

Microflora of Air in the Sewage Treatment Plant of Kapuściska in Bydgoszcz

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Abstract

The present study was aimed at evaluating the air microflora on the premises of the sewage treatment plant Kapuściska in Bydgoszcz and in the surrounding area, and was conducted with the use of the sedimentation and impaction methods. The analysis reveals that mould fungi were the most abundant, followed by mesophilic heterotrophic bacteria. Actinomycetes and mannitol-positive staphylococci constituted the least abundant group. The highest counts of the studied microorganisms (with the exception of staphylococci) were determined at Site III, located between the aeration tanks and the bioreactor, which are considered to be the main emitters of microbial aerosol contamination. The concentration of microorganisms decreased considerably with distance from the source of microbial aerosol emissions.

The following genera prevailed among the mould fungi: *Penicillium* (54%), *Aspergillus* (23%), *Cladosporium* (11%), *Fusarium* (6%), *Alternaria* (3%), and others (3%).

Keywords: mould fungi, heterotrophic bacteria, actinomycetes, mannitol-positive staphylococci

Introduction

Municipal utilities may constitute a source of microbiological contaminants in the air. Sewage treatment plants, compost plants, and landfill sites that accumulate solid waste are a significant source of biological aerosol emission, posing serious health hazards for people and animals [1-3].

Bioaerosols are defined as airborne particles of biological origin, containing living organisms such as bacteria, fungi, fungal spores, viruses, and pollen and their fragments, including various antigens. Particles may range in size from aerodynamic diameters of ca. 0.5 to 100 μm [4-6]. The number of microbes in the air varies according to numerous atmospheric conditions.

In sewage treatment plants aerosols and microorganisms are emitted mainly during the process of aerating and

mixing the sewage, and subsequently during the process of distributing the effluent on biological units [3, 7].

The sewage, irrespective of its origin, contains pathogenic and potentially pathogenic saprophytic microorganisms [8-10]. The most diversified and abundant microflora is found in domestic and commercial sewage, which may include actinomycetes (*Mycobacterium tuberculosis*), viruses, fungi, protozoans [11], and bacteria of *Enterobacteriaceae*, *Pseudomonadaceae*, *Lactobacillaceae*, and *Micrococaceae* families [12, 13]. The emission range, amount, and survival rate of the airborne microorganisms are strictly connected with physicochemical and meteorological air conditions, the lay of the land, the time of the day, the season of the year, the origin of the sewage, and the applied sewage treatment technology [14]. On the other hand, the environmental impact of the sewage treatment plant itself depends on its capacity, technological solutions applied in the process of treating the sewage, sewage sludge treatment, care and maintenance, and many other elements [15, 16].

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A substantial number of local and global factors contribute to the influence that municipal utilities (including sewage treatment plants) have on the environment and people. Since public utilities may pose serious health and environmental hazards, regular monitoring is highly recommended. The main objective of this paper was discovering and evaluating the correlation between the biological sewage treatment plant with activated sludge and air quality.

Materials and Methods

The microbiological studies were carried out on the premises of the sewage treatment plant Kapuściska, located in the southeastern outskirts of Bydgoszcz. The plant, which combines mechanical and biological methods of sewage treatment, receives municipal sewage from the housing estates in the city of Bydgoszcz located along the right-bank of the River Brda, and from the town of Solec Kujawski, as well as industrial sewage from the Zachem and Nitro-Chem chemical plants. The current capacity of the plant is 43,000 m³ of municipal sewage per day and 29,000 m³ of industrial sewage per day.

Six sampling sites were established for the research, each representing a different stage of the technological process of treating sewage (Fig. 1).

Site I – at the bar screens and grit tanks

Site II – at the primary clarifier

Site III – between the aeration tanks and bioreactors

Site IV – at the final clarifiers

Site V – at the treated water discharge

Site VI – at the entrance to the plant

Sampling

The investigation was pursued in a seasonal cycle using two methods of air sampling, namely the sedimentation method according to Polish Norm [17] and the impaction method based on the MAS-100 air sampler by MERCK.

In the sedimentation method, open Petri dishes containing agar nutrient media were exposed at the measuring stations for 10 and 30 minutes, 150 cm above ground level.

In the impaction method 100 litres of air was filtered through the sampler's chamber containing a Petri dish filled with a suitable nutrient medium. The microflora in the air stream sucked in by the air sampler deposited on the surface of the medium.

At each station the air sampling based on the above-mentioned methods was conducted in three parallel repeats.

Both methods required transporting the air samples to laboratory, where they were inserted in a thermostat and incubated for a specific time at an appropriate temperature. After incubation the concentrations of growing colonies was counted as colony forming units (CFU), and the result was recalculated per cubic meter of air (CFU/1 m³). The sedimentation method was based on Omeliański's formula [18]:

$$A = \frac{a \cdot 100 \cdot 100}{\pi r^2 \cdot t^{\frac{1}{5}}}$$

...where:

A – total count of bacteria or fungi per 1m³ (CFU/1m³),

a – arithmetic mean count of bacteria /fungi on a dish,

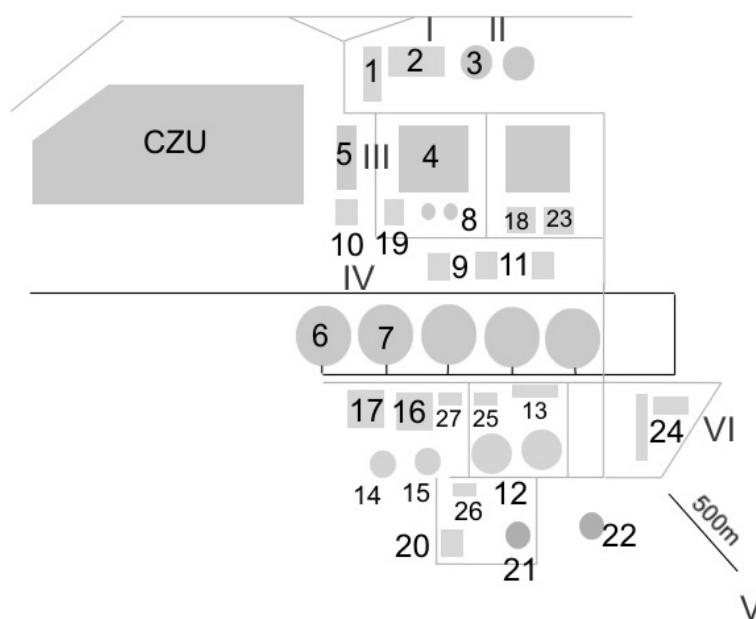


Fig. 1. The infrastructure and the location of sampling sites of the sewage treatment plant Kapuściska in Bydgoszcz.

1. Bar screen, 2. Grit tank, 3. Primary clarifiers, 4. Bioreactors, 5. Aeration tank, 6-7. Final clarifier, 8. Sludge thickeners, 9-11. Sludge pumping station, 12. Closed fermentation chamber, 13. Building to service the closed fermentation chamber, 14. Digested sludge tank, 15. Post-coagulation tank, 16. Separators building, 17. Sludge landfill, 18. Blowers stations, 19. Coagulation station, 20. Gas separator, 21. Gas tank, 22. Gas torch, 23. Energy building, 24. Building, 25. Sewage pumping station, 26. Sewage pouring, 27. Boiler room CZU – Main reservoir, I-VI – Sampling sites.

r – radius of a Petri dish in cm,
 πr^2 – area of a Petri dish,
 t – exposure time of a dish (in minutes)
 100; 1/5 – constant values

The impaction method involved using a measuring table by Feller [19], enclosed in the Manual for the MAS-100 Air Sampler by MERCK.

$$Pr = N [1/N + 1/N - 1 + 1/N - 2 + 1/N - r + 1]$$

...where:

Pr – most probable number of microorganisms in the tested air volume,

N – number of holes in the perforated base of the sampler (400),

r – colony forming units (CFU).

The following meteorological parameters were measured during sample collection: air temperature, relative humidity, and wind velocity. These measurements were carried out using a Nielsen-Kellerman anemometer, Kestrel 3500 (Table 1).

Microbiological Research

The microbiological research on the premises of the sewage treatment plant Kapuściska investigated the following:

- 1) the total count and morphology of heterotrophic bacteria
- 2) the concentration of mannitol-positive bacteria of *Staphylococcus* genus
- 3) the concentration of *Pseudomonas fluorescens*
- 4) the count of actinomycetes
- 5) the concentration of mould fungi and their identification

The total count of heterotrophic bacteria was determined using TSA agar medium. The bacteria were incubated at 37°C for 48 hours, then the count of growing colonies was measured as colony forming units per cubic meter of air (CFU/1 m³).

To determine the morphology of airborne bacteria, cultured bacterial strains were isolated and analyzed using Gram staining method. The study focused on detecting cocci, endospore forming rods, and nonsporulating rods.

The presence of mannitol-positive staphylococci was detected in accordance with the Polish Norm [20] using Chapman's nutrient medium. The bacterial cultures were incubated at 37°C for 48 hours, then the colonies of mannitol-positive bacteria were counted. Light, yellow zones around a developed colony indicated a positive result.

The count of *Pseudomonas fluorescens* was detected in accordance with the Polish Norm PN 89/Z-04111/02 [20] using King's B nutrient medium. The bacterial cultures were incubated at 26°C for 5 days.

The count of actinomycetes and mould fungi was determined using Pochon's and Sabouraud's nutrient medium accordingly. Their incubation was conducted at 26°C for 5 days, then concentrations of colonies were counted (CFU) and the results were calculated per 1 m³ of air. Identification of mould fungi was determined based on macro- and microscopic features [21-24].

Table 1. Meteorological parameters during sample collection.

Date of sampling	Temperature (°C)	Humidity (%)	Wind speed (km/h)
13.05.2009	16	36	14.4
01.07.2009	27	80	5
03.10.2009	18	28	16
18.02.2010	-1	5	8

On the basis of the final results, the level of air contamination on the premises of the sewage treatment plant Kapuściska was evaluated in accordance with the Polish Norm [20, 25].

Results

The results of the investigation into the amount of each group of the studied microorganisms in the air on the premises of the sewage treatment plant Kapuściska are presented in Tables 2 and 3. As can be seen from the tables, comparable results were obtained with the use of either of the two methods applied, (i.e. the sedimentation and the impaction methods) with the higher counts most frequently determined with the use of the sedimentation method.

On average, the highest counts of heterotrophic bacteria were determined using the sedimentation method (2,800 CFU/m³) and the impaction method (1,830 CFU/m³), noted at Site I (located at the bar screens and grit tanks) and at Site III (located between the aeration tanks and the bioreactors), 2,720 CFU/m³ (the sedimentation method) and 1,400 CFU/m³ (the impaction method) (Table 2).

The lowest counts of heterotrophic bacteria were noted at Site V near the treated water discharge, where the results obtained with the sedimentation method amounted to 1,260 CFU/m³ on average, and the results obtained with the impaction method amounted to 710 CFU/m³ on average (Table 2). Compared to acceptable amounts of heterotrophic bacteria in the air defined by Polish Norms PN-89/Z-04111/02 and 03, the air on the premises of the sewage treatment plant Kapuściska was estimated as moderately contaminated (with heterotrophic bacteria) at all sampling sites. However, at Site IV (located at the final clarifies) and at Site V (located at the treated water discharge) the amount of heterotrophic bacteria determined with the use of the impaction method never exceeded 1,000 CFU/m³, which indicates no contamination with these microorganisms (Table 2).

Remarkably, figures reveal seasonal fluctuations in the distribution of heterotrophic bacteria – the highest counts were noted in spring and summer, whereas a considerable decrease was observed during the winter season (Table 3).

Endospore forming rods and cocci were the prevailing morphological forms, constituting 44% and 48% of all bacterial forms, respectively, whereas nonsporulating rods constituted the least abundant microbial group, making up to 8% of the entire microflora.

Table 2. The count and the level of air contamination by microorganisms at the different sampling sites on the premises of the sewage treatment plant Kapuściska according to Polish Norms PN-89/Z-04111/02 and PN-89/Z-04111/03.

Research site	Concentration of microorganisms in 1 m ³ air									
	Heterotrophic bacteria		Staphylococci		<i>Pseudomonas fluorescens</i>		Actinomycetes		Mould fungi	
	A	B	A	B	A	B	A	B	A	B
I – at the bar screens and grit tanks	2,800.0	1,830.0	10.0	3.0	471.0	325.0	83.0	33.0	3,440.0	2,000.0
	**		**		***		**		*	
II – at the primary clarifier	1,750.0	1,130.0	20.0	10.0	410.0	353.0	134.0	32.0	4,440.0	1,770.0
	**		**		***		***	**	*	
III – between the aeration tanks and bioreactors	2,720.0	1,400.0	0.0	0.0	989.0	534.0	230.0	30.0	7,050.0	2,090.0
	**		*		***		***	**	**	*
IV – at the final clarifiers	1,580.0	880.0	0.0	0.0	212.0	236.0	125.0	11.0	5,070.0	1,830.0
	**	*	*		***		***	**	**	*
V – at the treated water discharge	1,260.0	710.0	15.0	8.0	202.0	187.0	118.0	34.0	2,770.0	1,630.0
	**	*	**		***		***	**	*	
VI – at the entrance to the plant	1,570.0	1,110.0	3.0	1.0	304.0	190.0	80.0	15.0	2,900.0	1,790.0
	**		**		***		**		*	
Polish Norm of air:										
No pollution*	<1000		No		No		10		3,000-5,000	
Medium pollution **	1,000-3,000		< 25		< 50		10-100		5,000-10,000	
Heavy pollution ***	> 3,000		> 25		> 50		>100		> 10,000	

A – sedimentation method, B – impaction method, N (number of parallel determinations) – 3

Furthermore, the research into *Staphylococcus* bacteria provides evidence that they were the most abundant at Site II (located at the primary clarifier), where their population amounted to 20 CFU/m³ on average (measured with the use of the sedimentation method) and 10 CFU/m³ (measured with the use of the impaction method). Mannitol-positive staphylococci were not noted at Site III (located between the aeration tanks and the bioreactors) and Site IV (located at the final clarifiers). At the remaining stations the contamination with staphylococci reached a moderate level – their amount never exceeded 25 CFU/m³ (Table 2). The most abounding in summer, the population of staphylococci was smaller in the remaining seasons (Table 3).

The results of the investigation into the concentration of *Pseudomonas fluorescens* bacteria show that the air on the premises of the sewage treatment plant Kapuściska is heavily contaminated with these microorganisms. The highest average amount was observed at Site III (located between aeration tanks and the bioreactors) and equalled 989 CFU/m³ (the sedimentation method) and 534 CFU/m³ (the impaction method). The lowest amount was noted at Site V (located at the treated water discharge) and equalled 202 CFU/m³ (the sedimentation method) and 187 CFU/m³ (the impaction method) (Table 2). The analysis of the seasonal cycle shows that the population of *Pseudomonas fluo-*

rescens was thriving from spring to autumn but declined slightly during winter (Table 3).

The population of actinomycetes varied considerably and ranged from 11 CFU/m³ (the impaction method) at Site VI (located at the entrance to the plant) to 230 CFU/m³ (the sedimentation method) at Site III (located between the aeration tanks and the bioreactors). Compared to acceptable amounts of actinomycetes in the air defined by Polish Norms PN-89/Z-04111/02 and 03, the air on the premises of the sewage treatment plant Kapuściska was estimated as moderately contaminated at all sampling sites (the results obtained with the use of the impaction method). According to the results obtained with the use of the sedimentation method, the air at Site I (at the bar screen and grit tanks) and Site VI (at the entrance to the plant) was moderately contaminated while at the remaining stations it was classified as heavily contaminated (Table 2). The highest percentage of actinomycetes in the air was determined in spring, whereas in the remaining seasons a decline was observed (Table 3).

Similarly to other microorganisms, mould fungi were distributed the least abundantly during the winter season but their population grew from May to reach a peak in the autumn season (Table 3). According to the results obtained with the sedimentation method and including the Polish Norm only Site III (located between the aeration tanks and

Table 3. An average number of microorganisms in 1 m³ of air on the premises of the sewage treatment plant Kapuściska, depending on the season.

Date of sampling	Heterotrophic bacteria		Staphylococci		<i>Pseudomonas fluorescens</i>		Actinomycetes		Mould fungi	
	A	B	A	B	A	B	A	B	A	B
13.05.2009	2,040.0*	1,170.0*	7.0*	295.0*	816*	295*	202.0*	40.0*	3,560.0*	1,040.0*
	761-3,307	400-2,989	0-39	250-320	98-1,120	250-320	105-394	13-180	1,365-6,037	990-1,990
01.07.2009	2,240.0*	1,320.0*	33.0*	339.0*	400*	339*	159.0*	30.0*	4,440.0*	1,560.0*
	945-3,648	340-3,252	0-79	289-500	192-777	289-500	79-341	10-145	1,601-8,531	1,282-2,410
03.10.2009	2860.0*	700.0*	10.0*	291.0*	318*	291*	151.0*	20.0*	8,850.0*	1,960.0*
	629-7,559	112-1,521	0-27	50-410	170-860	50-410	26-236	4-110	3,596-13,819	920-2,561
18.02.2010	96.0*	69.0*	10.0*	207.0*	278*	207*	2.0*	0.0*	249.0*	149.0*
	26-289	18-189	0-42	40-330	53-394	40-330	0-8	0-0	56-1,007	68-178

A – sedimentation method, B – impaction method, *average values, N (number of parallel determinations) – 3

the bioreactors) and Site IV (located at the final clarifiers) showed moderate contamination with mould fungi (7,050 CFU/m³ and 5070 CFU/m³, respectively), while the remaining stations were assessed as uncontaminated (Table 2). Making up 54% of the entire fungal population, *Penicillium* prevailed, followed by *Aspergillus* and *Cladosporium*, which constituted 23% and 11% of the fungal microflora, respectively. *Fusarium* (6%), *Alternaria* (3%), and others (3%) were less abundant (Fig. 2).

Discussion

Although sewage treatment plants, similarly to other public utilities, play an important role in environmental protection, they may also negatively influence the quality of the air [12, 26], surface water, and underground water in the surrounding area, and in consequence affect the health of people living in the vicinity. The amount of microorganisms in bioaerosols is determined by numerous factors, including climatic conditions, the origin of sewage, and sewage treatment machinery and equipment used in the process of treating sewage [27, 28].

The results of the microbiological analysis carried out on the premises of the sewage treatment plant Kapuściska

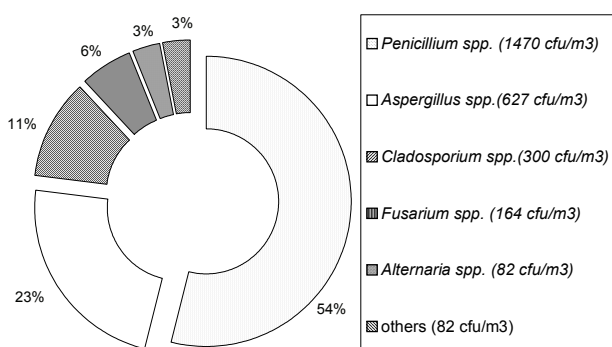


Fig. 2. Dominant genera of moulds.

with the use of two research methods, namely the sedimentation method and the impaction method, show that higher amounts of microorganisms in the air were always distinguished with the sedimentation method in a majority of tests, regardless of the sampling site, season, and atmospheric conditions.

Similar results were obtained during the air examinations carried out on the premises of the sewage treatment plant with planted soil filters [29], as well as on the premises of the sewage treatment plant with the system of aerated basins and stabilization ponds [14]. The percentage of the studied group of microorganisms in the air samples collected with the sedimentation method and converted into 1 m³ is between 3 and 6 times higher than measured with the impaction method [18]. This may be linked to the fact that in the impaction method a part of microorganisms bounce from the agar surface when the air is sucked through the jet. Additionally, a part of microorganisms may be captured by a strong air current created by the air sampler, as a result of which some of them are unable to settle on the medium [12].

It must be accentuated that the results of the air tests are only of temporary value, i.e. they are reliable at the exact moment of obtaining samples. Owing to physical and chemical properties of the air, sudden changes of the contamination level at a specific spot may be observed. Thus the results presented in this thesis provide only an approximate idea of the amount of microorganisms in the air; they primarily help to define whether the amount is low or high [30].

In air microflora on the premises of the sewage treatment plant Kapuściska, mould fungi constituted the most abundant group, followed by mesophilic heterotrophic bacteria and *Pseudomonas fluorescens* bacteria, whereas the populations of actinomycetes and staphylococci were sparse. Similar results were obtained during the investigation conducted on the premises of the landfill site in Żółwin-Wypaleniska [31].

A large population of mould fungi observed on the premises of municipal utilities including sewage treatment plants Kapuściska in Bydgoszcz, Kujawy, and Płaszów in Kraków [26], as well as the landfill site Żółwin-Wypaleniska [31] indicates their remarkable ability to spread in the air [32].

Mould fungi of *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, and *Mucor* genera constitute more than 90% of the entire population of microorganisms in the air. On the premises of the sewage treatment plant Kapuściska, *Penicillium* constituted 54%, *Aspergillus* 23%, *Cladosporium* 11%, *Fusarium* 6%, and *Alternaria* 3% of the microbial community. A similar composition was noted during research carried out on the premises of the sewage treatment plant in Ciechocinek and in the surrounding area [33], as well as in the greenhouse air [34].

Mould fungi present in the air may be highly pathogenic for humans, i.e. they may cause serious illnesses. They are linked to numerous medical conditions such as congenital malformations, low birth mass, miscarriages, headaches, liver dysfunction, neurological ailments, skin infections, mucous membrane irritation, respiratory problems, oncological diseases, allergies, mycosis, and toxic reactions [26]. Allergies are mainly associated with *Cladosporium*, *Alternaria*, *Penicillium*, and *Aspergillus* genera.

In the air on the premises of the Kapuściska sewage treatment plant, all investigated groups of microorganisms (with the exception of mannitol-positive staphylococci) were the most abundant at Site III, located between the aeration tanks and bioreactors, which are considered the main source of airborne contaminants.

This observation is confirmed by research conducted in the sewage treatment plant in Bartoszyce [35]. A high count of microorganisms present in the sewage pumped into aeration tanks is released into the air in the process of aerating the sewage with the use of surface aerators, which stimulate the circulation of the sludge in the tank. The rotation of the blades in the sewage causes the splashing of drops of the liquid, and subsequently an emission of numerous microorganisms into the air. Furthermore, the drops, returning to the surface of the sewage instigate the rebounding of new particles, whose sizes diminish successively. This phenomenon, referred to as secondary contamination, is observed during aeration.

In bioreactors, air bubbles formed during the process of aerating the sewage burst violently in the sewage liquid, releasing small particles that contain microorganisms from the sewage. The concentration of microorganisms in the air at this station is between 10 to 100 times higher than the concentration observed in the sewage itself [9].

As has been demonstrated above, areas immediately surrounding the aeration tanks are most heavily affected by biological aerosols. The concentration of microbiological contaminants generated by the aeration tanks may range from a dozen thousands in 1 m³ [36]. The concentration of microorganisms emitted from the aeration tanks and remaining stations diminish with the distance from the emitters [37], where they are replaced with the autochthonous microflora and pigmented bacteria. The count of bac-

teria released to the air decreases significantly with the distance from their emitters [15, 16]. Remarkably, the present research seems to confirm this observation.

Compared to acceptable amounts of *Pseudomonas fluorescens* bacteria in the air defined by Polish Norms [20, 25], the air at all sampling stations was heavily contaminated (with *Pseudomonas fluorescens* bacteria). Similarly, the concentration of actinomycetes was high and ranged from 11 to 230 CFU/m³, which classifies the air as moderately or heavily contaminated with these microorganisms. Comparable amounts of actinomycetes were noted on the premises of the sewage treatment plant in Pasłek [28], whereas a high level of contamination with actinomycetes was noted at all sampling stations on the premises of the Żółwin-Wypaleniska landfill site [31].

Being a constant component of the air in urban areas as well as in the vicinity of municipal utilities [38], actinomycetes should not be considered as indicators of the environmental impact of municipal utilities on air quality in the surrounding area.

The amounts of heterotrophic bacteria, mannitol-positive staphylococci, and mould fungi determined during the research classifies the air (on the premises of the Kapuściska sewage treatment plant) as moderately contaminated or uncontaminated.

Notably, climatic conditions must be regarded as an important determining factor affecting the population of microorganisms in the air [39, 40]. During winter, with weather conditions unfavourable for microbial growth, the populations of studied microorganisms were distinctly smaller. During spring and summer favourable atmospheric conditions accelerated the growth and subsequently the spread of microorganisms in the air. A similar seasonal distribution pattern of microorganisms was observed on the premises of different sewage treatment plants [14, 29, 41].

Conclusions

1. Mould fungi prevailed in the air microflora on the premises of the Kapuściska sewage treatment plant in Bydgoszcz, followed by mesophilic heterotrophic bacteria and *Pseudomonas fluorescens* bacteria; actinomycetes and staphylococci were sparsely distributed.
2. According to Polish Norms PN-89/Z-04111/02 and PN-89/Z-04111/03, air contamination by different microbial groups did not exceed permissible standards. In a majority of tests the air contamination reached a medium level or no microbial contamination was noted. However, serious contamination with *Pseudomonas fluorescens* bacteria and actinomycetes was observed.
3. The aeration tanks constituted the main source of bioaerosols on the premises of the investigated sewage treatment plant.
4. A higher percentage of microorganisms belonging to each particular microbial group in the air was determined with the use of the sedimentation method rather than the impaction method.

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