age of the spore and nutrient medium, humidity, differences in aggregation rates of the spores, and type of particles they are associated with such as mist or dust. It should be noted that bioaerosol particles can occur in air as single cells or aggregates of cells as well as in fragments. They are often transported attached to other

particles, such as skin flakes, soil, dust, saliva, or water droplets (Nasir and Colbeck 2010).

Since establishing the continuous monitoring of the bioaerosols level indoors, including the children's indoor environments is practically impossible, a great number of case studies on this subject are needed. Although many papers have been published on the exposure to bioaerosols in the residential indoor environments, there are still no studies on bioaerosols size distribution in nursery schools where preschool children spend substantial time.

The present study was carried out in the urban nursery school in Gliwice, a town in the southern Poland to find the total concentration of airborne bacteria and their size distribution and to identify culturable bacterial genera. In particular, we analysed the impact of the bacterial flora present in outdoor air on the bacterial aerosols in the indoor air. The relationship between winter and spring seasons as well as younger and older children classrooms was also examined.

## 2 Materials and methods

Gliwice is a typical city in the industrial region of Upper Silesia (4.5 million people in the region). The city is home to 36 nursery schools, 72.2 % of which are public. The study was carried out in nursery school located in residential and traffic area during the winter (from 7th to 17th January) and spring (from 7th to 17th April) 2014.

### 2.1 Sampling site and building

The nursery school is located in an urban traffic area. The front facade of the building is located/sited 50 m from the street with heavy traffic reaching 2400–2800 vehicles per hour (Kozielska 2013). Between the building and the street, there is parking space available, which enables the flow of air from the traffic in the street.

Nursery school is located in detached building with two floors. The building underwent the process of thermal efficiency improvement, which was completed in 2007. The process included thermal insulation of the exterior walls and the installation of airtight windows. Inside the buildings, the heating system modernization included new radiators and pipes and in

the building the replacement of a low-class heat centre. In the building, heat circulation is equipped with a weather-compensated control system. During the thermal insulation process, the natural ventilation using the air duct systems of the buildings was left unchanged. Consequently, the indoor air quality is mostly ensured by means of stack ventilation and airing through open and unsealed windows.

Children attending the nursery school range from 3 to 6 years old divided by age into six different classrooms. The daily schedule is as follows: breakfast occurs at about 9:00, lunch at 12 o'clock, and at 14:00 dessert. The classrooms are usually subjected to airing for a few minutes, with the children leaving the classrooms for toilet activities prior to and after meals. In the nursery school, younger children rest on sleeping mats covered by nursery school blankets. During the resting time, they are usually watching fairy tale stories on a television. For the duration of an afternoon nap, one or two windows are usually unsealed.

In all classrooms, floors were predominantly covered with vinyl floor covering and partly covered with carpet. Cleaning inside the classrooms occurs in the morning or at the end of the afternoon, when children are not in the classrooms, while daily cleaning in corridors and common spaces is conducted during children occupying the classrooms. The nursery school has a spacious outdoor playground.

The study was conducted in two nursery school classrooms, with the volume of 210 m<sup>3</sup> each. The first of these was the room of children aged 5–6 years (classroom I), and the second room of children aged 3–4 years (classroom II). The classroom for older children (I) is situated above the classroom of younger children (II). In each group, there were 25 children and 1–2 nursery staff. The samples of bacterial aerosols were collected in the centre of each classroom at a height of about 1 m from the ground, which is in the breathing zone of children. Three sets of measurements were performed in every classroom and outdoors. All 108 Petri dishes with biological material were analysed. Furthermore, the number of children was recorded (on average 18 younger and 20 older children were present in the classroom).

## 2.2 Sampling and analytical methods

Air samples were collected by six-stage Andersen cascade impactor (with aerodynamic cut—size

diameters of 7.0, 4.7, 3.3, 2.1, 1.1 and 0.65 µm). The impactor is also composed of a pump, which provides a constant flow rate (28.3 L min<sup>-1</sup>) during measurement. Sampling time was 10 min. Microorganisms were collected on nutrient media in Petri dishes located on all impactor stages. Tryptic soy agar (TSA) was used for bacteria, with cycloheximide added to inhibit fungal growth. Field blanks were also tested during the sampling. The Petri dishes were incubated 48 h at 36 ± 1 °C. It is important to note that although direct measurement of the concentration of living airborne bacteria is extremely difficult, the commonly used substitute of the concentration of living microorganisms present in the air is the number of colony-forming units in the volume of air CFU m<sup>-3</sup>.

The next step was the identification of collected bacteria, which took place in two stages. The first stage involved an analysis of morphological and microscopic colonies of grown cells stained with Gram. In the second stage API biochemical tests were carried out, which allowed the differentiation of the bacterial strains—on the basis of their metabolic properties.

Bacterial identification was based on morphology, Gram staining, and endospore formation. Bacteria were grouped as Gram-positive cocci, nonsporing Gram-positive rods, endospore-forming Gram-positive rods bacilli, and Gram-negative bacteria, according to their microscopic morphology.

## 2.3 Quality control

Quality control was practiced throughout the analyses to avoid any interference and minimize the risk of error. The bioaerosol analyses were continuously performed on the basis of the PN-EN12322 standard (2005), which recommends an adequate number of culture media from each series in order to test the microbial contamination. Sterility was ensured by incubating the culture medium at a temperature appropriate for the method used for at least 3 days (>72 h). The standard PN-EN 12322 does not specify how often the culture media must be controlled for sterility or the temperature to incubate them. Therefore, the sterility testing was based on another standard, ISO 11133 (2014). The testing of blank plates was performed per batch of sample at the temperature used during the performed procedure. The sampling (Andersen Impactor) and laboratory (laminar flow cabinet, autoclave, incubators, and

microscope) equipment are regularly checked and have current certificates.

## 2.4 Statistical analysis

All statistical calculations were performed using the statistical package Statistica 10 (StatSoft). The non-parametric Mann–Whitney  $U$  and Wilcoxon matched-pairs tests were used. The Mann–Whitney  $U$  test was used to analyse whether the total concentrations of bioaerosols were different between seasons and locations (outdoor and inside the classrooms). Meanwhile, the Wilcoxon test was performed in order to compare the size distributions of bioaerosols between different seasons in outdoor and indoor (older and younger children's classrooms) air. A statistical significance level of  $\alpha = 0.05$  was used throughout the study.

## 3 Results and discussion

### 3.1 Total bacterial aerosols

The first remark is that the average concentration of the total bacterial aerosols in the indoor and outdoor environment differed significantly (Table 1). The level of bacterial aerosol concentration in the indoor air ranged from 2500 to 3000 CFU m<sup>-3</sup> and exceeded 6–8.7 times the level recorded in the outdoor air. These results indicate the significant role of internal emission sources and agree well with the literature data. The studies on indoor air quality in nursery school conducted by Yang et al. (2009) in South Korea from July to December 2004 in 55 schools, reported that the average concentration of bacterial aerosols was

1300 CFU m<sup>-3</sup>, and the maximum concentration reached the value of 4700 CFU m<sup>-3</sup>.

Similar studies were carried out in three schools in Portugal, and the results showed that the concentration of bacteria in the indoor air in schools was >500 CFU m<sup>-3</sup> (Pegas et al. 2010). The values obtained in nursery school in Gliwice are also comparable to the data obtained in the studies conducted in one of the schools in Lublin, Poland, where the concentration of airborne bacteria was 3500 and 2000 CFU m<sup>-3</sup>, in winter and spring, respectively (Dumała and Dudzińska 2013). Significantly lower concentration level of bacterial aerosols was found in the Upper Silesian homes (1021 CFU m<sup>-3</sup>) and offices (300 CFU m<sup>-3</sup>) (Pastuszka et al. 2000), while in the sport hall in an elementary school in this area the concentration of airborne bacteria was very high even during the first class (almost 5500 CFU m<sup>-3</sup>) and rapidly increased with lesson hours, finally reaching the level higher than 12,000 CFU m<sup>-3</sup> (Pastuszka et al. 2004).

In Poland and in many other countries the legislation governing microbiological standards for air pollution have not been developed and implemented. The main reason for this is a huge variety of air microflora and a large variety of methods. Therefore, there are no generally accepted criteria for assessing exposure to biological agents. Polish proposals for regulatory levels of bacterial aerosols residences and public buildings were presented by Górný et al. (2011). A wide review of these propositions for non-industrial workplaces can be summarized according to the following proposal, for the total number of bacteria up to 7000 CFU m<sup>-3</sup>. It can be seen that the

**Table 1** Average concentration  $C$  (CFU m<sup>-3</sup>) of total culturable bacteria in the studied outdoor and indoor air; (OUT)—outdoor; (I)—older children's classroom; (II)—younger children's classroom

	Average concentrations of bacterial aerosols				Indoor/outdoor ratio	
	Winter		Spring		Winter	Spring
	C	SD	C	SD		
(OUT)	354	7.5	427	144.2	–	–
Classroom (I)	3098	353.0	2545	440.1	8.75	5.96
Classroom (II)	2915	745.7	2890	785.3	8.23	6.77

SD standard deviation; The number of collected samples for each season was three in outdoor air, as well as three in classroom I and three in classroom II. Each sample included six impaction stages with Petri dishes

concentration levels of airborne bacteria obtained in our study were below the proposed standard.

The high value of the obtained indoor/outdoor concentration ratio points to share of internal sources of bacteria in the formation of indoor air pollution. It should be noted that especially high I/O ratio was observed in winter. There are probably two reasons of this phenomenon. First, in this cold season, the lowest concentration of bacteria in the outside is generally reported. The second reason is certainly the formation of microbiota in indoor air during the use of the central heating system. The heated air circulates around the room arousing dust and lifting it together with biological particles. This resuspension process can elevate the level of different kinds of airborne particles, including bacteria.

Detailed analysis of Table 1 indicates that no significant differences ( $p > 0.05$ ) between total CFU counts were found between the older (I) and younger (II) children's classrooms in the nursery school under study. Season also did not influence the total concentration of bacteria ( $p_{OUT} = 0.66$ ,  $p_I = 0.38$  and  $p_{II} = 0.98$ ). However, differences between indoor and outdoor total bacteria concentrations were significant ( $p < 0.1$ ) in each season of measurement. The indoor-to-outdoor ratio suggests the significant role of indoor factors, especially the activity of children. Significantly, the higher number of culturable bacteria indoors could also be due to ingress of outdoor dust particles and their further resuspension from different indoor surfaces.

### 3.2 Size distribution of bacterial aerosols

Figure 1 shows examples of the size distribution of bacterial aerosols in the atmospheric air in the two seasons of research—winter and spring. No significant differences in size distribution ( $p_{OUT} = 0.13$ ,  $p_I = 0.12$  and  $p_{II} = 0.87$ ) were found between the seasons. Bacterial aerosols in the atmosphere, both in spring and winter, reached a maximum concentration of bacteria in the air at the same range of diameters (4.7–7  $\mu\text{m}$ ).

High contribution of the bacterial particles having aerodynamic diameter  $> 2.1 \mu\text{m}$  may be partially caused by rafting of some fine bacterial cells on the surface of coarse particles. Figure 2 shows such solid particles located on the colony of airborne bacteria collected from the atmospheric air in Gliwice on the

stage 1 (cutoff diameter  $> 7 \mu\text{m}$ ). It is possible that one or some small bacterial cells were attached to this coarse solid particle and formed this agglomerate. During the sampling process, this agglomerate was classified by the impactor as one big bacterial particle, and after incubation such picture as in Fig. 2 appears. On the contrary, the microorganisms present in the nursery school in Gliwice are mostly fresh, therefore, like in the other indoor air [example:(Pastuszka et al. 2000)] the fine bacterial particle dominated there (Figs. 3, 4).

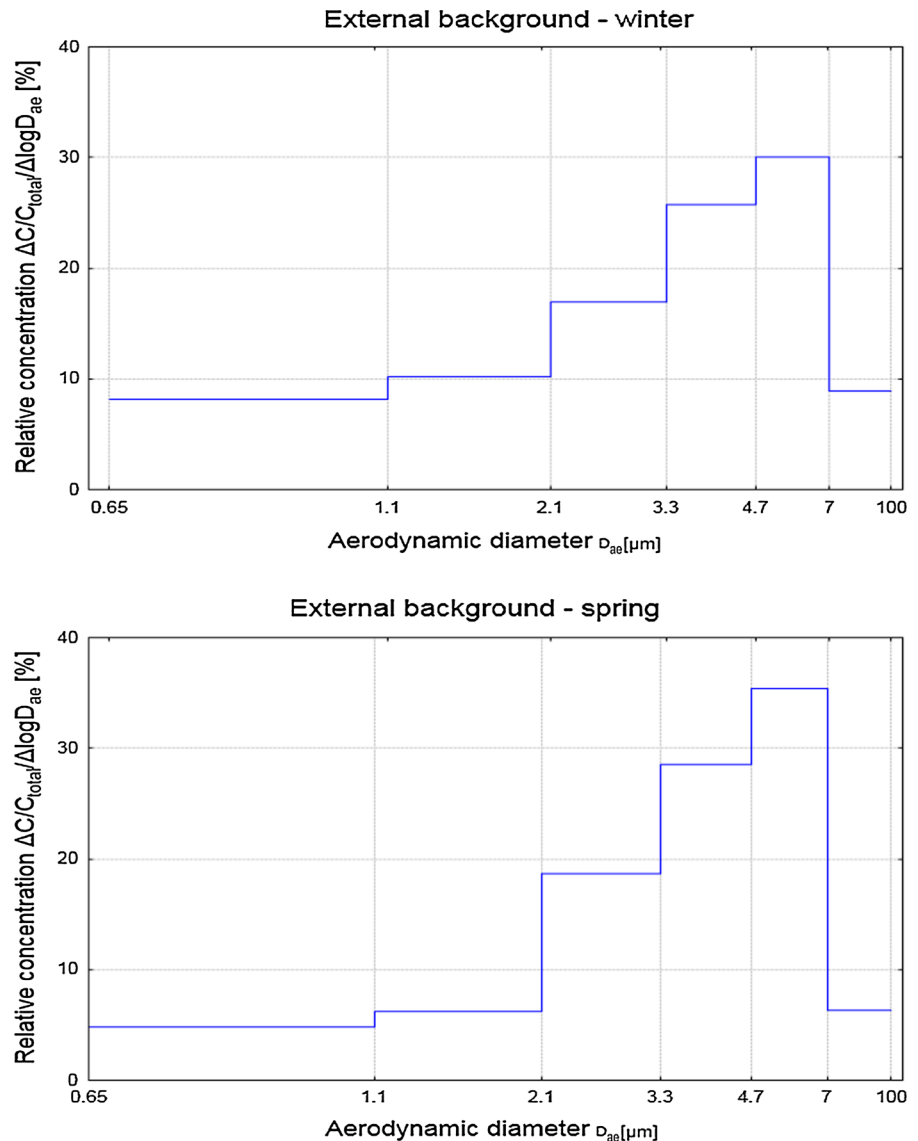
In fact, Figs. 3 and 4 show that small particles,  $< 4.7 \mu\text{m}$ , are more prevalent in indoor air. These respirable particles mostly can deposit in either the tracheal, bronchial, or alveolar region of the lungs. In the older children's classroom (I), the concentration peak appeared for the bacterial particles ranged from 1.1 to 2.1  $\mu\text{m}$ . In the younger children's classroom (II), the peak was shifted into the bigger particles: 2.1–3.3  $\mu\text{m}$  and 3.3–4.7  $\mu\text{m}$ , during the winter and spring, respectively.

### 3.3 Identification of bacterial aerosols

Four groups of culturable bacteria were identified: Gram-positive cocci, nonsporing Gram-positive rods, sporing Gram-positive rods, family *Bacillaceae*, and Gram-negative rods (Table 2). Airborne Gram-positive bacteria were the most abundant, accounting for 90 % of the measured population. Gram-negative bacteria were present in  $< 10$  % of outdoor samples in our study. Somewhat similar observations have been made in which Gram-negative bacteria were found in lower counts (Aydogdu et al. 2010). Among the Gram-negative bacteria, only *Pseudomonas spp.* was found in our study.

The results of bacterial species identification for aerosols in the outdoor environment revealed that the largest percentage relative to the total bacterial flora were Gram-positive bacilli-forming endospores; during winter 43 % and in the spring 58 %. Aerobic and facultative anaerobic Gram-positive bacilli are commonly found in soil and water habitants, and in many part of the normal skin and mucous membrane flora of humans and various animals. The virulence of Gram-positive bacilli is highly variable. Many of them have the potential to be opportunistic pathogens, capable of producing disease only in persons with compromised host resistance (Aydogdu et al. 2010), characteristic

**Fig. 1** Size distribution of the bacterial aerosols in the outdoor air



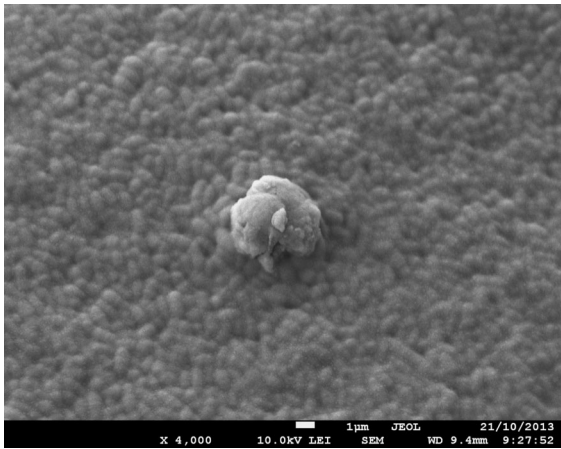
especially of small children. The second most frequently isolated group of bacteria in winter were Gram-positive nonsporing rods (28 %), while in spring this was Gram-positive cocci (20 %).

Our results show that Gram-positive cocci were dominant in the indoor air of both classrooms, which is in agreement with other research (Aydogdu et al. 2010; Dumała and Dudzińska 2013; Pastuszka et al. 2000). Gram-positive bacteria, particularly the cocci, are microorganisms that are widespread in nature and can be isolated either from the environment or from the skin, mucous membranes, and other body sites in

humans and animals as commensal inhabitants (Aydogdu et al. 2010).

The activity of children is normally high, especially—older children, and thus Gram-positive cocci can be transmitted to the air from children's bodies and respiratory tracts. The results of qualitative analysis obtained in the nursery school in Gliwice are comparable to those obtained in the studies conducted in schools in Lublin, Poland, where the dominant bacteria isolated from indoor air samples were: *Micrococcus* spp., *Staphylococcus*, *Bacillus cereus*, *Bacillus pumilus*, and *Bacillus lentus* (Dumała and Dudzińska





**Fig. 2** Micrograph of the part of bacterial colony incubated on the TSA agar after sampling on the first stage of the Andersen impactor (cut-size diameter > 7 µm). Sample was collected in the atmospheric air in Gliwice

2013). Similar results were also obtained by Kim et al. (2007) in the indoor environment of elementary schools in Ulsan, South Korea; they found that 84 % of identified bacteria were observed as Gram-positive, and *Micrococcus* spp. was the most abundant group with 61 % of tested isolates. The study carried out by Stryjakowska-Sekulska et al. (2007) in various rooms of the university buildings in Poznań, Poland, also shows that the predominant airborne bacteria were *Staphylococcus* spp. and *Micrococcus* spp.

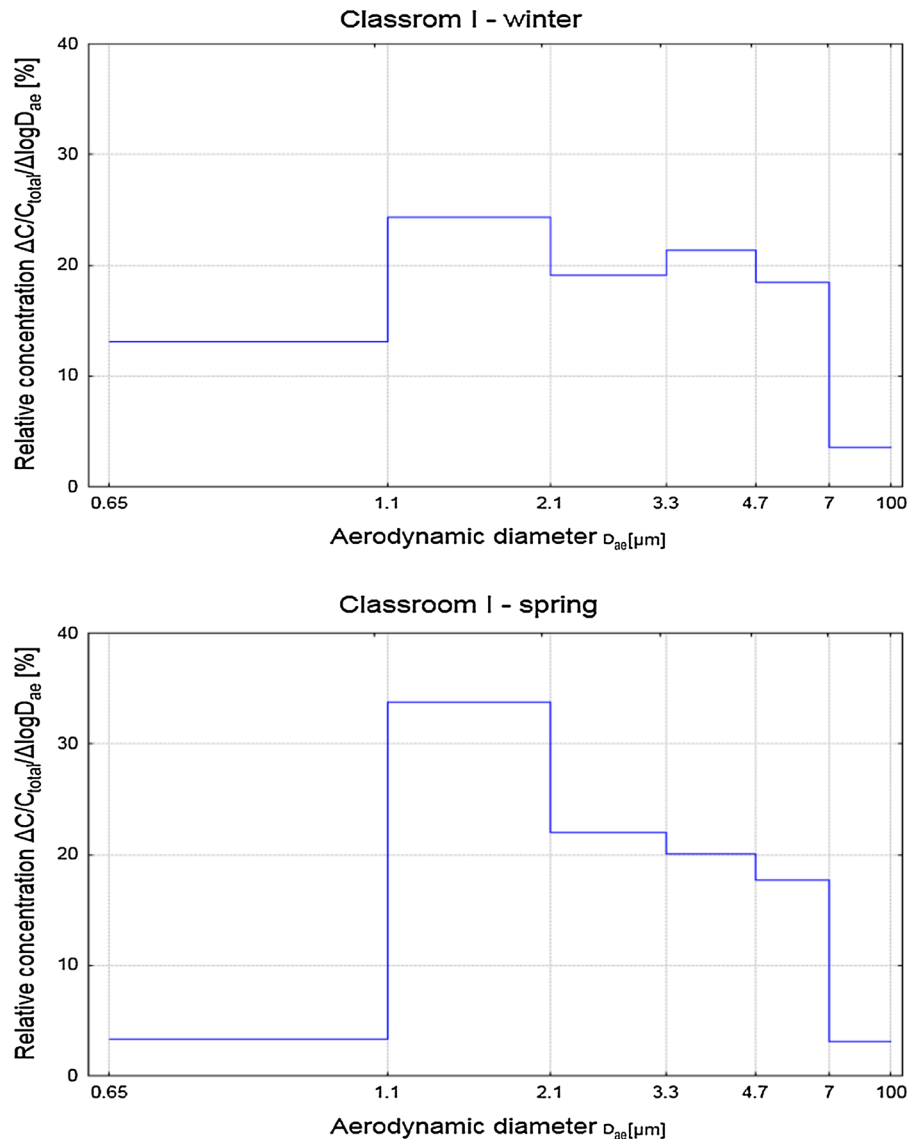
Generally, in the air of the studied nursery school, exposure to the bacterial aerosols does not create an immediate risk of any acute health effects; however, the long-term inhalation of such high doses of airborne bacteria can cause some adverse health effects, especially among sensitized persons. Such persons may have greater susceptibility to diseases of the upper respiratory tract and allergic symptoms such as headache, watery eyes, itchy skin, coughing, and others (Daisey et al. 2003). Although the level of the microbial pollution inside the studied kindergarten should be classified as safe, some action to improve the indoor air quality is needed. It can be expected that if some sick children are present in the kindergarten, the level of pathogenic bacteria will rapidly elevate in this building, especially in the rooms with younger children. Therefore, the increase in the air exchange rate there is strongly recommended. Certainly, such recommendation can be addressed to huge number of the kindergartens in Poland.

### 3.4 Nursery school exposure dose (*NSED*)

The interest in exposure to bioaerosols has increased over the last few decades because they are associated with a wide range of health effects with major public health impacts, including infection by diseases, acute toxic effects, allergies and cancer. However, even as regards only infection diseases, no clear correlation has been found between concentrations of culturable microorganisms in the air and infection (Henningson et al. 1997). One reason for this could be that infection should be correlated with the dose rather than the concentration. Although dose–response relationships still have not been established for most biological agents (Douwes et al. 2003; Walser et al. 2015), the bioaerosols expert network concluded recently (Walser et al. 2015) that the measurement of bioaerosols should be performed according to a protocol representative of exposure patterns and duration, which means—in fact—the dose. This is not an entirely new idea. For example, in 1994 Alekseev et al. reported their study on the dynamics of *P. pseudomallei* infection and its antigens in white rats after aerogenic infection with a dose equal to 941 CFU. Pastuszka (2001) estimated that children living in healthy homes in Upper Silesia, Poland, inhaled daily doses of 1780 and 560 CFU of airborne bacteria and fungi, respectively. Therefore, estimating the dose of culturable bacteria inhaled by those in the nursery school of the present study seems to be important for possible future exposure analysis. We think, however, that in the future assessment of the risk of infection for children and staff depends not only on the number of inhaled culturable bacteria important but so too on their body mass. According to this hypothesis, we calculated the doses of culturable bacteria per mean body weight.

The nursery exposure dose (*NSED*) was calculated on the basis of the EPA's Child-specific Exposure Factors Handbook (US EPA 2002) and other publications (Johnson-Restrepo and Kannan 2009; Ott 2006; US EPA 2004). Total concentration levels were used in the calculations of absorbed dose of airborne bacteria although particles with aerodynamic diameter > 10 µm usually cannot be inhaled. However, in the studied indoor environment the contribution of bacterial particles >10 µm is extremely low and may be neglected (see the Fig. 3, 4). Based on the measured concentration of bacterial aerosols as well as on additional data including children and staff activity

**Fig. 3** Size distribution of the bacterial aerosols in the indoor air of classroom I



pattern and the general characteristics, received from questionnaires, the dose inhaled by the adult, in this case nursery staff, as well as by the older and younger children was estimated. The calculations were based on the following equation:

$$NSED = \frac{C \cdot IR \cdot IEF}{BW} \quad (1)$$

where:

*NSED*—nursery school exposure dose for indoor environment,  $\text{CFU kg}^{-1}$

*C*—bacterial aerosols concentration,  $\text{CFU m}^{-3}$

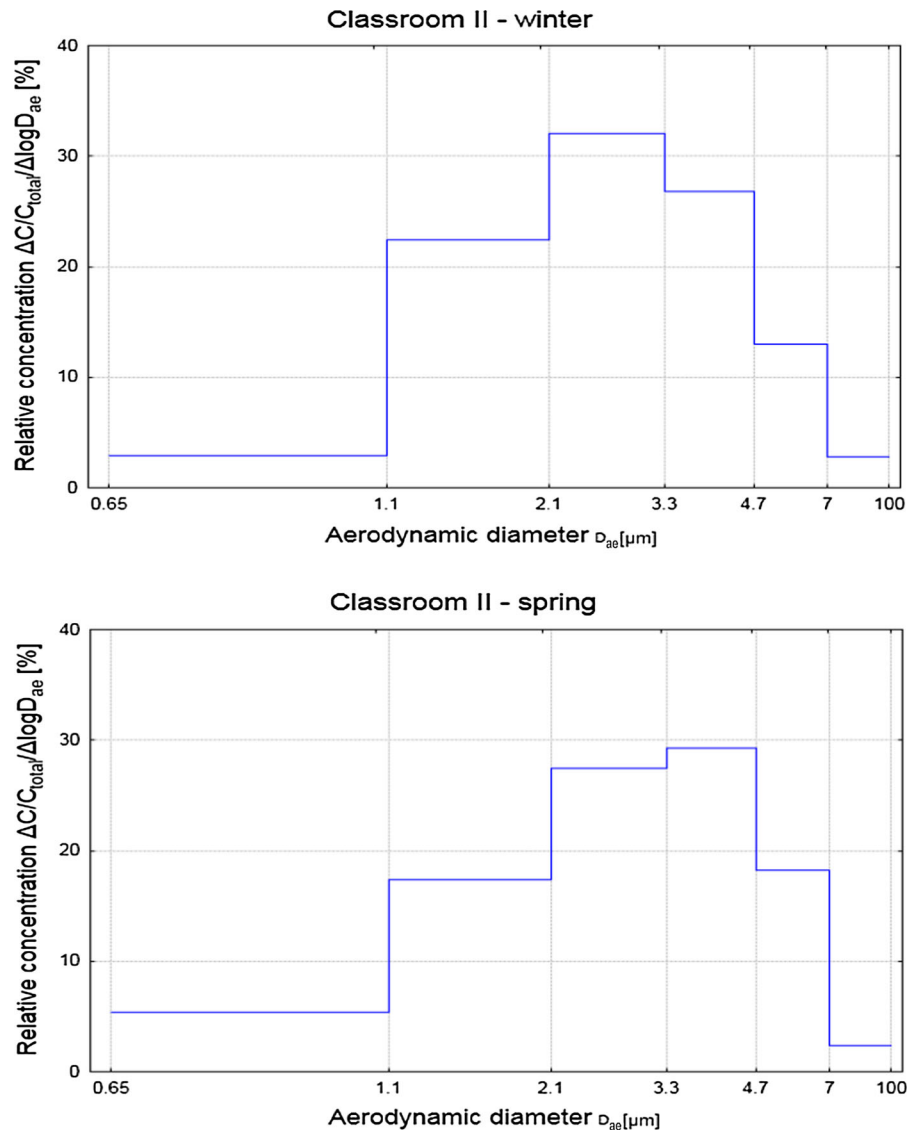
*IEF*—indoor exposure fraction—hours spent over a day in nursery school, concerning diverse activity patterns hour (in sum on average: children 7 h, adults 8 h)

*IR*—inhalation rate coefficient, characteristic for selected activity levels. It was assumed that the weighted average of *IR* was  $0.72 \text{ m}^3 \text{ h}^{-1}$  for children and  $0.79 \text{ m}^3 \text{ h}^{-1}$  for staff (U.S. EPA 2011).

*BW*—mean body weight, kg. The measured mean body weight was equal to 23.5 kg and 15.8 kg for older and younger children, respectively, while for the staff was 59 kg.



**Fig. 4** Size distribution of the bacterial aerosols in the indoor air of classroom II



The detailed values of *IR* and *IEF* obtained in our study, depending on the children and staff physical activity, are contained in Table 3, while in Table 4 the calculated results of the inhaled dose of culturable airborne bacteria are shown. Generally, it can be concluded that the higher dose in case of children compared to staff was attributed to the higher inhalation of air relative to body weight.

It can be seen that both children and staff inhaled the highest dose (*NSED*) of bacterial aerosols in winter. However, the most important is to note that younger children inhaled significantly higher *NSED* than the older ones. Although the bacterial dose

inhaled by the staff in the studied kindergarten is 1.5–1.9 times higher than the dose absorbed by adults inside apartments in Katowice, Upper Silesia ( $175.4 \text{ CFU kg}^{-1}$ ) (Brągoszewska 2014), their *NSED* is three times lower than the dose inhaled by younger children. It could be one of the reasons that children, especially these who started their preschool education in the kindergarten, are frequently sick because adverse health effects strongly depend on the absorbed dose of air pollutants. Additionally, the infection diseases among children spread faster than among adults. In this age group, there is no proper awareness of the importance of hygiene in the prevention of

**Table 2** Culturable bacterial genera identified outdoors—during winter and spring, and indoors in the classrooms during winter

Bacteria	Percentage of species in total bacteria concentration (%)			
	Outdoor air (OUT)		Classroom	
	Winter	Spring	Older children (I) Winter	Younger children (II)
Gram-positive cocci, including:	19	20	90	82
<i>Micrococcus spp.</i>	19	11	36	48
<i>Staphylococcus epidermidis</i>	n.i.	n.i.	14	22
<i>Staphylococcus sciuri</i>	n.i.	9	19	12
<i>Staphylococcus lentus</i>	n.i.	n.i.	21	n.i.
Nonsporing Gram-positive rods, including:	28	14	n.i.	n.i.
<i>Brevibacterium spp.</i>	10	14	n.i.	n.i.
<i>Corynebacteriu auris</i>	18	n.i.	n.i.	n.i.
Sporing Gram-positive rods, family Bacillaceae, including:	43	58	10	18
<i>Bacillus subtilis</i>	11	28	n.i.	n.i.
<i>Bacillus cereus</i>	24	14	10	18
<i>Bacillus pumilus</i>	8	11	n.i.	n.i.
<i>Bacillus circulans</i>	n.i.	5	n.i.	n.i.
Gram-negative rods, including:	10	8	n.i.	n.i.
<i>Pseudomonas spp.</i>	10	8	n.i.	n.i.

n.i. not identified

**Table 3** Dependence of the inhalation rate (IR) and indoor exposure fraction (IEF) on children's and staff's activity

Activity of the occupants of the nursery school	Children				Nursery school	
	Older (I)		Younger (II)		Staff	
	IR, m <sup>3</sup> /h	IEF, h	IR, m <sup>3</sup> /h	IEF, h	IR, m <sup>3</sup> /h	IEF, h
Sleep or nap	0.26	–	0.26	2	0.30	–
Sedimentary passive	0.27	2	0.27	–	0.29	3
Light intensity	0.66	3	0.66	3	0.78	3
Moderate intensity	1.26	2	1.26	2	1.68	–
High intensity	2.22	–	2.22	–	3.12	–

**Table 4** Calculated exposure dose (NSED) of bacterial aerosols inhaled by children and staff

Season	NSED—nursery school exposure dose [CFU kg <sup>-1</sup> ]			
	Children		Nursery school staff	
	Older (I)	Younger (II)	(I)	(II)
Winter	664.4	929.9	331.9	312.3
Spring	545.8	921.9	272.6	309.6

infection, and in terms of preschool, children often only learn good hygiene. Inadequate hand washing, direct contact during the game, and being in constant

motion favour the spread of bacterial infections, which increases the risk of exposure educators to harmful microorganisms (Górny et al. 2014).

#### 4 Conclusions

Simultaneous study of bacterial aerosols in the indoor and outdoor environment was carried out in the nursery school in Gliwice, Poland.

The level of culturable bacterial aerosols indoors was about 3000 CFU m<sup>-3</sup>—six to eight times higher than outdoors. The obtained result indicates the

significant role of indoor emission sources in this kindergarten.

Culturable bacterial particles  $<4.7 \mu\text{m}$  contributed up to 85 % of the total concentration of the bacterial aerosols inside the studied building. This result additionally increases the health risk for exposed children.

The highest values of the dose of bacterial aerosols inhaled by the staff and the nursery's children were recorded in the winter; the older (I) and younger children (II) absorbed 664.47 and 929.9 CFU  $\text{kg}^{-1}$ , respectively. The higher *NSED* of younger children is connected with the higher inhalation rate relative to body weight. The level of *NSED* for children is up to three times higher than for the staff.

The indoor air is dominated by Gram-positive cocci, including *Micrococcus* spp. These bacteria migrate from the respiratory system and human body. The outdoor environment is dominated by Gram-positive rods forming endospores. The main sources of these bacteria are soil, plants, and water.

Although the obtained results seem to be typical for a great number of urban nursery schools in Poland, future studies on the exposure to bioaerosols in kindergartens located in other regions, especially in rural areas are needed.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

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