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BIOAEROSOL-FORMING ACTINOMYCETES AT THE SELECTED SITES OF KRAKOW

PROMIENIOWCE TWORZĄCE BIOAEROZOL W WYBRANYCH MIEJSCACH KRAKOWA

Abstract: Krakow is a city where, due to mostly so-called low-stack emissions and intensified traffic, air dustiness continues to increase and often, especially in winter, the threshold values of dustiness are exceeded. Air dustiness increases the number of airborne microorganisms, as they are associated with particles of dust to form bioaerosols. Actinomycetes are one of the most significant components of bioaerosol. Their presence in bioaerosol is dangerous to human health, since even very low concentrations of these microorganisms can cause allergic reactions.

The aim of this study was to verify the relationship between the concentration of actinomycetes in the air of Krakow depending on spatial variation of thermal conditions and the degree of air dustiness. The study was conducted using a cascade impactor for bioaerosol particle separation, including the respirable fraction. Moreover, the measurements of air dustiness were performed simultaneously, separating 4 dust fractions. The research was conducted over 12 months (full calendar year) and the measurements were performed once per month, within one day, at 16 research sites.

The lowest average numbers of actinomycetes were recorded in places characterized by little urban green space (*eg* Main Square or crossroads). On the other hand, the highest numbers were found in green areas (*eg* Blonia Park meadows, green area at the Krakow University of Technology).

Based on the normative classification of air quality (Polish Standard PN-89/Z-04111/02) the number of actinomycetes in 75 % measurements showed that the air was polluted, including heavily polluted air in 16 % measurements.

The most common actinomycetes belonged to the genus *Streptomyces* (*St. albus, St. badius, St. globisporus*).

Keywords: actinomycetes, bioaerosol, air dustiness, Krakow

Actinomycetes acquired the unique ability to colonize the so-called solid surfaces. They can survive on rocks, plants, animals, clothing, food and other bare surfaces. This is due to the properties of their spores, which are resistant to long periods of drying and capable of survival at low moisture content of the substrate on which they grow [1].

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Actinomycetes are one of the important components of bioaerosol. Actinomycetes, mostly in the form of spores, have been identified in the air for a few decades. Until now, the mechanism of their release into the atmosphere has not been fully understood. It is suggested that they can be released into the air as a result of mechanical damage to surface on which they grow, *eg* during agrotechnical operations or strong wind they can be raised from soil surface, in which they develop extensively. Due to their smaller size, it is more difficult for actinomycete spores to transform into bioaerosol phase than for fungal spores [1, 2].

The share of actinomycete spores significantly increases in the bioaerosol fraction below 3.3 μm [3].

The airborne spores of at least several actinomycete species (*eg Streptomyces albus*) have proven their allergic effects. Research has shown that actinomycete spores stimulate macrophages in the lungs, resulting in inflammation and tissue damage [4, 5].

Dusts have also a large share in atmospheric pollution. They originate in the processes of combustion of solid fuels (municipal and household sector), liquid fuels (car engines), in industrial processes and in natural phenomena (*eg* volcanic eruptions). Dust particles with a diameter below 10 micrometers are considered as one of the most toxic components of air; therefore in the European Union dust PM 10 is currently the primary indicator of air quality [6].

Krakow is a city, where due to mostly so-called low-stack emissions and intensified traffic, air dustiness continues to increase and often, especially in winter, the threshold values of dustiness are exceeded. Air dustiness stimulates the increase in the number of airborne microorganisms, since they associate with dust particles to form bioaerosol [7].

The aim of this study was to verify the relationship between the concentration of airborne actinomycetes in Krakow, spatial variability of thermal conditions and the air dustiness level.

Material and methods

The study was conducted over 12 months (full calendar year) during the period from March 2010 to February 2011 in order to detect both the changes in temperature and in the quantitative and qualitative composition of microflora between the seasons of the year – once per month, during one day, at 16 research sites. At the same time, dustiness and microclimatic parameters (temperature, atmospheric pressure, wind energy, relative humidity) were measured at the designated sites to correlate all measured values.

The research sites were selected based on the deviations from the mean annual temperature in Krakow, including:

– 7 sites characterized with temperatures below the average temperature in Krakow,

– 7 sites, where the temperature is above the average,

– 2 sites, which the results from the above-mentioned sites were related to: the first one, with the temperature equal to the average recorded in Krakow and the second one: control site outside Krakow (Table 1).

Table 1

Comparison of research sites

Control site (outside Krakow) (C)	
C	Control site Libertow – 400 m from the "Zakopianka" road – above the basin, where Krakow is located
The research sites with temperatures below the average temperature in Krakow (B1–B7)	
B1	The Kosciuszko Mount – at the top of the Mount
B2	The Krakus Mount – at the top of the Mount
B3	The Nowa Huta Centre of Culture
B4	Blonia Park – in the middle of Blonia Park
B5	Vistula Czerwienski Boulevard – next to the Wawel Dragon
B6	Water reservoir in Nowa Huta – Bulwarowa Street
B7	Botanical Garden – Kopernika Street
The research site in which temperature is equal to the average temperature in Krakow (AVG)	
AVG	The School of Sports Championship area
The research sites with temperatures above the average temperature in Krakow (A1–A7)	
A1	Krakow University of Technology, Jan Paweł II Street – by the new building
A2	Nearby the crossing with Fabryczna Street
A3	The Teatralne Estate in Nowa Huta – behind the building of The Ludowy Theatre at Obroncow Krzyza Street
A4	Main Market Square – nearby the Town Hall Tower
A5	Mickiewicza Avenue – between the University of Agriculture and the AGH – University of Science and Technology
A6	ArcelorMittal Poland – Ujastek Street – in front of the administrative building
A7	ArcelorMittal Poland – Igolomska Street, nearby the crossing with Dymarek Street

The air samples were collected using a 6-stage Graseby-Andersen impactor. Application of this instrument allowed for the separation of the examined bioaerosol particles depending on their aerodynamic diameters into fractions: over 7.0 μm, 7.0–4.7 μm, 4.7–3.3 μm, 3.3–2.1 μm, 2.1–1.1 μm, and 1.1–0.65 μm. The term respirable fraction means the total number of bacteria from fractions below 4.7 μm [8, 9]. The sampling lasted 5 minutes; the air flow through the impactor was 28.3 dm^3 per minute. The Gauze's medium was applied and the incubation was carried out for 7 days at 28 ^oC to isolate actinomycetes. Dustiness and microclimatic parameters were measured simultaneously to correlate the measured values. Dust concentration measurements were performed with a DustTrak II Aerosol Monitor 8530 (TSI Inc.) which operates based on laser photometry (measurement time is 60 seconds – for each fraction). In this research project, the dust concentration was measured simultaneously for 4 dust fractions: PM10, PM4, PM2,5 and PM1. The PM10 dust is composed of solid and liquid particles suspended in the air, both of organic and inorganic substances. The suspended dust may contain toxic substances, such as policyclic aromatic hydrocarbons (*eg* benzo-α-pyrene), heavy metals, dioxins and furanes as well as microorganisms deposited on solid and liquid particles. Dust measured as PM4 is the fraction of respirable dust, which corresponds to the respirable fraction with the aerodynamic diameter below 4.7 μm measured using a cascade impactor. Kestrel 4000 Pocket Weather Meter (Nielsen-Kellerman) was applied to determine the microclimatic parameters (temperature, wind energy and relative humidity).

The correlation between the examined factors was analyzed using Statistica 9.1 software.

Results and discussion

Actinomycetes form a group of microorganisms, which normally occurs in the air in much lower concentrations than bacteria and fungi, but their comparable quantities can cause much more serious health effects. The number of actinomycetes was 20 % higher at a control site outside than within Krakow. The average actinomycete bioaerosol concentration at the sites with temperature below the mean (indicated in Table 1 as B) was 66 CFU · m^{-3}, at the control site (marked as C) located outside Krakow it was 61 CFU · m^{-3}, at the site with average temperature (AVG) – 60 CFU · m^{-3} and at the sites with temperature above the average (A) – 56 CFU · m^{-3}. If assumed that B equals 100 %, the percentages amount to: C – AVG – A: 92.5 % – 90.9 % – 84.8 %, respectively. This means that the numbers of this microbial group were about 15 % lower at the sites with temperature above the average (Table 2).

Table 2

Comparison of minimum, maximum, average and total numbers of actinomycetes [CFU · m^{-3}]

Research site	Minimum	Mean	Maximum	The sum of annual measurements
C	0	**61**	212	733
B1	14	72	488	858
B2	0	51	127	616
B3	0	66	262	787
B4	9	99	665	1193
B5	0	49	290	583
B6	0	63	219	760
B7	0	86	361	1037
Mean from **B1–B7**		**66**		
AVG	0	**60**	290	718
A1	18	110	431	1322
A2	0	26	64	316
A3	7	62	290	742
A4	7	32	99	385
A5	0	41	177	495
A6	0	64	332	768
A7	0	57	375	689
Mean from **A1–A7**		**56**		

Table 3

Comparison of the number of actinomycetes at the research sites during the entire study period with the determination of microbial air pollution according to the Polish Standard PN-89/Z-04111/02 [CFU · m^{-3} of air]

Research sites	Measurement period												Measurements when the air was polluted [%]	Including heavily polluted [%]
	03.'10	04.'10	05.'10	06.'10	07.'10	08.'10	09.'10	10.'10	11.'10	12.'10	01.'11	02.'11		
C	**7**	**99**	**57**	**0**	**71**	**212**	**185**	**21**	**35**	**18**	**0**	**28**	75.0	16.7
B1	**28**	**14**	**28**	**14**	**85**	**488**	**44**	**35**	**44**	**18**	**18**	**42**	100.0	8.3
B2	**85**	**64**	**14**	**0**	**120**	**127**	**9**	**28**	**97**	**26**	**18**	**28**	83.3	16.7
B3	**21**	**35**	**71**	**71**	**78**	**262**	**150**	**28**	**0**	**18**	**26**	**28**	91.7	16.7
B4	**21**	**14**	**21**	**148**	**156**	**665**	**35**	**28**	**18**	**9**	**35**	**42**	91.7	25.0
B5	**35**	**14**	**85**	**21**	**57**	**290**	**18**	**7**	**18**	**0**	**18**	**21**	83.3	8.3
B6	**0**	**7**	**28**	**71**	**198**	**219**	**44**	**92**	**62**	**18**	**0**	**21**	75.0	16.7
B7	**78**	**7**	**7**	**148**	**361**	**346**	**9**	**0**	**0**	**9**	**44**	**28**	50.0	25.0
AVG	**28**	**0**	**7**	**7**	**113**	**290**	**158**	**14**	**0**	**18**	**62**	**21**	66.7	25.0
A1	**21**	**28**	**134**	**191**	**431**	**346**	**44**	**21**	**18**	**18**	**26**	**42**	100.0	33.3
A2	**0**	**21**	**28**	**35**	**57**	**64**	**26**	**7**	**9**	**26**	**35**	**7**	66.7	0.0
A3	**42**	**21**	**49**	**7**	**106**	**290**	**18**	**64**	**88**	**9**	**26**	**21**	83.3	16.7
A4	**7**	**7**	**35**	**7**	**49**	**99**	**9**	**21**	**53**	**53**	**9**	**35**	58.3	0.0
A5	**21**	**0**	**7**	**28**	**134**	**177**	**0**	**28**	**53**	**0**	**18**	**28**	66.7	16.7
A6	**14**	**0**	**28**	**0**	**276**	**332**	**35**	**21**	**35**	**26**	**0**	**0**	66.7	16.7
A7	**156**	**7**	**7**	**7**	**7**	**375**	**0**	**42**	**0**	**35**	**53**	**0**	41.7	16.7

Legend: air pollution levels with actinomycetes according to Polish Standard PN-89/Z-04111/02

< 10	Unpolluted air
10–100	Average air pollution
> 100	Heavy air pollution

The lowest average numbers of actinomycetes were recorded in places characterized by little urban green space (*eg* Main Square or crossroads). On the other hand, the highest numbers were found in green areas (*eg* Blonia Park meadows, green area by the Krakow University of Technology) (Table 2).

Regarding the classification of air quality based on the Polish Standard PN-89/Z-04111/02 (Table 3) the number of actinomycetes exceeded the admissible values in 144 of 192 conducted measurements, which constitutes 75 %, of samples which were considered to be polluted, including 16 % of the measurements, when the air was heavily polluted.

Table 4

Average number of actinomycetes in different seasons of the year [CFU · m^{-3}]

Research site	Spring	Summer	Autumn	Winter
C	9	16	13	3
B1	4	33	7	4
B2	9	14	7	4
B3	7	23	10	4
B4	3	54	5	5
B5	7	20	2	2
B6	2	27	11	2
B7	5	48	0	5
AVG	2	23	10	6
A1	10	54	5	5
A2	3	9	2	4
A3	6	22	9	3
A4	3	9	5	5
A5	2	19	5	3
A6	2	34	5	1
A7	9	22	2	5
Average for research sites B1–B7+A1–A7+AVG	5	27	6	4

Table 5

Percentage of fractions, including the respirable fraction of actinomycetes

Fraction [μm]	The sum of microorganisms from all research sites and year-long measurements [CFU · m^{-3}]	% share of fraction
0.65–1.1	561	4.7
1.1–2.1	1055	8.8
2.1–3.3	2302	19.2
3.3–4.7	3360	28.0
4.7–7.0	3116	26.0
> 7.0	1608	13.4
Share of the respirable fraction		60.6

Table 6

The predominant airborne actinomycete species

Actinomycete species	C	B1	B2	B3	B4	B5	B6	B7	AVG	A1	A2	A3	A4	A5	A6	A7	Frequency of the species occurrence
Streptomyces albo-griseus			+		++	+				+++	+					+	**9**
Streptomyces albus	+	+++	+		+	+	++	+	++	++		+++	+		+	+	**20**
Streptomyces anulatus	+			++				+			+		+		+		**7**
Streptomyces badius	++		+		++	++			+	++		+	+	++		+	**15**
Streptomyces collinus		++		+				++			+			++			**8**
Streptomyces fradiae					+	+		++	++				+		+	++	**10**
Streptomyces globisporus	++				+		+	+++		++	+	+		+	++		**14**
Streptomyces lavendulae		++	+	++			+	+				+					**8**
Streptomyces longisporus	++		+	+	++		++	+	+	+					+	+	**13**
Streptomyces mirabilis		++	+				+					+					**5**
Streptomyces roseogriseolus					+				+	+					+	+	**5**
Streptomyces sp.		+			+					+							**3**
Streptomyces violaceus-niger				+	+		+			+					+		**5**
Streptosporangium sp.				+				+									**2**

Legend: C, B1, B2 – research sites, as in Table 1.
Frequency of the occurrence of a species (strain): +++ very frequently, ++ frequently, + rarely.

Considering seasons, the average numbers of actinomycetes (Table 4) at the research sites (B1–B7, AVG and A1–A7) rank in the following order: summer (27 CFU · m^{-3}), autumn (6 CFU · m^{-3}), spring (5 CFU · m^{-3}) and winter (4 CFU · m^{-3}).

Table 5 presents total numbers of actinomycetes measured during the year at all research sites to estimate the shares of different bioaerosol fractions. The fraction with the smallest aerodynamic diameter (0.65–1.1 μm) took the lowest share – 4.7 %, while the fraction with the greatest diameter (3.3–4.7 μm) – 28 %. The share of the respirable fraction of actinomycetes was 60.6 %.

A total of 40 strains were isolated, which were subsequently classified into 2 genera – the vast majority of species belonged to the genus *Streptomyces*. Within the genus *Streptomyces* the isolates were classified into 12 species. The most frequently occurring species were: *Streptomyces albus, St. badius* and *St. globisporus.* Strains of the genus *Streptosporangium* could not be assigned to any of the species (Table 6).

A strong correlation was found between temperature and the abundance of actinomycetes from the fraction 2.1–7.0 μm. On the other hand, there was a negative correlation between air humidity and all actinomycete fractions above 1.1 μm. A statistically significant correlation was observed between dust fractions PM2.5 and PM1 and bioaerosol forming actinomycete fractions from 2.1 to 7.0 μm (Table 7).

Table 7

Correlations between the number of actinomycetes and microclimatic factors and dust concentration

Factors	Actinomycetes – fractions					
	>7.0	4.7–7	3.3–4.7	2.1–3.3	1.1–2.1	0.65–1.1
Air temperature	**0.22**	**0.49**	**0.46**	**0.45**	0.35	**0.11**
Relative humidity	**–0.18**	**–0.43**	**–0.44**	**–0.43**	**–0.32**	–0.01
Wind energy	–0.06	–0.09	–0.11	–0.07	–0.05	**0.04**
Dust – fraction PM10	–0.05	0.09	**0.15**	0.07	–0.03	–0.11
Dust – fraction PM4	–0.10	0.07	0.12	0.06	0.00	–0.09
Dust – fraction PM2.5	–0.07	**0.18**	**0.24**	**0.15**	0.02	**–0.14**
Dust – fraction PM1	–0.08	**0.14**	**0.18**	**0.17**	–0.02	–0.13

Legend: bolded – results significant at $p < 0.01$.

Conclusions

Based on the year-long study, conducted from March 2010 to February 2011 at 15 research sites in Krakow and one control site outside Krakow, the following conclusions may be drawn:

1. Based on the normative classification of air quality (Polish Standard PN-89/Z-04111/02) the number of actinomycetes showed that the air was polluted in 75 % of measurements, 16 % measurements, when heavy pollution of the air was detected.

2. Particles of the respirable fraction – with the aerodynamic diameter in the range 3.3–4.7 μm represented the most numerous group, the share of fraction 2.1–3.3 μm was

less numerous. The smallest number of microorganisms was detected in the extreme fractions (below 1.1 μm and above 7 μm).

3. The following statistically significant correlations were found:

– positive between temperature and the number of actinomycetes,

– negative between the number of actinomycetes and air humidity,

– positive between actinomycetes from the fractions 2.1–7 μm and dust with the diameter below 2.5 μm (PM2.5 and PM1).

4. Actinomycetes of the genus *Streptomyces* were most frequently observed (*St. albus, St. badius, St. globisporus*).

5. Green urban spaces nearby the Krakow University of Technology and at the Kosciuszko Mount were most heavily contaminated with actinomycete spores.

6. Significant differences in microbial numbers were recorded between research sites with low and high average air temperature. There was a negative correlation between actinomycete numbers and the average air temperature – in "cold" locations (B) the numbers of these microorganisms was from 13 to 15 % higher than in other sites.

References

[1] Marcinowska K. Charakterystyka, występowanie i znaczenie promieniowców w przyrodzie. [In:] Aktywność drobnoustrojów w różnych środowiskach. Kraków: AR Kraków; 2002:121-129.

[2] Reponen TA, Gazenko SV, Grinshpun SA, Willeke K, Cole EC. Characteristics of airborne actinomycetes spores. Appl Environ Micr. 1998;64(10):3807-3815.

[3] Polymenakou PN. Atmosphere: a source of pathogenic or beneficial microbes? Atmosphere, 2012;3:87-102, DOI: 10.3390/atmos3010087.

[4] Kagen SL, Fink JN, Schlueter DP, Kurup VP, Fruchtman RB. Streptomyces albus: A new cause of hypersensitivity pneumonitis. J Allergy Clin Immunol. 1981;68:295-299.

[5] Hirvonen M-R, Nevalainen A, Makkonen M, Mönkkönen J, Savolainen K. Streptomyces spores from mouldy houses induce nitricoxide, TNFx and IL-6 secretion from RAW264.7 macrophage cell line without causing subsequent cell death. Environ Toxicol Pharmacol. 1997;3:57-63.

[6] Boreson J, Dillner AM, Peccia J. Correlating bioaerosol load with PM2.5 and PM10cf concentrations: a comparison between natural desert and urban-fringe aerosols. Atmos Environ. 2004;38:6029-6041.

[7] Pająk B, Czarnecka L, Dębska B. Ocena jakości powietrza w województwie małopolskim w 2004 roku. Kraków: WIOŚ; 2005.

[8] Pastuszka JS. Narażenie na aerozole ziarniste, włókniste i biologiczne (bakterie i grzyby mikroskopijne) populacji generalnej Górnośląskiego Okręgu Przemysłowego. Prace Naukowe Inst. Inż Ochrony Środow Polit Wrocł. Seria: Monografie. Wrocław: Ofic Wyd Polit Wrocł; 2001.

[9] Górny RL. Cząstki grzybów i bakterii jako składniki aerozolu pomieszczeń, właściwości, mechanizmy emisji, detekcja. Sosnowiec: Wyd. Inst Med Pracy i Zdrow Środow; 2004.

[10] Polska Norma PN-89/Z-04111/02. Ochrona czystości powietrza. Badania mikrobiologiczne. Oznaczanie liczby bakterii w powietrzu atmosferycznym (imisja) przy pobieraniu próbek metodą aspiracyjną i sedymentacyjną.

PROMIENIOWCE TWORZĄCE BIOAEROZOL W WYBRANYCH MIEJSCACH KRAKOWA

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Abstrakt: Kraków jest miastem, w którym głównie na skutek tzw. niskiej emisji oraz wzmożonego ruchu kołowego zapylenie powietrza wciąż wzrasta i nierzadko szczególnie w okresie zimowym są przekraczane progowe wartości zapylenia. Zapylenie powietrza wpływa na zwiększenie się ilości drobnoustrojów w powietrzu, gdyż te ostatnie wiążą się z cząstkami pyłów, tworząc bioaerozol. Jednym z ważnych składników bioaerozolu są promieniowce. Ich obecność w bioaerozolu jest groźna dla zdrowia ludzkiego, gdyż nawet ich bardzo niskie stężenia mogą działać alergizująco.

Celem niniejszych badań była weryfikacja zależności pomiędzy stężeniem promieniowców w powietrzu na obszarze Krakowa, przestrzennym zróżnicowaniem stosunków termicznych oraz stopniem zapylenia powietrza.

Do badań użyto impaktora kaskadowego w celu uzyskania rozdziału cząstek bioaerozolu, w tym frakcji respirabilnej. Jednocześnie wykonywane były pomiary stopnia zapylenia z podziałem na 4 frakcje. Badania były wykonywane przez 12 miesięcy (pełen rok kalendarzowy), raz w miesiącu, w ciągu jednego dnia, na 16 stanowiskach pomiarowych.

Najniższe średnie liczebności promieniowców notowano w miejscach, gdzie jest mało zieleni (np. Rynek Główny, czy też skrzyżowanie ulic). Najwyższe liczebności stwierdzono na terenach zielonych (np. Błonia, teren zielony przy Politechnice Krakowskiej).

Promieniowce zgodnie z klasyfikacją jakości powietrza atmosferycznego (Polska Norma PN-89/Z-04111/02) powodowały, że w przypadku 75 % wykonanych pomiarów powietrze było zanieczyszczone, w tym silnie zanieczyszczone dla 16 % pomiarów.

Najczęściej stwierdzano występowanie promieniowców z rodzaju *Streptomyces* (*St. albus, St. badius, St. globisporus*).

Słowa kluczowe: promieniowce, bioaerozol, zapylenie, Kraków