

Assessment of microbiological quality of air in the selected sites situated by the main roads of Kraków

Ocena jakości mikrobiologicznej powietrza w wybranych punktach usytuowanych przy głównych szlakach komunikacyjnych Krakowa

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ABSTRACT

Introduction. Kraków is one of the most beautiful but also one of the most crowded Polish cities with large numbers of cars, pedestrians and cyclists travelling each day. There has been an increasing concern about the human exposure to bioaerosols, which can occur, among others, at the sites characterized by increased dustiness, such crowded streets. The aim of this study was to evaluate the microbiological quality of air in the selected sites situated by the main roads in Kraków. **Material and methods.** Air samples were collected in 10 sites located by the main roads, using a MAS-100 impactor four times per year. Four microbial groups were enumerated: mesophilic bacteria, fungi, actinomycetes and staphylococci. The results were expressed as colony forming units per m³ of air and compared with Polish Standards concerning microbiological air quality. **Results.** The greatest bacterial and fungal aerosol concentration was observed in autumn, when atmospheric conditions could have promoted abundance of those microorganisms. In general, fungi were the most numerous group of airborne microorganisms, while staphylococci were the least numerous. The number of actinomycetes was alarmingly high in all sites. It was found that seasonal differences in the bioaerosol concentration were statistically significant and the recorded differences could have been affected by atmospheric conditions. **Conclusions.** The conducted studies showed that in terms of airborne bacteria and fungi, the air in the examined locations was microbiologically unpolluted. However, high numbers of airborne actinomycetes were observed in all sites, which can cause adverse health effects in pedestrians or cyclists who are frequently exposed to

bioaerosols by the main routes in Kraków. Statistically significant variability was found in the prevalence of the examined microorganisms in different seasons of the year.

Keywords: air quality, Kraków, bacteria, fungi, actinomycetes, staphylococci

STRESZCZENIE

Wstęp. Kraków jest jednym z najpiękniejszych i zarazem jednym z najbardziej zatłoczonych polskich miast, w którym każdego dnia po ulicach przemierzają się tysiące samochodów, a także przechodniów i rowerzystów. Coraz większe zaniepokojenie budzi problem narażenia ludzi na aerozol biologiczny, którego podwyższone stężenie może wystąpić między innymi w zapyłonych miejscach, takich jak zatłoczone ulice. Z tego powodu, celem badań była ocena jakości mikrobiologicznej powietrza w wybranych punktach przy głównych szlakach komunikacyjnych Krakowa. **Materiał i metody.** Próbkę powietrza pobierano w 10 punktach umiejscowionych wzdłuż głównych dróg, czterokrotnie w ciągu roku, przy użyciu impaktora MAS-100. Oznaczono liczebność czterech grup mikroorganizmów – bakterii mezofilnych, grzybów, promieniowców i gronkowców. Wyniki wyrażono jako jednostki tworzące kolonie w m³ powietrza i porównano z Polskimi Normami dotyczącymi jakości mikrobiologicznej powietrza atmosferycznego. **Wyniki.** Najwyższe stężenie bioaerozolu bakteryjnego i grzybowego stwierdzono jesienią, gdy warunki atmosferyczne były korzystne dla zwiększonej liczebności drobnoustrojów w powietrzu. Ogółem, grzyby były najliczniejszą grupą drobnoustrojów, a najmniej liczną były

gronkowce. We wszystkich badanych lokalizacjach liczebność promieniowców była niepokojąco wysoka. Stwierdzono istnienie istotnej statystycznie zmienności sezonowej w stężeniu bioaerozolu, przy czym różnice te mogły być wynikiem zmiennych warunków atmosferycznych. **Wnioski.** Przeprowadzone badania wykazały, że pod względem stężenia bakterii i grzybów powietrze w badanych lokalizacjach było mikrobiologicznie czyste. Jednakże we wszystkich lokalizacjach stwierdzano wysokie

stężenie promieniowców, co może prowadzić do niekorzystnych skutków zdrowotnych np. u pieszych i rowerzystów, którzy często narażeni są na kontakt z bioaerozolem, podróżując wzdłuż głównych dróg w Krakowie. Stwierdzono istotną statystycznie zmienność liczebności badanych drobnoustrojów w różnych porach roku.

Słowa kluczowe: jakość powietrza, Kraków, bakterie, grzyby, promieniowce, gronkowce

INTRODUCTION

Kraków is one of the biggest and most beautiful Polish cities. Each year it is visited by a vast number of tourists and it is also one of the biggest academic centers in Poland. Most of the Kraków inhabitants eagerly walk or travel either by cars or use the public transport to travel around the city. The main communication routes are among the most crowded places in the city, with thousands of cars. At the same time, thousands of pedestrians pass by the bus or tram stops each day. Also, there are numerous bicycle paths routed along the main roads in Kraków.

Air is one of microbial habitats, but it is a hostile environment for them due to lack of proper amount of nutrients, water and due to excessive solar radiation. Unlike water and soil, air is only the place where microorganisms are present periodically, where they can be transferred and retain their infectious character, but they are unable to grow and divide [1]. Airborne microorganisms occur in the form of bioaerosols, which comprise various groups of microorganisms, but also cell fragments, connected with dust particles and water drops [1]. There has been an increasing concern about the human exposure to bioaerosols, due to the associated health effects, such as asthma and rhinitis, hypersensitivity pneumonitis or infections [2]. In urbanized areas, the sources of airborne microorganisms outdoors include people and animals, soil, leaf surfaces or even fecal material, most likely dog feces [3]. Additionally, the levels of airborne microorganisms outdoors is associated with the concentration of dusts [4], including PM₁₀ (particulate matter of diameter less than 10 µm), which is produced, among others, by motor vehicles [5]. According to the studies conducted by the Voivodeship Inspectorate of Environmental Protection in Kraków (PIOŚ) [6], in recent years the amount of particulate matter PM₁₀ in Kraków significantly exceeds the admissible limits, particularly in winter. Increasing

frequency of respiratory diseases are a challenge to take some preventive measures. Such actions are not problematic, when the diseases are transmitted by water or food, as in this case easy preventive measures, such as e.g. cooking, are sufficient. However, in the case of diseases spread through the air when breathing, prevention of infections is much more difficult [7].

Considering the problem of more and more rapidly spreading civilization-related diseases, caused among others by air pollution, this study was undertaken to examine the microbiological quality of air along the main roads of Kraków, which are frequented every day by motorists, pedestrians and cyclists.

MATERIAL AND METHODS

The air sampling was conducted in 10 sites located by the main communication routes in Kraków (fig. 1, tab. I).

All of the sites, selected for the analyses are characterized by heavy traffic during the whole day, with bus and tram stops where numerous people cross streets, at the same time having bicycle paths, therefore all of the examined sites are significantly crowded. The air sampling was conducted four times per year, once in each season:

- I – May 21st 2013 (spring)
- II – July 12th 2013 (summer)
- III – October 25th 2013 (autumn)
- IV – January 16th 2014 (winter).

The measurements were performed using a single-stage MAS-100 impactor (Merck, Switzerland). Air volume was 100 liters. The sampling was performed during the day, when the traffic was heavy. While sampling, the impactor was placed at a height of 1.0–1.5 m above the ground to simulate aspiration from the human breathing zone. The samples were collected on Petri dishes containing microbiological media used for enumeration of mesophilic

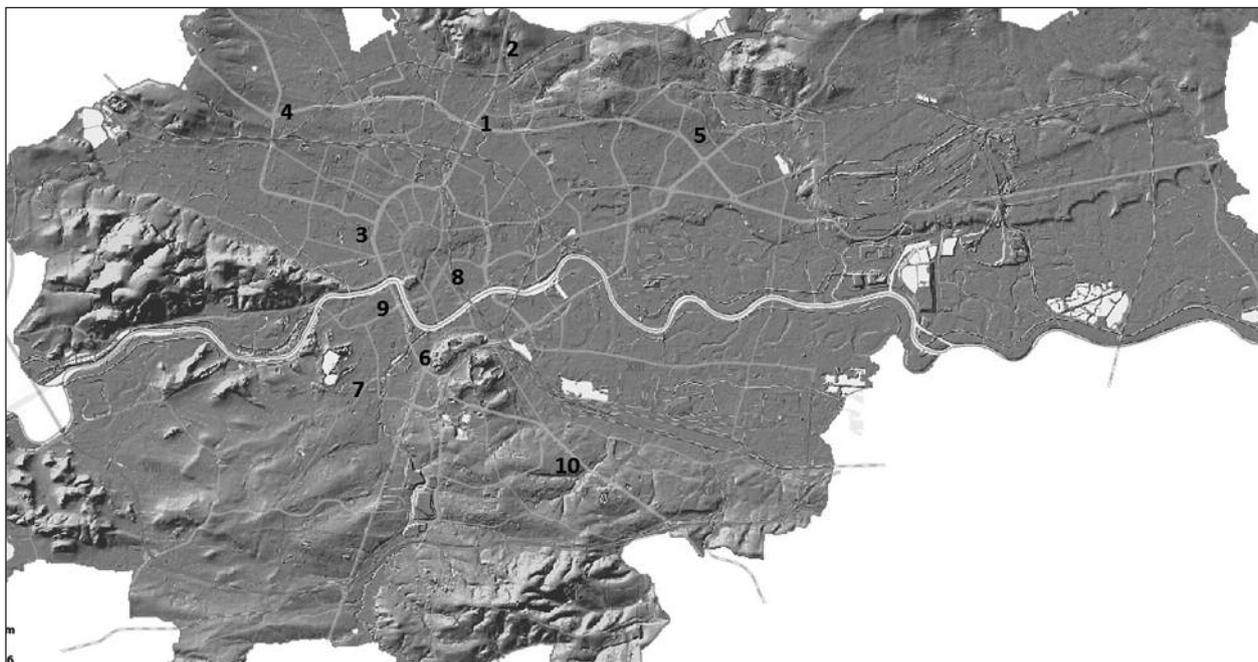


Fig. 1. The location of the examined sampling sites [8]

Ryc. 1. Lokalizacja punktów badawczych [8]

bacteria (Trypticasein Soy Lab Agar, Biocorp, Poland), fungi (Oxoid, Great Britain), actinomycetes (Actinomycete Isolation Lab Agar, Biocorp, Poland)

Table I. Description of the sampling sites

Tabela I. Charakterystyka punktów badawczych

No.	Location	GPS coordinates
1	Crossing between 29 listopada Ave. with Opolska and Lublańska Street	N50°5'8.985" E19°57'16.732"
2	Warszawska Street	N50°7'27.271" E19°58'3.718"
3	Mickiewicza Ave (by the Faculty of Animal Sciences, University of Agriculture)	N50°3'39.348" E19°55'26.423"
4	Ofiar Katynia Roundabout	N50°5'15.725" E19°53'30.551"
5	Kocmyrzowskie Roundabout	N50°4'46.716" E20°1'38.502"
6	Matecznego Roundabout	N50°2'10.364" E19°56'25.551"
7	Kobierzyńska Street (Ruczaj Estate)	N50°1'52.615" E19°55'13.522"
8	Starowiślna Street (Kazimierz Estate)	N50°3'17.274" E19°56'49.999"
9	Grunwaldzkie Roundabout	N50°2'55.069" E19°55'56.434"
10	Wielicka Street	N50°1'4.482" E19°59'24.950"

and staphylococci (Chapman agar, Biocorp, Poland). All measurements were conducted in triplicates and the data presented in tables are the mean values from those replicates. The air temperature and humidity were recorded onsite using an HT-9213 Thermohydrometer (ATM, China), wind speed was acquired from an online weather archive [9], while the atmospheric pressure and particulate matter (PM10 and PM2.5) were derived from the Voivodeship Inspectorate of Environmental Protection in Kraków (PIOŚ) website [6]. After sampling, Petri dishes were incubated in proper conditions (i.e. total number of mesophilic bacteria 37 °C [10, 11], 48 hrs; fungi 25 °C [11], 3–5 days; actinomycetes 25 °C, 5–7 days; staphylococci 37 °C, 48 hrs). After incubation, the number of colonies characteristic for different microbial groups were counted and expressed as colony forming units per cubic meter of air (CFU/m³). The actual colony count per each culture plate was corrected according to the positive hole correction table [12]. The obtained results were compared with the limits specified by the Polish Standards [13, 14] to evaluate the microbiological quality of air. Statistica v. 10.0. software (StatSoft, US) was used to calculate the basic descriptive statistics and one-way analysis of variance was applied to verify the significance of differences in the number of airborne microorganisms between different locations.

RESULTS

Meteorological conditions prevailing during the sampling dates are presented in tab. II. The highest concentration of both particulate matter PM10 and PM2.5 was recorded in January.

The recorded numbers of mesophilic bacteria are presented in tab. III and fig. 2. Overall, the mean concentration \pm standard deviation of airborne bacteria was 97 ± 72 CFU/m³. The greatest concentration of those microorganisms was observed at the site No. 2 in autumn (327 CFU/m³ of air). Similarly, the greatest mean annual concentration of airborne bacteria was recorded at this site. On the other hand, the smallest mean concentration of airborne bacteria was observed at the site No. 9, i.e. 40 CFU/m³. Also, the range of bacterial bioaerosol concentration at this site was the smallest.

The overall mean concentration of fungi \pm standard deviation was 863 ± 867 CFU/m³. During the examined period, the mean number of fungi ranged from 226 to 1111 CFU/m³ of air (fig. 3). The greatest mean number of fungi was recorded at the site No. 3 (Mickiewicza Ave), where the greatest concentration was recorded in autumn (3447 CFU/m³, tab. IV). On the other hand, the smallest mean concentration of fungal aerosol was observed at the site No. 9 (Grunwaldzkie Roundabout) with the smallest number of airborne fungi recorded in summer (i.e. 60 CFU/m³, tab. IV).

Overall, the concentration of actinomycetes \pm standard deviation was 438 ± 457 CFU/m³. The concentrations of actinomycete bioaerosol were high at all examined sampling sites, except for the measurements conducted in summer, when their maximum numbers reached 20 CFU/m³ and in four locations

Table II. Meteorological conditions prevailing at the sampling dates

Tabela II. Warunki meteorologiczne panujące podczas przeprowadzania badań

Parameters \ Sampling date	May 21 st 2013	July 12 th 2013	October 25 th 2013	January 16 th 2014
Temperature [°C]	13.8 (range 11.2–15.2)	17.2 (range 14.0–19.7)	10.8 (range 8.1–13.3)	3.2 (range –1.2 – +5.0)
Wind speed [km/h]	5.5	8	3.75	5.5
Humidity [%]	57 (range 43–62)	79 (range 62–85)	55 (range 48–66)	80 (range 66–87)
Pressure [hPa]	988	995	998	989
Particulate matter PM10 [$\mu\text{g}/\text{m}^3$]	26	30	60	81
Particulate matter PM2.5 [$\mu\text{g}/\text{m}^3$]	17	20	37	52
General weather conditions	Cloudy and light showers	Variable, storms alternating with sun	Sunny, dry, no clouds	Sunny, dry, light fog

Table III. Number of mesophilic bacteria (CFU/m³) in each sampling site

Tabela III. Liczebność bakterii mezofilnych (jtk/m³) w badanych lokalizacjach

Sampling date \ Location	May 21 st 2013	July 12 th 2013	October 25 th 2013	January 16 th 2014
1	180	90	247	40
2	80	140	327	73
3	120	150	63	61
4	40	70	77	30
5	60	120	30	87
6	190	190	23	23
7	180	140	11	47
8	70	80	63	80
9	20	50	57	33
10	100	10	247	23

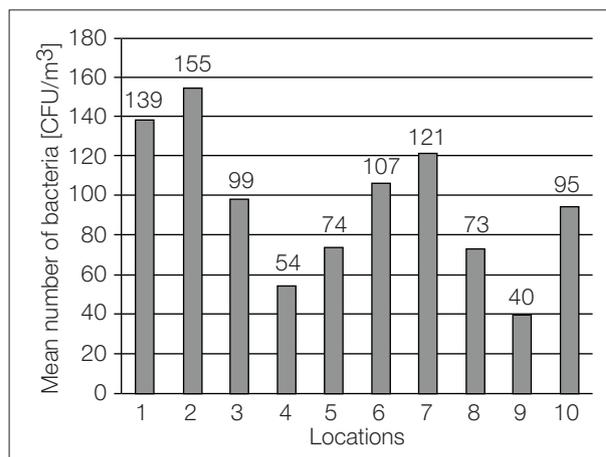


Fig. 2. Mean annual number of mesophilic bacteria (CFU/m³) for each sampling site

Ryc. 2. Średnia roczna liczebność bakterii mezofilnych (jtk/m³) w każdym z badanych punktów

the presence of these microorganisms was not recorded at all (tab. V). Mean concentration of actinomycetes at the examined sites ranged from 179 to 692 CFU/m³ (fig. 4). The greatest concentration of airborne actinomycetes was observed at the site No. 7 (Kobierzyńska Street), while the smallest – at the site No. 9 (Grunwaldzkie Roundabout).

The recorded concentrations of airborne staphylococci are shown in tab. VI and in fig. 5. In general, the mean concentration ± standard deviation of staphylococci was 22±40 CFU/m³. Mean annual number of staphylococci ranged from 10 CFU/m³ at the site No. 5 (Kocmyrzowskie Roundabout) to 69 CFU/m³ at the site No. 1 (Crossing between 29 listopada Ave. with Opolska and Lublańska Street).

In terms of seasonal variation in the microbial

aerosol, the greatest concentration of fungi and mesophilic bacteria was recorded in autumn, actinomycetes – in spring and staphylococci – in summer. On the other hand, the smallest concentration of fungi and mesophilic bacteria was recorded in winter, staphylococci – both in winter and spring, while actinomycetes – in summer (fig. 6).

Statistical analysis of the results revealed that the differences in the concentration of bioaerosol between the examined locations are statistically significant only in the case of mesophilic bacteria. On the other hand, the seasonal differences in the bioaerosol concentration are statistically significant for all of the microbial groups except for staphylococci (tab. VII).

Table IV. Number of fungi (CFU/m³) in each sampling site
Tabela IV. Liczebność grzybów (jtk/m³) w badanych lokalizacjach

Sampling date / Location	May 21 st 2013	July 12 th 2013	October 25 th 2013	January 16 th 2014
1	720	500	2277	313
2	570	200	2090	123
3	350	190	3447	457
4	120	70	1823	440
5	400	140	2043	133
6	1310	160	1170	100
7	330	160	1223	220
8	260	190	1883	413
9	160	60	563	120
10	420	1280	1770	140

Table V. Number of actinomycetes (CFU/m³) in each sampling site

Tabela V. Liczebność promieniowców (jtk/m³) w badanych lokalizacjach

Sampling date / Location	May 21 st 2013	July 12 th 2013	October 25 th 2013	January 16 th 2014
1	310	20	577	180
2	710	20	857	180
3	740	0	390	370
4	340	0	183	227
5	510	10	297	173
6	1540	20	503	97
7	700	10	1647	410
8	1030	20	883	127
9	420	0	177	120
10	1010	0	207	130

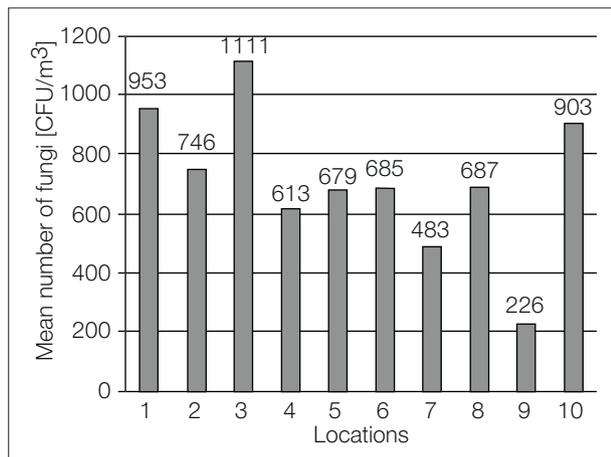


Fig. 3. Mean annual number of fungi (CFU/m³) for each sampling site

Ryc. 3. Średnia roczna liczebność grzybów (jtk/m³) w każdym z badanych punktów

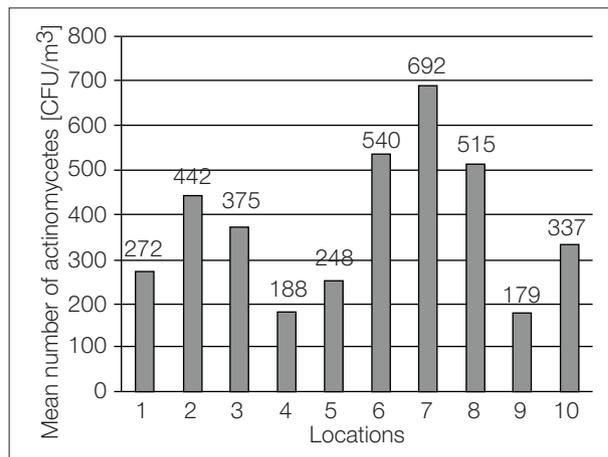


Fig. 4. Mean annual number of actinomycetes (CFU/m³) for each sampling site

Ryc. 4. Średnia roczna liczebność promieniowców (jtk/m³) w każdym z badanych punktów

Table VI. Number of staphylococci (CFU/m³) in each sampling site

Tabela VI. Liczebność gronkowców (jtk/m³) w badanych lokalizacjach

Sampling date / Location	May 21 st 2013	July 12 th 2013	October 25 th 2013	January 16 th 2014
1	0	250	26	0
2	0	10	25	20
3	20	0	26	7
4	10	10	28	3
5	10	0	28	3
6	50	20	29	10
7	10	10	30	13
8	20	10	25	3
9	20	0	25	90
10	10	10	26	7

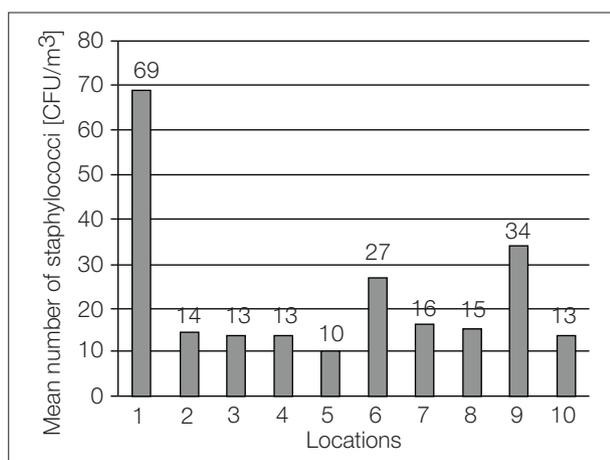


Fig. 5. Mean annual number of staphylococci (CFU/m³) for each sampling site

Ryc. 5. Średnia roczna liczebność gronkowców (jtk/m³) w każdym z badanych punktów

DISCUSSION

The concentration of microbial aerosol, observed in this study, varied depending on both the sampling location and the season. The recorded numbers of airborne microorganisms were compared with the admissible levels given in the Polish Standards [13, 14]. The concentration of bacterial aerosol recorded in each of the sites did not exceed the threshold of 1000 CFU/m³ in any of the sampling periods, therefore in terms of the bacterial aerosol the air can be regarded as microbiologically unpolluted. Similar observation was made in the case of fungi. Only in one sampling site (No. 3 – Mickiewicza Ave) the

Table VII. Results of the analysis of variance concerning the spatial and temporal variations in the bioaerosol concentration

Tabela VII. Wyniki analizy wariancji dotyczącej czasowego i przestrzennego zróżnicowania stężeń bioaerolu

Microorganisms	F value (location)	F value (season)
Mesophilic bacteria	3.05*	5.35*
Fungi	0.96	43.85*
Actinomycetes	1.53	12.70*
Staphylococci	1.24	1.36

* values are significant with $p < 0.05$

* wartości są istotne przy $p < 0,05$

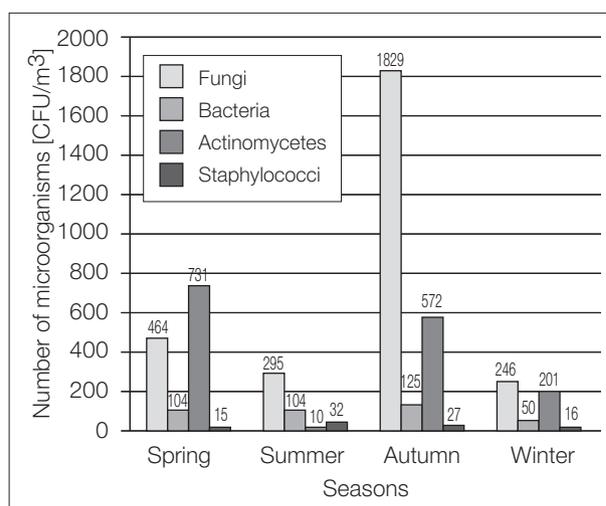


Fig. 6. Seasonal changes in microbial bioaerosol concentrations – mean values from all examined locations

Ryc. 6. Sezonowe zmiany stężenia bioaerolu – wartości średnie ze wszystkich badanych lokalizacji

number of airborne fungi exceeded 3000 CFU/m³ (in autumn). In contrast, the concentration of actinomycetes exceeded the threshold values (i.e. >100 CFU/m³) in almost each site in spring, autumn and winter (tab. V). Only in summer, when the weather conditions were unfavorable, 4 sites were considered unpolluted, while in the remaining 6, the pollution was average (between 10 and 100 CFU/m³).

In the case of particulate matter concentrations, their levels were compared with the limits specified by the Regulation of the Ministry of Environment of 24 August 2012 concerning evaluation of substance levels in the air [15]. It was shown that in autumn and winter the permissible levels of both PM₁₀ (50 µg/m³) and PM_{2.5} (25 µg/m³) in the air was exceeded. This might be one of the factors affecting the concentrations of bacterial and fungal

aerosol, which were the highest in autumn (fig. 6). On the other hand, those differences could result from a number of other factors, as the concentrations of particulate matter were not measured at each of the sampling sites. In our study, the temporal variation in the number of airborne microorganisms could be related to the fact that both during winter and summer the weather conditions were unfavorable for microbial proliferation. The lowest concentration of actinomycetes was recorded in summer, when the weather was very variable, with showers that could result in sedimentation of these microorganisms [16, 17]. The concentration of bacterial and fungal aerosol was the smallest in winter, when on the other hand the temperature could have been too low for those microorganisms [1]. The number of staphylococci did not vary significantly between different seasons, which was also shown by the statistical analysis (tab. VII). The mean number of bacteria varied from 50 CFU/m³ in winter to 125 CFU/m³ in autumn (fig. 6), when the greatest numbers of those microorganisms were recorded in half of the sites (tab. III). The mean number of mesophilic bacteria is lower than the one recorded by Donderski et al. [10] in the city of Toruń, where the maximum numbers of heterotrophic bacteria occurred in July and reached 372 CFU/m³. However, the maximum number of mesophilic bacteria during the whole study was recorded in autumn at the site No. 2 (Warszawska Street) and reached 327 CFU/m³. The maximum number of mesophilic bacteria in summer was observed at the site No. 6 (Matecznego Roundabout) and was 190 CFU/m³ (tab. III). Those numbers were on the other hand smaller than the ones observed by Grzyb et al. [18] on the marketplaces in Kraków, where the mean value of airborne bacteria was 890 CFU/m³ and the maximum numbers reached 2305 CFU/m³.

On the other hand, the concentrations of fungal aerosol, recorded in our study were much higher than the ones reported by Donderski et al. [10], as the maximum number of those microorganisms was recorded by these authors in June and reached 285 CFU/m³ of air. Also in the study conducted by Grzyb et al. [18], the mean number of fungi was 513 CFU/m³ of air, although the concentration of fungal aerosol in the most polluted site recorded by these authors reached 3460, which was similar to the maximum value observed in this study (i.e. 3447, recorded in autumn, at the site No. 3 – Mickiewiczza Ave). In our study, in the course of a year, the maximum mean number of fungi was observed in autumn and it was 1829 CFU/m³.

The mean concentration of airborne actinomycetes in our study ranged from 179 CFU/m³ at the site No. 9 (Grunwaldzkie Roundabout) to 692 CFU/m³ at the site No. 7 (Kobierzyńska Street). The maximum number recorded by Grzyb et al. [18] at the Kraków marketplaces was 140 CFU/m³, but in some of the examined sites the actinomycetes were absent. Large numbers of actinomycete spores can be released into the air by mechanical disturbance of the substance they are growing on [19]. Their numerous presence in the air is linked with the amount of airborne dust or soil particles. Lloyd [20] stated that any action that disturbs the soil surface and launches soil particles into the air, even gusty wind, can increase the concentration of airborne actinomycete propagules. They are known to be important air contaminants and have been related to the incidence of allergic alveolitis and other severe health effects [19, 21]. Hirvonen et al. [22] showed that the spores of *Streptomyces* spp. can stimulate lung macrophage reactions, which can cause inflammation and tissue injury.

The statistical analysis showed that, except for mesophilic bacteria, the differences in the concentration of microbial aerosol between the examined sites were statistically insignificant. All of the examined sites are crowded with heavy traffic, sometimes during the whole day. These factors can significantly affect the abundance of microorganisms, as increased traffic raises dust particles, which also contain microorganisms [10]. Moreover, the presence of buildings or trees by the roads, which are covered with microorganism-containing dust is not without significance [23].

CONCLUSIONS

The presented study showed that in terms of bacterial and fungal aerosol the air in all of the examined sites was microbiologically unpolluted (with values below the threshold of 1000 CFU/m³ and 5000 CFU/m³ of air, respectively for bacteria and fungi). The concentration of airborne bacteria, fungi and actinomycetes was the smallest at the site located by the Grunwaldzkie Roundabout. Alarming high number of airborne actinomycetes was observed in all of the examined sites, which can cause some adverse health effects in pedestrians or cyclists who are frequently exposed to bioaerosols by the main routes in Kraków. The conducted study indicated that the differences between microbial concentrations in various seasons of the year were significant, whereas the greatest

numbers of bacteria and fungi were observed in autumn, when the atmospheric conditions were most favorable.

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