

Evaluation of Microbial Air Quality in a Forest Recreation Park

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Abstract

Bioaerosols significantly influence indoor and outdoor air quality and may cause numerous allergies and diseases. Our study presents results of an evaluation of air quality in the forest recreation park in Myślęcinek.

In the forest recreation park in Myślęcinek, microbiological air tests were conducted with the use of two methods of air sampling, namely the sedimentation method and the impaction method. The analysis indicate that mould fungi were the most abundant, constituting nearly 66% of all the microbial forms according to the sedimentation method, or 75% according to the impaction method. Heterotrophic bacteria were less numerous, making up to 32% of all microorganisms (the sedimentation method) or 24% (the impaction method). Actinomycetes and mannitol-positive staphylococci occurred in minimal concentrations. The highest count of microorganisms was recorded at Site V located on the verge of the forest near the tram terminus in Rekreacyjna Street, though staphylococci were the most abundant at Site I in the Polish Fauna Zoo Garden. The air was of high quality (containing the smallest number of microorganisms) at Site III located near Lake Myślęcińskie.

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

Introduction

In addition to various gases and condensed vapour, air also includes various biological elements such as seeds, mould spores, yeast, bacteria and viruses, insect eggs, small worms and protozoan cysts [1].

Air frequently serves as a temporary habitat for a variety of microorganisms. Although it does not offer particularly favourable living conditions for the development of microorganisms, it produces a microclimate where they may stay alive and active. They may drift attached to dust particles of mineral and organic origin, attached to vegetal and animal remains and immersed in water and secretion particles constituting so-called bioaerosols [2]. Bioaerosols consist of all airborne particles of biological origin, i.e. bac-

teria, fungi, fungal spores, viruses, and pollen, and their fragments, including various antigens. Particle sizes range from aerodynamic diameters of ca. 0.5 to 100 μm [3-5]. The number of microbes in the air varies according to atmospheric conditions.

The lifespan of air microbes is directly connected with their resistance to desiccation and ultraviolet radiation, which is different for various species. The longest lifespan is typical of bacterial spores, actinobacterial conidia and fungal spores adapted for surviving for extended periods of time even in unfavorable conditions. The microbial cells that produce a mucous halo or pigments protecting them from harmful UV radiation can also live longer in the air [6].

Mould fungi from *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Mucor* and *Rhizopus* genera, together with yeast from *Saccharomyces*, *Torulopsis* and

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Rhodotorula genera constitute more than 90% of all microbial forms in the air. Bacteria, the majority of which belong to the actinomycetes, occur in relatively small numbers (less than 10% of all airborne microorganisms). Less than 1% of the microbial community comprises Gram-positive cocci of *Micrococcus* and *Staphylococcus* genera, Gram-negative non-spore-forming rods of *Alcaligenes* genus, aerobic endospore-forming rods of the *Bacillus* genus and irregular, non-spore-forming gram-positive rods of the *Corynebacterium* genus [7]. The air is also a dispersal medium for pathogenic bacteria such as *Streptococcus pyogenes*, *Mycobacterium tuberculosis*, *Legionella pneumophila* and viruses, which can cause numerous diseases of the respiratory system [8].

Mould fungi present in the air may cause numerous mycoses, allergies and toxic reactions in humans. Since the number of respiratory allergies is still increasing, the 21st century has been branded as the age of the allergy epidemics [9]. Allergies develop mostly in organisms attacked by mould fungi of *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus* genera; *Cladosporium* and *Alternaria* are typical of the outdoor microflora, while *Penicillium* and *Aspergillus* are mainly found indoors [10, 11].

The forest recreation park in Myślęcinek is regularly visited by large numbers of Bydgoszcz residents and tourists, therefore it is exposed to pathogenic microflora in the air. Regular outdoor air quality monitoring, including bacteriological testing, is of great importance.

The aim of the present study was to estimate the concentration of heterotrophic bacteria, mannitol-positive staphylococci, actinomycetes and mould fungi in the air in the forest recreation park in Myślęcinek near Bydgoszcz, where the inhabitants of Bydgoszcz and the surrounding area spend their leisure time.

Materials and Methods

The research was carried out in the forest recreation park in Myślęcinek, near Bydgoszcz. The sampling sites were set in the most distinctive areas of the park, i.e. in the Polish Fauna Zoological Garden (Site I); the Botanical Garden (Site II); near the biggest lake, Myślęcińskie (Site III); in the centre of the walking area (Site IV); and on the edge of the forest near the tram terminus on Rekreacyjna Street (Site V) (Fig. 1).

Sampling

The investigation was pursued in a seasonal cycle using two methods of air sampling, namely the sedimentation method according to Polish Norm [12] 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 30 minutes, 150 cm above ground level.



Fig. 1. The location of sites.

In the impaction method 100 litres of air were 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 were deposited on the surface of the medium.

On 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 the laboratory, where they were inserted in a thermostat and incubated for a specific time at an appropriate temperature. After incubation the concentration 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 [2]:

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

...where:

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

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

r – radius of a Petri dish in cm,

πr^2 – area of a Petri dish,

t – exposure time of a dish (in minutes).

The impaction method involved using a measuring table by Feller [13], enclosed in the manual of the MAS-100 Air Sampler.

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

...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).

Microbiological Research

The microbiological research in the Forest Recreation Park in Myślęcinek near Bydgoszcz investigated the following:

- 1) the total count and the morphology of heterotrophic bacteria,
- 2) the count of mannitol-positive bacteria of *Staphylococcus* genus,
- 3) the count of actinomycetes,
- 4) the count of mould fungi.

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/1m³).

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

The presence of mannitol-positive staphylococci was detected in accordance with the Polish Norm [14] 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 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 the colonies were counted (CFU) and the results were calculated per 1 m³ of air.

On the basis of the final results, the level of air contamination in the Forest Recreation Park in Myślęcinek near Bydgoszcz was evaluated in accordance with the Polish Norm [14, 15].

Results

The findings of the survey into the concentration of different groups of airborne microorganisms in the forest recreation park in Myślęcinek near Bydgoszcz are presented in Tables 1 and 2, and Figs. 2 and 3. There is evidence that both methods applied in the research brought about similar results; the highest counts of heterotrophic bacteria were detected at Site IV, located in the centre of the walking area and at Site V located near the tram terminus in Rekreacyjna Street. For Site IV, the count determined by the sedimentation method was 3,912.0 CFU/1m³ and the count determined by the impaction method was 4,104.0 CFU/1m³, while for Site V the count determined by the sedimentation method was 3,373.3 CFU/m³, and the count determined by the impaction method was 3,353.0 CFU/1m³ (Table 1). Medium pollution by microorganisms were found at Site I (Polish Fauna Zoological Garden) and Site II (Botanical Garden), where the count of heterotrophic bacteria amounted to around 2,000 CFU/1m³. The smallest concentration of heterotrophic bacteria was observed near Lake Myślęcinińskie – 592.3 CFU/1m³ according to the sedimentation method and 341.0 CFU/1m³ according to the impaction method (Table 1).

The analysis of different seasons reveals that the highest count of heterotrophic bacteria in the air of the investigated site was recorded during summer and autumn, while the lowest was in wintertime (Table 2).

Cocci were dominant morphological bacterial forms, making up 67% of the airborne bacterial community on arithmetic mean. Endospore-forming rods and non-spore-forming rods had similar populations, which constituted between 15 and 18% of the bacterial community (Fig. 2).

The collected data suggest that bacteria of *Staphylococcus* genus were the most numerous at Site I (Polish Fauna Zoological Garden). With the arithmetic mean values ranging from 50.0 CFU/1m³ (the impaction method) to 78.5 CFU/1m³ (the sedimentation method), this station was considered highly contaminated. At the other sampling sites only medium contamination (up to 25 CFU/1m³) by staphylococci was observed (Table 1). Staphylococci were the most abundant during summer and autumn, while they were not detected during winter (Table 2).

Table 1. The count and the level of air contamination by microorganisms at different sampling sites in the forest recreation park in Myślęcinek near Bydgoszcz [14, 15].

Sampling site	Concentration of microorganisms in 1m ³ air							
	Heterotrophic bacteria		<i>Staphylococci</i>		<i>Actinomycetes</i>		Mould fungi	
	A	B	A	B	A	B	A	B
I.	2,451.5	2,266	78.5	50	78.8	36.3	8,531.8	8,103
	**		***		**		**	
II.	2,005.0	1,826.0	19.8	22.5	78.8	95.0	9,269.3	10,736.0
	**		**		**		***	
III.	592.3	341.0	19.8	15.0	78.5	35.5	1,238.5	2,134.0
	*		**		**		***	
IV.	3,912.0	4,104.0	19.8	5.0	78.8	50.0	6,742.8	7,515.0
	***		**		**		***	
V.	3,373.3	3,353.0	19.8	25.0	216.3	135.0	10,884.3	11,683.0
	***		**		***		***	
Polish Norm:								
not pollution *	< 1,000		0		10		3,000 - 5,000	
medium pollution **	1,000 - 3,000		< 25		10 - 100		5,000 - 10,000	
heavily pollution ***	> 3,000		> 25		>100		> 10,000	

A - sedimentation method, B - impaction method.

The figures show that contamination by actinomycetes according to the Polish Norms was highest at Site V (near the tram terminus in Rekreacyjna Street). The total count was 216.3 CFU/1m³ (the sedimentation method) and 135.0 CFU/1m³ (the impaction method) (Table 1). The remaining stations were only moderately contaminated by actinomycetes with their count stretching between 10 and 100 CFU/1m³. The highest percentage was observed in spring, similar values were noted during summer and autumn, and their count decreased significantly in winter (Table 2).

Similarly to other microorganisms, mould fungi prevailed during the summer and autumn seasons, while in the winter season a minimal count was noted (Table 2). Fungi were the least numerous at Site III (near Lake Myślęcinińskie) - the count established by the sedimentation method was 1,238.5 CFU/1m³ and count established by the impaction method was 2,134.0 CFU/1m³. In relation to the Polish Norms, Site I (Polish Fauna Zoological Garden) and Site IV (in the centre of the walking area) were presumed moderately contaminated (Table 2), while the remaining stations were described as heavily contaminated by fungi whose concentrations exceeded 10,000 CFU/1m³. (Table 1).

In the microflora of the air in the park mould fungi were the most numerous and formed around 66% of the local microbial community (measured by the sedimentation method) or 75% (measured by the impaction method). Less numerous heterotrophic bacteria constituted around 24% of

all the microorganisms (the impaction method) or 32% (the sedimentation method). The concentrations of actinomycetes and staphylococci were fractional (Fig. 3).

Discussion

Air, an essential element of the natural environment, is being contaminated by a growing number of different pollutants. Rapid industrial and transport development together with aggressive urban expansion has led to a menacing concentration of the air pollutant emission sources within relatively small areas. The atmosphere must absorb substantial amounts of harmful contaminants, including differ-

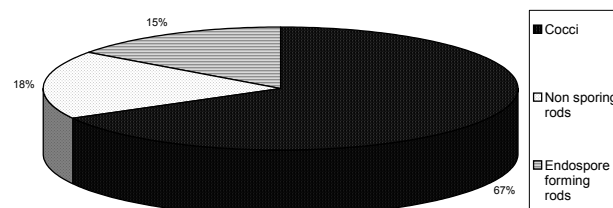


Fig. 2. Percentage fraction of morphological forms of bacteria on the premises in the forest recreation park in Myślęcinek near Bydgoszcz.

Table 2. An arithmetic mean count of microorganisms per 1m³ of air on the premises in the forest recreation park in Myślęcinek, depending on the season.

Date of sampling	Heterotrophic bacteria		<i>Staphylococci</i>		<i>Actinomycetes</i>		Mould fungi	
	A	B	A	B	A	B	A	B
24.06.2006	6,207.0*	5,624.0*	94.6*	74.0*	94.2*	72.4*	5,506.6*	8,780.0*
	1,100-13,761	220-13,700	79-157	20-100	0-157	0-180	1,730-19,252	5,900-16,600
03.10.2006	2,997.6*	3,180*	31.4*	20*	94.6*	80*	12,352.2*	15,360*
	315-6,809	400-7,100	0-157	0-100	0-236	0-100	1,179-35,388	12,800-39,500
24.02.2007	190.8*	108*	0*	0*	15.8*	4*	31.6*	40.8*
	0-718	4-444	0-0	0-0	0-79	0-20	0-158	32-60
04.05.2007	471.8*	600*	0*	0*	220.2*	125*	2,186*	5156*
	236-708	400-900	0-0	0-0	79-550	25-300	1,651-3,067	980-8,700

A - sedimentation method, B - impaction method, * - arithmetic mean values.

ent powders, organic compounds, non-organic compounds of nitrogen, sulphur, coal and other compounds, as well as various microorganisms including bacteria, viruses, fungal spores, etc. Since the air's ability to self-clean is vastly limited, it is necessary to supervise its quality and take measures that would protect it from excessive pollution [16].

It must be remembered, however, that this research presents only transient values, i.e. values lasting for a markedly brief time, credible at the time of obtaining the samples. As the values are time and space-variable owing to the physicochemical properties of the air, they give no more than an approximate idea of the concentration of microorganisms, roughly defining the count as low or high at a given moment [17].

Mould fungi made the highest percentage (66-75%) of studied airborne microorganisms in the air in the park, heterotrophic bacteria were less numerous (24-32% of studied microflora) while actinomycetes and staphylococci occurred in very small concentrations (only 0,5-1,5% of of studied microflora). A similar pattern was observed during the examination of the air at the landfill site in Żółwin-Wypaleniska [18], where mould fungi prevailed and constituted around 60-61% of the entire airborne microflora. Less numerous heterotrophic bacteria constituted about 30% of

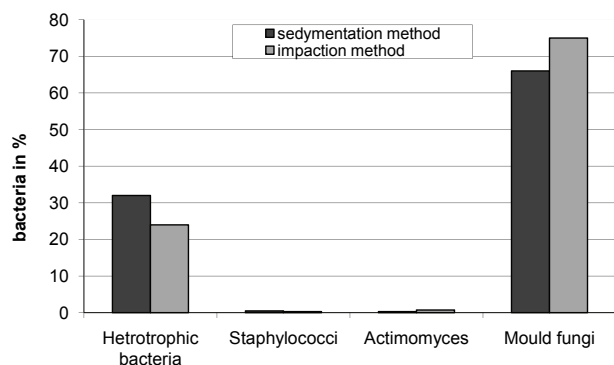


Fig. 3. Percentage fraction of microorganisms in the air of the forest recreation park in Myślęcinek.

studied airborne microorganisms, actinomycetes made up to 8-9% and mannitol-positive staphylococci only 1% of studied airborne microorganisms.

A large amount of mould fungi is confirmed by the fact that these microorganisms are well adjusted to spreading in the air [19] and to surviving in unfavourable conditions owing to the production of spores resistant to the following atmospheric factors: UV radiation and low humidity. As they can also easily develop on the soil surface and on the shrubs and trees [20], their high count in the park is justifiable.

A range of other factors influences the count of microorganisms in the park air, including its surroundings, traffic and tourist flow, local flora and fauna, and microbes that cover buildings and trees and are caught by the wind and transferred in the air [21]. The high contamination of the air by mould fungi, heterotrophic bacteria and actinomycetes detected at Site V near the tram terminus in Rekreacyjna Street is in direct connection with all the factors mentioned here.

The concentration of heterotrophic bacteria in the park air varied considerably. The smallest count was determined at Site III near Lake Myślęcienie, namely 592.3 CFU/1m³ measured by the sedimentation method and 341.0 CFU/1m³ measured by the impaction method. Yet the air was highly contaminated by heterotrophic bacteria at Site IV (in the centre of the walking area) and at Site V (near the tram terminus in Rekreacyjna Street), where the count exceeded the permissible dose of 3,000 CFU/1m³. This unusually increased count of heterotrophic bacteria may be directly linked to a big count of visitors to the zoo and the inflow of the microflora from different environments including the soil, plants' surface and animals. Surprisingly, a lower count of heterotrophic bacteria was found even in the air around the Old Town in Toruń (111-189 CFU/1m³) [22].

Staphylococci are used as air quality indicators as they usually point to the pathogenic microbes present in the air [11]. Although staphylococci do not produce spores, they have the ability to survive in the air for a long time, which

means spreading infections through the air [23, 24]. In the park, mannitol-positive staphylococci were the most abundant at Site I, where they exceeded the permissible dose, i.e. 25 CFU/1m³, while the remaining stations showed only medium-high contamination by these bacteria. The high contamination at Site I may be a natural consequence of a large number of animals and a large number of visitors as well.

Only Site V turned out to be highly contaminated by actinomycetes (more than 100 CFU/1m³). At the remaining stations their count ranged from 10-100 CFU/1m³, which can be described as an arithmetic mean contamination by this microbial community. In the air around the landfill site Żółwin-Wypaleniska, contamination by actinomycetes was high at all research stations [18]. Since actinomycetes are always present in urban air and around public sanitation facilities, they should not be regarded as air quality indicators [25].

Weather conditions obviously have a great influence on the count of microorganisms in the air of a specific area [26, 27]. Temperature rise accompanied by rain scarcity can lead to a sudden increase in the concentration of microorganisms in the air. Consequently, in summer and early spring, with the weather conditions most friendly for the spread and development of numerous microorganisms, heterotrophic bacteria, mannitol-positive staphylococci and mould fungi were the most abundant in the park air.

In winter, however, when the weather conditions were highly unfavourable for the growth of all microorganisms, only minimum counts of all microbes were noted. Similar results were obtained during air examination in Toruń [22] and Poznań [8], where the highest concentrations of bacteria and fungi were noted in summer months while the lowest concentrations were noted in winter months. The seasonal analysis of the air in the park in Myślęcinek also indicates that actinomycetes were the only microbial community whose maximum count was noted in spring. A similar pattern was observed in the sewage treatment plant in Łęguty and its surroundings [28].

Research into the morphology of the bacteria in the air in the Myślęcinek park shows that Gram-negative bacteria, rather poorly adapted to surviving outside their natural environment, were sparse, making up only 18% of all bacterial forms. On the other hand, Gram-positive bacteria formed the predominant part of all bacterial forms (67% cocci, 15% endospore-forming rods). Similar results were obtained during the examination of the air at the Żółwin-Wypaleniska landfill site in Bydgoszcz [18] and at the Old Town in Toruń [22], where Gram-positive bacteria constituted as much as 80-90% of airborne microflora.

Conclusions

The following conclusions may be formed on the basis of the detailed tests:

1. In the microflora of the air in the Forest Recreation Park Myślęcinek near Bydgoszcz, mould fungi were the most numerous followed by heterotrophic bacteria,

whereas mannitol-positive staphylococci and actinomycetes were the least numerous. The prevalence of mould fungi in the air in the Bydgoszcz park may be linked to the fact that the fungal spores are more resistant to unfavourable conditions such as UV radiation and low air humidity.

2. The highest concentration of microorganisms was recorded at Site V located on the edge of the forest near the tram terminus in Rekreacyjna Street, though staphylococci were the most abundant at Site I in the Fauna Zoo Garden. The air was of the best quality (containing the smallest number of microorganisms) at Site III near Lake Myślęcińskie. The concentration of different microorganisms in the Forest Recreation Park in Myślęcinek is strictly connected with the park's fauna and flora, intense tourist traffic within the area, and with the park's surroundings, particularly with nearby traffic.
3. In winter the count of airborne microorganisms decreased markedly, while in summer and autumn the count increased significantly, it may be connected with physico-chemical conditions to which microorganisms are exposed.
4. Cocci were a prevailing morphological form of bacteria detected in the air, while non-sporing rods and endospore-forming rods were less numerous.

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