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Airborne Bacteria in Gliwice—The Industrialized City in Poland

Michał Kowalski [1,2], Jozef S. Pastuszka [1,†], Agnieszka Brąszewska [3], Josef Cyrys [2] and Ewa Brągoszewska [4,*]

1 Department of Air Protection, Silesian University of Technology, 44-100 Gliwice, Poland
2 Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, 85764 Neuherberg, Germany
3 Plant Cytogenetics and Molecular Biology Group, Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice, 40-032 Katowice, Poland
4 Department of Technologies and Installations for Waste Management, Silesian University of Technology, 44-100 Gliwice, Poland
* Correspondence: ewa.bragoszewska@polsl.pl
† Deceased.

Abstract: The results of the study on the characteristics of the viable (culturable) and total bacterial particles in the ambient air in Gliwice, Poland, are presented. The concentration of viable bacteria in the air ranged from 57 CFU m^{-3} (Colony Forming Units per cubic meter) during winter to 305 CFU m^{-3} in spring, while the concentration of all bacteria (live and dead) in the air, measured in selected days, ranged from 298 cells m^{-3} in winter to over 25 thousand per m^3 in autumn. A field study was also carried out to find out the level of the sterilization rate (k) for airborne bacteria. The obtained value of k for viable bacteria exposed to UV solar radiation in Gliwice was approximately 10 cm^2 W^{-1}s^{-1}. The patterns of the size distributions of viable bacteria found in three seasons, spring, summer, and autumn, were similar, showing a peak in the range of 3.3–4.7 μm. In the winter season, the main peak was shifted into the smaller particles with an aerodynamic diameter ranging from 2.1 to 4.7 μm. The dominant group of culturable bacteria within the studied period was Gram-positive rods-forming endospores (34–55%), while the least frequent were Gram-negative rods (2%). This research can be used to assess the health effects of exposure to bacterial aerosols in people living in this area.

Keywords: bioaerosol; culturable bacteria; non-culturable bacteria; size distribution; airborne bacteria identification; solar radiation; sterilization rate

1. Introduction

Bioaerosols are a mixture of particles of biological origin, such as viable and inactive forms of bacteria and fungi (both pathogenic and non-pathogenic), their metabolites, endotoxins and mycotoxins, peptidoglycans, β(1,3)-glucans, viruses, high molecular weight allergens and pollens [1,2]. These bioaerosol particles can cause respiratory diseases and increase mortality and morbidity [3]. Especially, elevated microbial concentrations in the air have a greater impact on causing infectious diseases [4,5], respiratory diseases [6,7] and even cancer [8,9].

Additionally, it should be noted that not only the inhalation of viable microorganisms can be dangerous [9]. For example, Gram-negative bacteria contain endotoxins, which can be secreted even from dead bacterial cells [10]. Generally, for many reasons, knowledge of both the viable and total amount of bacterial particles in the atmospheric air (containing alive and dead cells) is needed.

Due to their wide prevalence in the environment and specific emission sources, bioaerosols present a high variation in composition, dominant fractions in size distribution, mostly occurring species and live/dead organisms' ratio. Because of their biological

character and undergoing different processes in the ambient air, bioaerosols present strong seasonal variability in the case of concentration and composition. Usually, the significant seasonal variability is observed in winter, obtaining a few times lower concentrations, compared to other seasons [11–13].

Research on bacterial and fungal aerosol has been developed for many years; the first published data on the subject of biological aerosols dates back to the XIX century [14–16]. However, information on the concentrations and influencing factors of viable bacteria is still poorly understood [17]. Multiple intensive studies on the concentration levels of bioaerosols and their characteristics in the indoor environment have been conducted (also in Poland, for example [18,19]). Based on that, researchers' attention shifted again to the problem of bacteria and fungi in the atmospheric air due to the dependence of the structure of these bioaerosols from meteorological parameters. This dependence is much more complex than in the case of dust and gaseous air pollutants. Additionally, as a result of the progressive climate changes, the structure of bioaerosols in the atmospheric air should be expected [20–22]. The climate is becoming more temperate, and the differences between seasons are blurring [23]. Frequent atmospheric fronts and extreme phenomena, such as heavy downpours, tornadoes and strong storms, accompanied by relatively long periods of even drought, cause the meteorological parameters to change dynamically, strongly influencing the biological/species composition of airborne biological particles, its concentration in air (including live particle concentration) and particle size distribution. These meteorological factors are, among others, air temperature, relative air humidity, intensity of solar radiation and ozone concentration in the air. The movement of air masses from remote geographic areas can, in turn, cause the migration of pathogenic microbes from southern Europe and even from Africa [24]. Therefore, it is becoming increasingly urgent to predict changes in the characteristics of bioaerosols, especially bacterial aerosols, which will occur over the next two decades. The first step to achieving this goal would be to establish the influence of meteorological parameters on bioaerosol characteristics. Unfortunately, no relevant algorithms strongly link the concentration, size distribution and species composition of bacterial aerosol with basic meteorological parameters, such as temperature and air humidity, wind speed and amount of atmospheric precipitation. The situation becomes even more complicated when attempting to describe the impact of a simultaneous change in several meteorological parameters, which is a situation often occurring in real conditions. This combined simultaneous interaction of various meteorological factors implies that the only method of prognostic modeling seems to work using artificial neural networks [25]. For this to happen, however, it is necessary to collect a large amount of measured data concerning the characteristics of bacterial aerosols with selected meteorological parameters in specific areas with specific topographic characteristics and a specific network of bioaerosol emission sources.

Due to the complex formation of biological particles present in ambient air, different sampling techniques to estimate the bioaerosol concentration have to be used. Originally, measurements of viable biological aerosol concentration were conducted using culturable techniques [26]. It should be noted that inactive microorganism or their fragments are not able to grow colonies on nutrient media. Furthermore, not all metabolically active bacteria can grow colonies on basic culturable media [27,28]. It is known that non-culturable organisms can cause several adverse health effects, such as inflammation or hypersensitivity [1,29–31]. For this reason, new techniques are being developed to determine the total concentration of bioaerosols [32], implementing systems of continuous emission monitoring and a new generation of samplers. These are usually based on measurements of luminescence and autoluminescence of biological particles [33], especially structural elements and metabolites, such as tryptophan, tyrosine or NAD(P)H [34–36]. Several new techniques for determining the species of collected bacterial particles have been also developed in the last thirty years, including X-ray photoelectron spectroscopy [37]. However, our study uses traditional methods for both collecting and analyzing airborne bacteria. The purpose is to

enable comparing the data obtained in the past with the current data, as well as with the future data that will be received over the next decade in the same area.

The research presented in this paper is aimed at examining the influence of the main meteorological factors on the basic characteristics of airborne bacteria in Gliwice—a medium-sized industrial city located in southern Poland. The conducted research can be used to assess the health effects of exposure to bacterial aerosols of the people living in this area. Their continuation will also allow for the future forecasting of the physical and biological properties of bacterial aerosols in urban areas based on the meteorological forecast. It will also provide a better understanding of the complex relationship between climate change and the characteristics of airborne bacteria. Therefore, the strategic goal is to collect data for the development of a prognostic model that would enable a comprehensive assessment of exposure to bacterial aerosols with altered characteristics due to climate change.

2. Materials and Methods

2.1. Study Site and Weather Conditions

The samples of bacterial aerosol were collected on the main campus of the Silesian University of Technology in Gliwice, Upper Silesia, Poland. Measurements were conducted in the outdoor air of the urban environment, a large city of the Upper Silesian conurbation (population of about 182 thousand inhabitants). A fixed monitoring point was established in an open area, in the vicinity of buildings of the Silesian University of Technology campus facilities (the building of the Centre for New Technologies, 22B Konarskiego Str., Gliwice). The exact location of the measurement point is shown on the map presented in Figure 1. The coordinates of the sampling points are the following: 50°17′34.3″ N 18°40′56.2″ E (50.292862, 18.682270).

Figure 1. Sampling site location in Gliwice, Poland. (Map data Maps ©2022 Google, Satellite image ©2022, CNES/Airbus, Maxar Technologies https://www.google.pl/maps (accessed on 16 January 2022). OpenStreetMap https://www.openstreetmap.org/ (accessed on 16 January 2022)).

It should be emphasized that the Gliwice campus is located just a 15 min walk from the city center (downtown) and is surrounded by residential and office buildings, whereas industrial plants are located on the outskirts of the city. For this reason, in all our studies, we assume that the results of air pollutant concentrations obtained at the measuring point on the campus in Gliwice are representative for the so-called urban background.

Measurements were carried out from April 2015 to September 2016. Bioaerosol samples were taken 1–2 times a week, in different meteorological/weather conditions. The meteorological factors characterizing the seasons of the year during sampling are shown in Table 1, while a detailed description of the weather conditions during this period is presented in Table 2.

Table 1. Meteorological factors characterizing seasons during sampling.

Season	Temperature of Ambient Air °C		Relative Humidity %		Atmospheric Pressure hPa		Wind Speed m s^{-1}		Solar Radiation Radiation W m^{-2}	
	Mean	STD *	Mean	STD *	Mean	STD *	Mean	STD *	Mean	STD *
Spring	20.9	6.3	33.1	14.4	991.3	6.3	1.3	1.3	446.0	232.5
Summer	28.8	4.8	36.2	10.7	996.2	4.2	1.4	0.9	502.9	193.2
Autumn	11.5	5.7	52.7	17.6	1000.7	5.2	1.4	0.8	147.2	165.5
Winter	3.7	4.7	61.2	15.2	996.6	13.5	1.3	0.6	144.3	78.8

* Standard deviation.

Table 2. Detailed description of the weather conditions during the measurements.

Meteorological Factor	Months in 2015								
	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Average temperature, °C	8.8	13	17.2	20.3	21.9	15	7.9	5.8	4.2
Deviation of air temperature, °C (average from the multi-year value)	−0.1	0.9	−0.7	−1.7	−4.0	−1.6	0.9	−2.2	−4.7
Average wind speed, ms^{-1}	2.6	2.0	2.0	2.3	2.3	2.0	2.2	2.9	3.6
Wind speed multi-year average, ms^{-1}	2.7	2.4	2.3	2.2	2.1	2.3	2.6	2.9	3.2
Wind stillness rate, %	2.9	7.7	5.0	6.0	8.1	9.6	9.7	4.2	4.0
Wind stillness multi-year average, %	9.2	11.1	11.1	12.5	15.2	13.6	11.7	7.5	6.8
Precipitation sum, mm	13.5	57.7	46.9	44.5	15.3	41.8	25.2	80.6	14.2
Number of days with precipitation	10.0	16.0	11.0	12.0	8.0	11.0	10.0	18.0	12.0
Multi-year average number of days with precipitation	13.0	15.0	16.0	14.0	13.0	13.0	14.0	16.0	18.0
Insolation, h	205.0	175.3	207.3	289.5	280.7	142.6	137.9	93.5	83.2
Multi-year average insolation, h	159.3	213.7	208.1	232.4	218.4	143.2	112.5	55.1	35.1
	Months in 2016								
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.
Average temperature, °C	−1.7	4.0	4.5	9.0	14.3	18.3	19.0	17.6	15.8
Deviation of air temperature, °C (average from the multi-year value)	0.1	−4.4	−1.2	−0.3	−0.4	−1.8	−0.4	0.3	−2.4
Average wind speed, ms^{-1}	2.8	3.4	2.3	2.1	2.1	1.8	2.0	1.7	17
Wind speed multi-year average, ms^{-1}	3.4	3.3	3.2	2.7	2.4	2.3	2.2	2.1	2.3
Wind stillness rate, %	6.3	3.6	5.4	8.2	9.1	12.3	8.2	12.8	18.6
Wind stillness multi-year average, %	6.3	5.2	5.7	9.2	11.1	11.1	12.5	15.2	13.6
Precipitation sum, mm	34.7	90.9	26.7	49.0	32.7	77.7	195.3	67.9	26.2
Number of days with precipitation	5.0	11.0	13.0	12.0	10.0	14.0	17.0	9.0	6.0
Multi-year average number of days with precipitation	18.0	17.0	15.0	13.0	15.0	16.0	14.0	13.0	13.0
Insolation, h	59.3	54.2	101.0	156.6	214.0	254.0	215.7	240.7	224.1
Multi-year average insolation, h	159.3	213.7	208.1	232.4	218.4	143.2	112.5	55.1	35.1

The meteorological data presented in Table 1 show the mean values of the parameters measured during sampling. These data, compared to the average meteorological parameters, such as ambient air temperature, relative humidity, wind speed or solar radiation, indicate high convergence and are representative of entire seasons. Additionally, we compared the average indicators obtained during sampling months with values for long-period meteorological observations and no significant deviations from the long-period averages for particular months were noted.

Meteorological parameters were characterized based on data available in the report State of the Environment in the Silesian Voivodeship in 2015 (WIOŚ Katowice, 2016) and through the website https://dane.imgw.pl/ (The source of these data is the Institute of Meteorology and Water Management—National Research Institute (http://powietrze.katowice.wios.gov.pl/ (accessed on 12 March 2017); https://powietrze.gios.gov.pl/ (accessed on 5 January 2022); https://dane.imgw.pl/ (accessed on 24 May 2017))).

During sampling, basic meteorological parameters (ambient air temperature and humidity, wind speed (at 2 m) and direction, precipitation rate and UV index) were measured, using on-site, portable Weather Station WMR 200 (Oregon Scientific, Portland, OR, USA). In addition, the intensity of solar radiation was obtained from the Davis Weather Station Vantage Pro 2 stationary measurement station (Davis Instrument Corporation, Hayward, CA, USA) and supplemented with measurement data generated by the Silesian Provincial Inspectorate for Environmental Protection. The solar radiation flux I, $\mu W\,cm^{-2}$ in the range from 250 nm to 400 nm was multiplied by the exposure time t, min to obtain the time dose of UV radiation per unit area D, $\mu W\,cm^{-2}$ min.

2.2. Sampling and Determination of the Concentration of Viable Bacteria

Sampling was carried out in various meteorological conditions in order to obtain information on the concentration of the bacterial aerosol in the broadest possible spectrum, at different times of the year. Detailed values of meteorological indicators in particular seasons are presented in Table 1. Seasons are treated according to the calendar months: winter (January–March), spring (April–June), summer (July–September) and fall (October–December).

The measurement of the bioaerosol concentrations was conducted using a six-stage Andersen impactor (Thermo Fisher Scientific, Waltham, MA, USA)—one of the most popular and frequently used reference devices in biological aerosol studies—with cut-off diameters of 7.0, 4.7, 3.3, 2.1, 1.1 and 0.65 µm. This sampler works by air aspiration through multiple-hole stage, below which is placed a Petri dish containing the appropriate agar media. Air drawn through a jet is deflected 90 degrees by the agar surface below the jet. Through the inertial impaction, microorganisms are transferred from the air onto the agar surface. Each stage has 400 round holes with decreasing (from stage to stage) diameters. During the measurements, the air flow was 28.3 l/min (1 ACFM) and the sampling time, calculated following Nevalainen et al. [26], was 10 min. Before and after sampling, the flow rate was measured using a rotameter. Microorganisms were collected on nutrient media in Petri dishes located at all impactor stages. The following culture media were used: TSA (Trypticasein Soy, LAB-AGAR™, BIOCORP Polska Sp. Z o.o., Warsaw, Poland) with addition Cycloheximide Actidion (95%, ACROS Organics, Morris Plains, NJ, USA). The medium was prepared and sterilized in an autoclave prior to being poured into the Petri dishes. Before and between sampling, the impactor was sterilized using methyl alcohol and periodically cleaned using an ultrasonic cleaner. Quality control was performed by using PN-EN12322 [38] and ISO 11133 [39] standards. This approach is still very popular among bioaerosol researchers, although the counting of culturable microorganisms has some drawbacks, including poor reproducibility and the fact that dead microorganisms, cell debris and microbial components are not detected, while they too may have toxic and/or allergenic proper [A].

Collected bacteria were incubated by 5–7 days at the temperature of 22 °C to determine the concentration of bacterial microorganisms. After sampling, grown colonies were

counted, using a positive hole correction method. It should be stressed that, although TSA or TYGA plates are often incubated at 37 °C for 48 h, incubation at lower temperatures may recover a greater number of species and provide the improved resuscitation of stressed bacteria [40]. Therefore, it is often recommended that the plates should be incubated at 20–25 °C and examined daily for several days [41]. Concentrations were calculated by counting the number of colonies grown divided by the volume of air taken up and expressed as colony-forming units per cubic meter of air (CFU m^{-3}) using a positive hole correction [26].

The study was performed over a hundred samplings (109 sampling sets) during 45 days of the study period. For sampling, the most representative days for each season were chosen and that covered the variable meteorological conditions.

2.3. Species Identification

After counting, bacterial strains were prepared for identification. We realized that the traditional identification of bacteria on the basis of phenotypic characteristics is not as accurate as identification based on genotypic methods, but at present, our laboratory does not allow us to perform more advanced methods. On the other hand, the benefit of a culturable method is that many different species can be identified using selective media, which is important in determining the risk of exposure to bioaerosols, as not all bacteria and their components have the same toxic potential. Therefore, in our study, bacteria were characterized in terms of their metabolic characteristics; using biochemical tests API, their analysis was supported using the proper application APIweb, which enables the identification of more than 700 species of bacteria. We also chose the API because of the ease of preservation and reliability of the results (this method is a benchmark all over the world) and is cheaper than the molecular method. Before using the API tests, we carried out a microscopic analysis of the collected bacteria based on Gram strain preparations and provided data on cell size and shape and the appearance of spores. We performed a microscopic analysis using Axio Imager A1 microscope (Carl Zeiss, Oberkochen, Germany), with 10 × 100 magnification, coupled with PC (AxioVision, Carl Zeiss, Oberkochen, Germany), which allowed us to measure specific colonies. According to the standard procedure, the selected bacterial colonies were isolated and grown on culturable growth media. Then, the isolated bacterial cellular morphologies were studied by the Gram-staining technique. Selected in Gram-classification and morphology bacteria (Gram-positive/Gram-negative, cocci, bacilli and staphylococci) were morphologically identified and with the use of suitable metabolic API tests (BioMérieux, Marcy L'etoile France). We used the following tests: API 20 E™ (*Enterobacteriaceae*); API 20 NE™ (*Pseudomonas, Acinetobacter, Vibrio, Aeromonas,* etc.); API 50 CH™ (*Bacillus, Enterobacteriaceae, Lactobacillus* metabolism); API Coryne™ (*coryneform*); API Staph™ (*Staphylococcus, Micrococcus, Kocuria*); API Strep™ (*Streptococcaceae*), according to the specific test manual, including culturing on specific growth media (MacConkey agar and blood agar) and catalytic reaction.

2.4. Total Bioaerosol Concentration

To determine the total bioaerosol concentration, including viable and non-viable bacteria, Air-O-Cell cassettes (Zefon International, Ocala, FL, USA) were used. The cassettes consist of a casing with a rectangular inlet nozzle, with an impaction plate covered with a special gel material placed inside. During sampling, the air goes through the cassette and impacts the impaction plate, where all particles having an aerodynamic diameter greater than the cut-size diameter are collected. The cassette is connected with a pump with a flow rate of 15 dm^3 min^{-1}. After sampling, the cassettes were sealed and transported to the laboratory. The cassettes were opened, placed on microscopic glass and the gel was stained using fluorescent DAPI dye in concentration 10 µg ml^{-1} for 10 min (Sigma-Aldrich, St. Louis, MI, USA), which intercalates DNA. Then, counting was performed using a ScanˆR (Olympus, Tokyo, Japan) system, based on wide-field microscope Olympus IX81, coupled with monochromatic camera CCD ORCA-ER (Hamamatsu Photonics, Shizuoka,

Japan) and 150 W xenon mercury lamp MT20. The automated system identifies (after providing boundary values) bacterial cellular nucleoid, based on fluorescent threshold values comparison of background–object. We used a 60× immersion lens, scanning field of 144 μm × 110 μm, in 150 scans.

2.5. Sterilization Phenomenon

It should be noted that, as early as the 19th century, it was discovered that microorganisms react to light [42]. This research has taken place over many decades (see, for example, [42,43]). However, quantitative relationships between the inactivation of a given microbial fraction and the dose of radiation obtained have been and are still obtained in laboratory tests with the use of artificial UV radiation (see, for example, Xu et al. [44]).

There is a common agreement that, assuming first order kinetic, the change in the number of viable bioaerosol particles, dN, is equal to the change in time of the suspended particles, dt, times a constant rate a, and microbe concentration N [45], i.e.,

$$\frac{dN}{dt} = -aN \tag{1}$$

Rearranging and integrating Equation (1) from $t = 0$ to period of time t yields

$$\frac{N_t}{N_0} = e^{-at} \tag{2}$$

where a—decay rate constant, s^{-1}, or min^{-1};
t—time of sampling, min;
N_0—original concentration of bioaerosol particles, CFU m^{-3};
N_t—concentration of bioaerosol particles at sampling time t, CFU m^{-3}.
Hence,
In our case, the process of reducing the level of live bacteria or fungi in the air is sterilization with UV radiation. Therefore, the decay rate constant a should be proportional to the UV radiation flux per unit area I, μW cm^{-2}:

$$a = kI \tag{3}$$

where k, cm^2 $μW^{-1}min^{-1}$, means the sterilization rate. This coefficient characterizes the survival of a given microorganism subjected to the process of sterilization by UV radiation.

However, the product of the radiation flux (I) and the radiation exposure time (t) represents the absorbed radiation dose per unit area (D). So, finally, we can write [46]:

$$\frac{N_t}{N_0} = e^{-kD} \tag{4}$$

2.6. Statistical Analysis

Statistical analysis was performed using Statistica 12 (StatSoft Inc., Tulsa, OK, USA) software. As the limit criterion of statistical significance, we assumed p-value < 0.05. Firstly, we checked the distributions of assessed variables with the Shapiro–Wilk test. Due to the skewed distributions of variables ($S - W = 0.77$, $p < 0.001$), non-parametric tests were used in further analysis. To assess the seasonal variability of bioaerosol concentration, we performed a Kruskal–Wallis non-parametric ANOVA test. Additionally, the Leven test indicated the lack of variance uniformity in particular seasons ($F = 2.51$, $p = 0.06$).

3. Results and Discussion

The concentration levels of viable bacteria in different seasons are presented in Table 3. According to the classical approach [26], it was assumed that the concentration of viable bacteria is equal to the count of colony-forming units in the volume of air (Colony-Forming Units per cubic meter).

Table 3. Concentration of viable airborne bacteria in particular seasons.

Season	Number of Samples	Geometric Mean	Concentration of Viable Bacteria, CFU m^{-3}					
			Min	Q1	Median	Q3	Max	IQR
Spring	24	305	124	226	269	481	1449	254
Summer	53	265	35	152	247	445	2880	293
Autumn	16	283	60	161	297	425	1780	265
Winter	16	57	11	31	57	121	198	90

Evidently, the highest concentration of viable bacteria was found in spring (geometric mean), but only slightly lower concentrations were recorded in summer and autumn. In winter, the bacterial concentration was lowered 5–6 times to the level of about 60 CFU m^{-3}. A more detailed analysis of the obtained data can be observed in Figure 2, and the results of the Kruskal–Wallis ANOVA test are presented below.
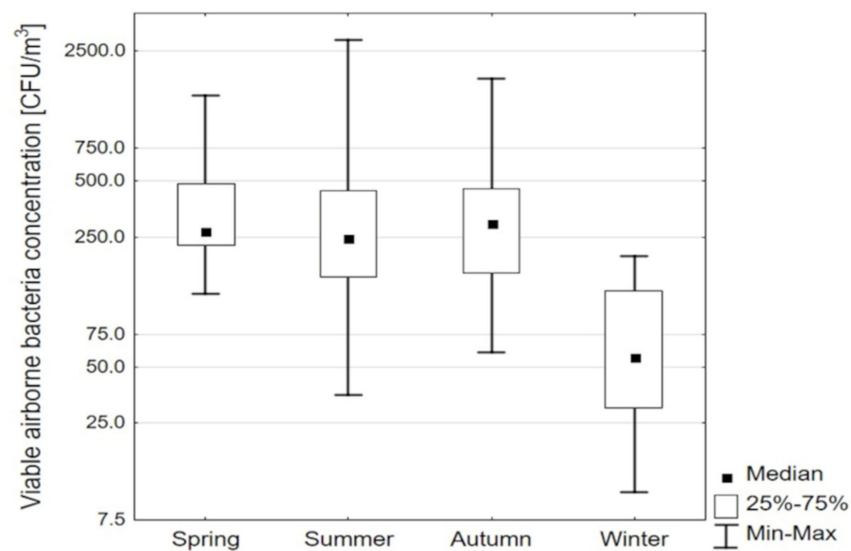
Figure 2. Variability of the concentration levels of bacterial aerosols in the studied seasons.

The result of the Kruskal–Wallis ANOVA test (H = 29.58; p = 0.00001) confirms the significant variability of airborne bacterial concentration through the seasons. In the winter season, the concentrations of bacterial viable particles were significantly lower than those in other seasons. These results agree well with the literature data. For example, Xi et al. [13] presented the seasonal variation of bioaerosol level in the Chinese Qingdao region. They found that the concentration of bacterial aerosols were several times lower during the winter compared to other seasons. Fang et al. [47] documented that, in China (Beijing), bioaerosol levels varied in the range from 5×10^3 CFU m^{-3} in summer and autumn to 286–380 CFU m^{-3} in winter. Further reports also confirmed seasonal changes in the prevalence of airborne microorganisms in the urban environment [11,13,48,49].

Figure 3 shows the seasonal mean size distributions of airborne bacteria obtained using the 6-stage Andersen impactor.
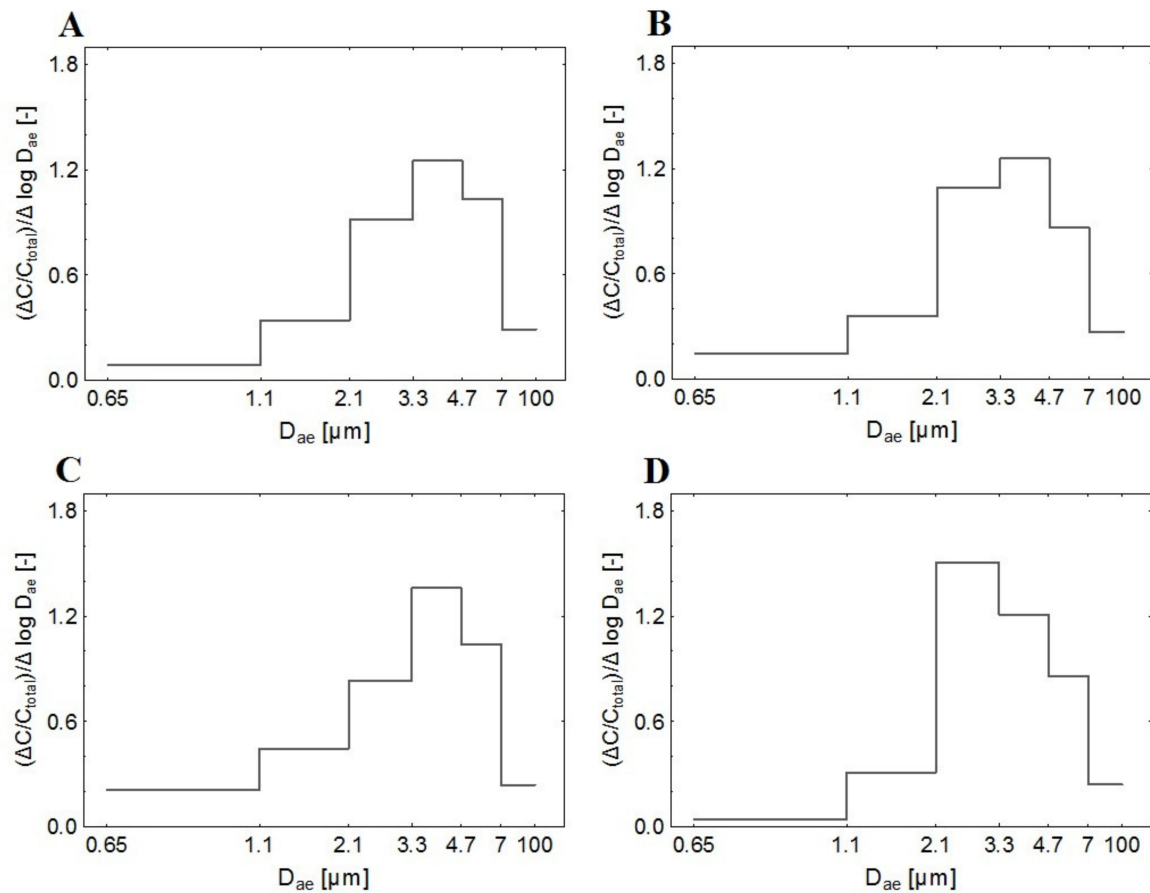
Figure 3. Size distributions for bacterial aerosols by season: (**A**)—Spring; (**B**)—Summer; (**C**)—Autumn; (**D**)—Winter.

It is noticeable that the shapes of the bacterial aerosol size distributions in the three seasons (spring, summer and autumn) were similar, indicating the peak in the range of 3.3–4.7 μm. In winter, the main peak was shifted into the smaller particles with the most occurrence of bacteria in an aerodynamic diameter ranging from 2.1 to 4.7 μm. This may suggest that coarse viable bacterial cells are more sensitive to cold air than fine cells and/or that the emission of these coarse live biological particles from soil and plants in winter is much weaker compared to the one of the fine cells.

The results of other studies also indicate the seasonal variability of the bioaerosols' size distributions. The research carried out near the coast (Chania, Greece) showed that, in the case of bacterial aerosols, the dominant fraction was particles in diameters ranging between 2.1 and 3.3 μm. The second most abundant fraction for this bioaerosol was particles sized within a range from 3.3 to 4.7 μm. On the other hand, in cold seasons (winter and early spring), a significant increase in the contribution of fine particles (1.1–2.1 μm) was observed [50]. Very similar results were also obtained by Li et al. [51] in the Qingdao region of China. The share of coarse bacterial particles was higher in the spring–summer months than in winter.

Figure 4 and Table 4 present the identified species composition of bacterial aerosols (viable particles only) in Gliwice in different seasons of the year.
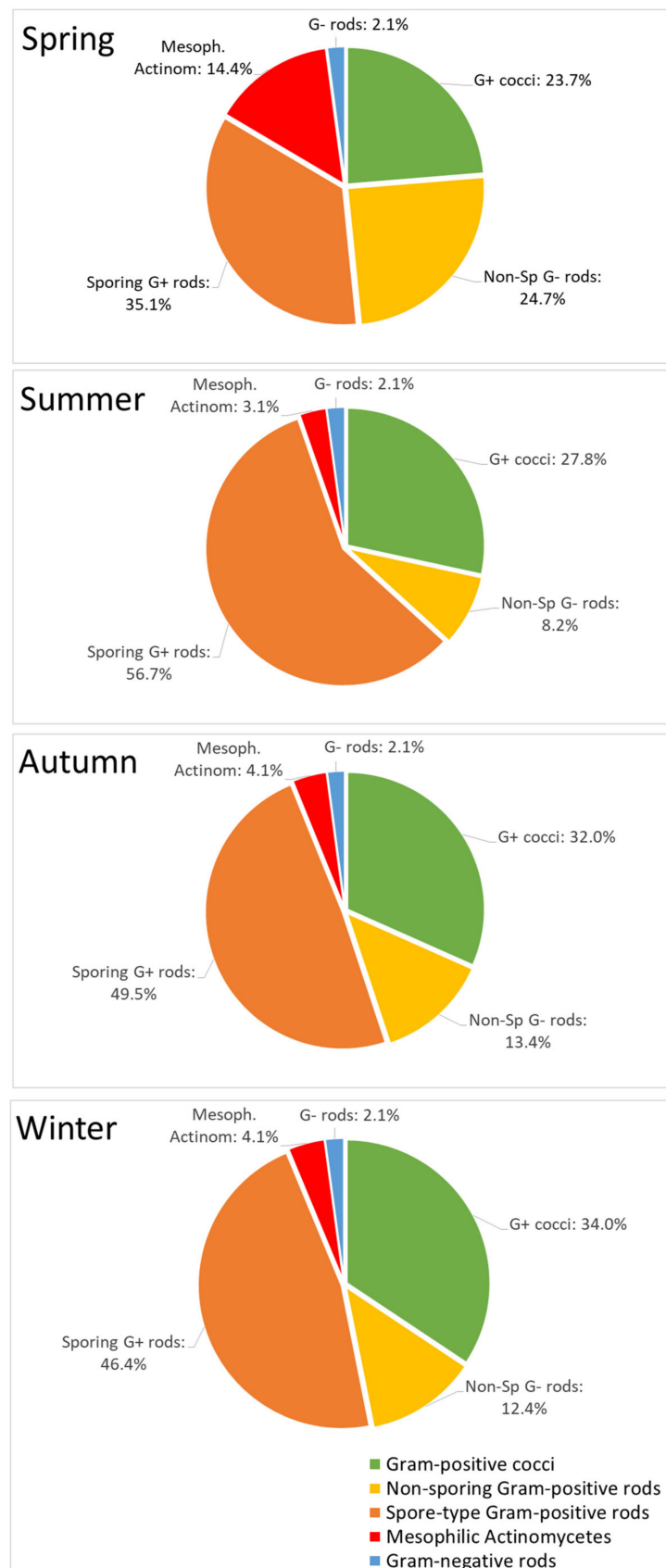
Figure 4. Bacteria genera isolated in consecutive seasons.

Table 4. Seasonal changes in viable bacteria genera and species.

Bacteria Species and Genera	Contribution to the Total Bacteria Genera in Season, %			
	Spring	Summer	Autumn	Winter
Gram-positive cocci	**23**	**27**	**31**	**33**
including: *Micrococcus* spp.	18	15	17	16
Staphylococcus sciuri	2	5	8	9
Staphylococcus lentus	2	6	4	8
Kocuria rosea	1	1	2	n.i.
Non-sporing Gram-positive rods	**24**	**8**	**13**	**12**
including: *Brevibacterium* spp.	14	5	3	12
Corynobacterium auris	10	3	7	n.i.
Arthorobacter	n.i	n.i.	3	n.i.
Spore-type Gram-positive roads, family Bacillacae	**34**	**55**	**48**	**45**
including: *Bacillus cereus*	17	14	8	15
Bacillus pumilus	14	17	16	14
Bacillus circulans	3	n.i.	11	n.i.
Bacillus subtilis	n.i.	243	9	14
Bacillus mycoides	n.i.	n.i.	4	2
Mesophilic Actinomecytes	**14**	**3**	**4**	**4**
including: Streptomyces spp.	9	n.i.	2	4
Rhodococcus spp.	5	3	2	n.i.
Gram-negative rods	**2**	**2**	**2**	**2**
including: Pseudomonas spp.	2	2	2	2

n.i.: not identified.

It can be seen that the dominant group of culturable bacteria in the ambient air in Gliwice in every season was that of Gram-positive rods that form endospores (35–57%). On the contrary, the contribution of Gram-negative rods (practically only *Pseudomonas*) to the total bacterial flora, equal to 2.1% in all seasons, was the lowest.

The most frequently isolated bacteria were *Micrococcus* sp. and *Bacillus* sp. Previous studies in reference to the identification of bacterial aerosols in Gliwice showed similar results [52]. Gram-positive bacterial forms constitute the vast majority of all isolated bacteria in the ambient air [53].

Table 5 presents the results of both the total bacterial particles counted as well as the concentration of viable bacterial particles (colony-forming units) selected for some representative days in particular seasons, during the research period, based on meteorological parameters similar to the seasonal mean. The concentration of total bacterial aerosol (including viable, non-viable, dead and fragmentary cells) was calculated using the fluorescent DAPI staining.

Table 5. Total and viable (culturable) bacterial aerosol concentration in selected days.

Season	Autumn	Winter	Spring 10 May 2016	Summer 8 July 2016
Day	14 October 2015	4 January 2016	Morning (9:30)	Noon (12:30)
Temperature [°C]	9.2	−3.7	20.9	21.6	25
Relative humidity [%]	82	63	35	39	32
Concentration of total airborne bacteria [m^{-3}]	25,520	298	24,266	13,968	14,148
Concentration of viable bacteria [CFU m^{-3}]	205	46	481	233	141
Viable/total ratio [%]	0.8	15.4	2.0	1.7	1.0

It can be seen that, in all the selected days, being representative for all seasons (regarding mean air temperature, relative humidity and other meteorological parameters), the concentration levels of the viable (precisely, culturable) airborne bacteria ranged from approximately 50 in winter to almost 500 CFU m^{-3} in spring. It is important to note that the levels of viable bacteria concentrations were close to the seasonal mean obtained during all experiments for each season. In the summer, the measurement was carried out in the morning and also at noon due to assess the diurnal changes in bacterial aerosol composition.

On the other hand, in spring, summer and autumn, the concentration of total bacterial particles in the ambient air in Gliwice was around 10^4 m^{-3} (cells m^{-3}). The same level was found over the grasslands of central Asia and North America [54]. Only in winter is the concentration significantly lower. However, Yin et al. [55] found recently that, in the coastal region of China, the concentration of total airborne bacteria was between 10^5 and 10^6 cells m^{-3}.

The second important conclusion is that culturable bacteria that can be treated as viable bacteria contribute between 1 and 2% of the total bacterial particles suspended in the ambient air. However, in winter, when the air temperature was about -4 °C, this contribution was elevated up to 15%. A similar ratio was obtained by Yin et al. [55] and by Gong et al. [14].

The important observation is that, with a similar concentration level of total aerosol concentration in the morning and afternoon (1.4 $\times$ 10^4 cells m^{-3}), there was a significant decrease in the concentration of viable forms at noon compared to the morning (i.e., from 233 CFU m^{-3} to 141 CFU m^{-3}). Therefore, the ratio of total to viable bacteria has changed significantly (in the morning 60:1 and noon 100:1). This may be caused by the significant influence of the phenomenon of sterilization occurring in the atmospheric air, strongly attributed to UV radiation.

Generally, it should be noted that, in the spring, summer and autumn, the total concentration of bacterial aerosols was in the order of 10^4 m^{-3} (cells m^{-3}), while in winter, it was only 10^2 m^{-3}, corresponding to a decrease of about 100 times. Meanwhile, the concentration of viable particles of this bacterial aerosol in the spring, summer and autumn was about 10^2 CFU m^{-3} but in winter 10 CFU m^{-3}, equaling to a decrease of about 10 times. Thus, in winter, the decrease in the total bacterial aerosols is ten times greater than the decrease in the concentration of viable particles of bacterial aerosols. The most probable phenomenon for the winter drop-in bioaerosol concentrations is the decrease in the emission of bacterial particles (from the soil, plants, etc.), which is much more significant than the dying out of living bacterial microorganisms in the air due to the low air temperature. However, the analysis of the ratio of viable concentrations to all airborne bacteria, presented in Table 4, requires a more detailed explanation. It turned out that the ambient air temperature has a stimulating effect on the concentration of biological aerosols, but only within a limited range of the temperature [56]. The obtained data indicate an important role of some phenomena that "kill" live bacteria. One such phenomenon is certainly solar ultraviolet radiation assisted by the ozone generated by this radiation. In fact, a synergistic effect was observed between the intensity of UV radiation and ozone concentration in the lower atmosphere [57]. That is why we conducted preliminary studies on the natural sterilization process of airborne bacteria.

Based on the results of a series of time measurements of bacterial aerosol concentrations carried out in the spring and summer in 2016, the sterilization rate k was estimated according to Equation 4, which characterizes the sensitivity of bacteria present in the atmospheric air to solar radiation in the ultraviolet range. Cloudless days were selected for the analysis, characterized by high values of total radiation, and at the same time for which the 24 h course of the total radiation flux was undisturbed and was close to the Gaussian distribution. These conditions were met for the following measurement days: 5 April, 23 June, 26 August, 1 September and 16 September. The values of the doses of UV radiation (D) in these days were determined as the sum of the products of the radiation flux I and the exposure time t. The obtained result is presented in Table 6.

Table 6. The sterilization rate (k) for airborne bacteria in Gliwice.

	k, cm^2 W^{-1}s^{-1}			
N	**Geometric Mean (SD)**	**Median (IQR)**	**Q1–Q3**	**Min–Max**
13	9.20 (7.58)	10.96 (14.78)	4.42–19.20	2.14–24.24

The table shows that the obtained sterilization rate k for airborne bacteria exposed to UV solar radiation in Gliwice is about 10 cm^2 W^{-1}s^{-1}. This result seems pioneering, but it should only be considered preliminary. In laboratory conditions, the k values were usually obtained several times higher, from 36 to 77 cm^2 W^{-1}s^{-1} [45]. In some studies, the reported results were one hundred and even a thousand times higher [12,58–60]. However, comparisons in this respect are difficult. In laboratories, the sterilization of bacteria exposed to artificial UV radiation is tested under well-controlled conditions. In addition, the results published in the literature concern sterilization tests of single, selected species of bacteria (e.g., various varieties of *Bacillus*, *Pseudomnas* and *Staphylococcus*). Nevertheless, the much lower k-value for bacteria suspended in the atmosphere compared to the data obtained in the laboratory tests suggests that they are more resistant to UV radiation than suggested by previous analyzes. This result may be explained based on the phenomenon of the attachment of fine bacterial particle/particles to a coarse solid particle (see, for example, [61,62]). The formation of such bacterial dust agglomerates can partially protect the living bacteria from the damaging effects of UV radiation [63].

4. Conclusions

The characteristics of viable bacteria in the ambient air in Gliwice, i.e., their concentration level, size distribution and species composition, shows significant seasonal variation. The concentration level in winter was six times lower than in the spring and summer. The size distributions of bacterial aerosols as well as the dominant genera of the identified bacteria also change in particular seasons. The patterns of the size distributions of bacterial aerosols in the three seasons (spring, summer and autumn) were similar, indicating the peak in the range of 3.3–4.7 μm. In winter, the main peak was shifted into the smaller particles, having an aerodynamic diameter in the range from 2.1 to 4.7 μm. This may suggest that coarse viable bacterial cells are more sensitive to cold air than fine cells and/or that the emission of these coarse live biological particles from soil and plants in winter is much weaker compared to that of fine cells.

The dominant group of culturable bacteria in the studied ambient air in every season was endospore-forming Gram-positive rods (34–55%), while the least frequently identified were Gram-negative rods (2%). The most frequently isolated bacteria were *Micrococcus* sp. and *Bacillus* sp. In winter, a greater decrease in total bacterial concentration level was noted compared to viable forms. An explanation for this observation can be the lowering of the emission of bacterial particles (from the soil, plants, etc.) in winter, which is much more important than the extinction of live bacterial particles in the air due to the low air temperature. Total bacterial concentration, obtained using the new, refined technique, ranged from 1.43×10^4 to 2.5×10^4 cells m^{-3} during the spring–summer–autumn season and 3×10^2 cells m^{-3} during winter. The estimated total/viable ratio for airborne bacteria varied from 125:1 in autumn to 6.5:1 during winter. In conclusion, the viable/total ratio ranged from 1 to 2%. Only in winter, when the air temperature was about −4 °C, this ratio was elevated up to 15%. Furthermore, the total bacterial aerosol concentration measured during sampling on the morning hours was similar to the afternoon, while in the case of viable (culturable) forms, the concentration significantly decreased between the morning and noon samplings. The ratio of total to viable bacteria also changed during day. To our knowledge, this is the first field study that investigated the level of the sterilization rate (k) for airborne bacteria in Poland. The obtained value of k for viable bacteria exposed to UV solar radiation in Gliwice was about 10 cm^2 W^{-1}s^{-1}. The comprehensive research we

initiated on the influence of various meteorological parameters, including UV radiation, on the characteristics of the bacterial aerosol in Upper Silesia should be repeated every few years. This would allow the quantification of the impact of progressive climate change on the bioaerosol exposure of inhabitants of this region, which is still one of the most polluted environments in Europe.

The authors agree that the counting of culturable microorganisms has some drawbacks, such as poor reproducibility and the fact that dead microorganisms, cell debris and microbial components are not detected, potentially having toxic and/or allergenic properties. The benefit of culturable methods is that many different species can be identified using selective media, which is important to determine the risk of exposures to bioaerosols, as not all bacteria and their components have the same toxic potential. The concentrations of airborne bacteria determined using this method allow us to obtain a considerable amount of important information about the microbial contamination of the air as well as the possible health effects of exposure to viable bacteria.

Author Contributions: M.K. conducted all field studies, including the sampling of airborne bacteria and the determination of the sterilization factor and prepared a first draft of the manuscript. J.S.P. prepared the research plan, led the research and prepared the final manuscript. A.B. performed the laboratory tests of samples to determine the total concentration of bacterial cells in the air. J.C. performed part of the statistical analysis and data validation. E.B. determined bacteria species and genera. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ACFM	Actual cubic feet per minute
ANOVA	Analysis of variance
API	Analytical profile index
CFU	Colony Forming Units
DAPI	$4'$,6-diamidino-2-phenylindole
ISO	International Organization for Standardization
NAD(P)H	Nicotinamide adenine dinucleotide
n.i.	not identified
STD	Standard deviation
S-W	Shapiro-Wilk test
TSA	Trypticase soy agar
TYGA	Tryptone Glucose Yeast Extract Agar
UV	Ultraviolet

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