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BIOAEROSOLS OF THE MUNICIPAL WASTE LANDFILL SITE AS A SOURCE OF MICROBIOLOGICAL AIR POLLUTION AND HEALTH HAZARD

BIOAEROZOLE SKŁADOWISKA ODPADÓW KOMUNALNYCH JAKO ŹRÓDŁO MIKROBIOLOGICZNEGO ZANIECZYSZCZENIA POWIETRZA I ZAGROŻENIA ZDROWOTNEGO

Abstract: The aim of this study was to determine the composition of bacterial and fungal aerosol on the premises of the Municipal Waste Disposal Complex at Zolwin-Wypaleniska near Bydgoszcz and to evaluate the degree of microbiological air pollution at the appointed stands. Microbial concentration in the air was determined at the waste sorting station and in the active sector of the landfill site as well as in the point situated 200 m beyond the facility. It was found that the total number of bacteria in the air ranged from 10^2 to 10^4 cfu · m^{-3} and many times strong air pollution occurred on the premises of that facility. High concentration level of actinomycetes and indicator bacteria *Pseudomonas flourescens* contributed also to strong or moderate air pollution at research stands 1 and 2. Potentially pathogenic bacteria of the family *Enterobacteriaceae* (such as bacilli of *Salmonella* sp. and *Escherichia coli*) as well as faecal streptococci occurred in the largest amounts also at stands 1 and 2, and their number reached up to 10^3 cfu · m^{-3}. The obtained values show that both the sorting station the active sector of the waste landfill are serious emission sources of dangerous bacterial aerosol. They can pose a health hazard to the workers staying at those workstands. The determined amounts of fungal aerosol prove that it did not pose a microbiological hazard, and the studied air was described as moderately clean throughout the research period, only in the area of the sorting station it was polluted at several times. Among the isolated fungi, species of the genera *Aspergillus* and *Penicillium* predominated, as well as *Sclerotinia sclerotorum*. However, also potentially pathogenic species were detected in the studied air, such as: *Aspergillus fumigatus, Cladosporium herbarium*, *Alternaria alternata* etc. Their presence may affect the health of people (workers of the facility and residents of neighbouring areas) and the risk of the environmental pollution, since those species are well-known for their production of mycotoxins and inducing allergic reactions. Based on the registered concentrations of bacteria and fungi at the point 200 m away from the facility, it should be assumed that the studied air was not polluted and bioaerosol transmission from the sources of emission to the surrounding areas did not occur.

Keywords: airborne bacteria, airborne fungi, actinomycetes, bioaerosol, municipal waste landfill site

Working municipal waste landfill sites are assumed to be proecological facilities, but through emission of both chemical and biological pollutions they may have a negative

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effect on the state of the environment. Municipal facilities may affect surrounding soils, surface and ground waters, pollute the atmosphere and through the atmosphere, distant agricultural, urban and recreational areas [1–4]. Main agents polluting the air are chemical substances (odorogenic) and microorganisms (bioaerosols) [5, 6]. Emission of gases, dusts and bioaerosol at landfill sites occurs during waste transport, unloading and processing, at sorting stations and composting plants, which are very often situated on the premises of the facility [7, 8]. The basic difference between biological factors and other dangerous substances consists in the ability of the former to reproduce. Under favourable conditions, even a small number of microorganisms can considerably increase in a short time. Biological factors pose a very serious health hazard to people employed in public utilities. They constitute a crucial and still more appreciated problem of both occupational health care and the public health. It is estimated that at least several hundred million people in the world are exposed to the action of those agents. The majority of biological particles do not pose a health threat under the natural environmental conditions, but a part of them show pathogenic, allergenic or toxic properties. Biological factors highly differ in respect of a degree of infection risk. They include bacteria, fungi, viruses, protozoans and compounds excreted by micro-organisms, such as: endotoxins, exotoxins, glucans, metabolites of fungi, mycotoxins, allergens etc [9–11].

In respect of the species composition of microorganisms contained in biological aerosol, we divide it into: saprophytic, infections and mixed, while none of them remains neutral for the environment. Live cells of biological aerosol often induce pathogenic infections, sometimes very serious, but also dead cells can provoke unfavourable health problems [12].

Both the knowledge of the bacterial and fungal composition, and also the level of concentration of bioaerosol particles in atmospheric air are essential for human health. Moreover, the fractional distribution of bioaerosol is a crucial element, since infections are caused by both a relatively high dose of large particles and a considerably smaller dose of small, submicronic particles. The respirable fractions, with the particle size up to 5 μm, is of particular importance in the epidemiology of infectious diseases, due to the easiness of spread and deep infiltrating the respiratory system, up to pulmonary alveoli [9, 13]. Contaminated air can be a cause of many illnesses. Depending on the composition of bioaerosol, it may cause occurring simple irritations and ailments, allergic reactions, infections, as well as dangerous infectious diseases and toxic reactions. The highest threat is posed by the components of bioaerosol transmitted by means of air – dust or air – droplet routes, which penetrate the organism through the skin, mucous membranes and food can also be the route of transmission [10].

Concentration of microorganisms in the environment of municipal waste landfill sites includes within a very wide range of values. Municipal wastes, and particularly their wet fraction, may contain bacteria, viruses, cysts of protozoans and eggs of worms, whose numbers can reach up to 10^9 per gram of wastes [14]. In the course of emptying cars transporting wastes, as well as their sweeping, compressing, closing sections, and sorting, bacteria and fungi spores are raised in the air. Gram-positive bacteria predominating among the determined bioaerosol belong to the genera: *Micrococcus*,

Enterococcus, S*taphylococcus*, *Bacillus*, *Mycobacterium*, whereas from Gram-negative bacteria: *Pseudomonas*, *Escherichia*, *Enterobacter* etc. [10, 14]. In the case of Gram-negative bacteria, produced enterotoxins and endotoxins occurring in their cell wall can cause various diseases (respiratory tract inflammation, toxic and allergic pneumonia). The presence of endotoxins in the air was observed at various workstations in the public utility facilities [9, 15]. For this reason, municipal waste landfill sites may be a potential source of many bacterial, viral or mycotic diseases for different organisms, the habitat of insects and rodents transmitting germs. Additionally, biological aerosol deriving from those facilities may contribute to pollution of nearby areas, as well as ground and surface waters [1, 16].

The aim of this study was to determine the quantitative and qualitative composition of the bioaerosol occurring on the premises and in the vicinity of the municipal waste landfill site. On the basis of the numbers of microorganisms, the level of micro-biological pollution of atmospheric air was determined, which allowed the assessment of the level of health hazard at the appointed stands.

Material and methods

Location of sampling stands

The study of the bioaerosol composition was carried out at the Municipal Waste Disposal Complex Sp. z o.o. The facility is located at Zolwin-Wypaleniska, 4 km south from Bydgoszczy (Fig. 1). The Complex takes about 150 000 Mg (ton) of wastes yearly

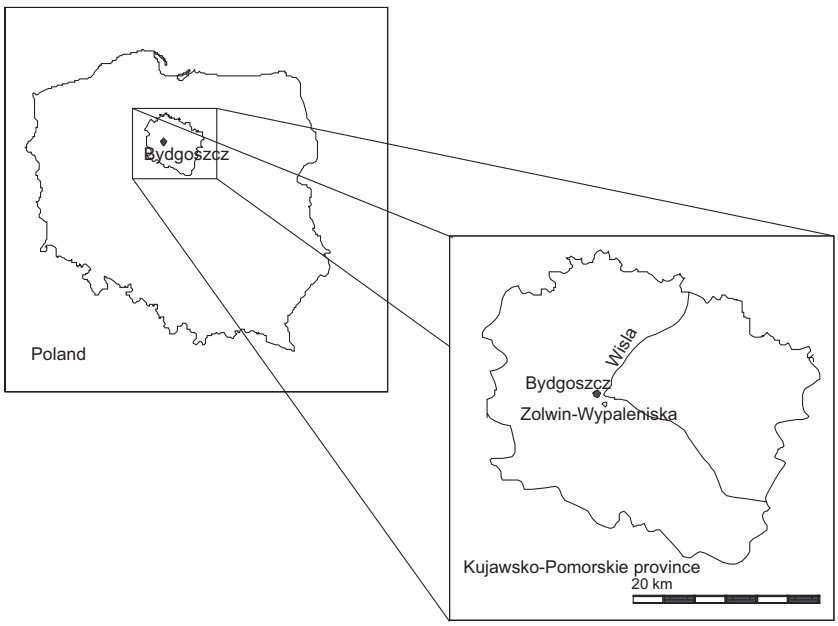

Fig. 1. Location of the studied area

from the city of Bydgoszcz, Solec Kujawski, communes Biale Blota and Nowa Wies Wielka. The wastes are partly neutralized and sorted. The Complex consists of three landfill sites, including one which is reclaimed (working in 1985–2003) and two working presently. The waste sorting station takes *ca* 50 thousand Mg (ton) of wastes. A total of 180 workers are employed in the facility [17].

Air samples were collected at three points, *ie*: in the area of the sorting plant (point 1), in the active sector of the waste landfill site (point 2) and at the point about 200 m to the east from the facility, in the vicinity of a housing estate (point 3). Analyses of the microbiological air pollution were made in the period from April to December 2008, taking into consideration the meteorological conditions prevailing in different times of the year.

Methods of the study

The air samples were taken with the impaction method using Microbiological Air Sampler MAS-100 Eco by Merck. Air samples were taken from the height of 1.5 m above the ground level. Through the head of the apparatus a strictly determined air volume was sucked on a Petri dish with agar medium, according to a season of the year and atmospheric conditions. The following groups of microorganisms were determined during the study, using the appropriate selective media:

– total number of bacteria on Standard nutrient agar (incubation 37 °C, 48–72 h),
– actinomycetes on the Pochon medium (28 °C, 7 days),
– bacteria *Pseudomonas fluorescens* on King B medium (26 °C, 48 h),
– mould and yeast-like fungi on wort agar (28 °C, 48–96 h),
– bacteria of the genus *Salmonella* on BPL agar media with brilliant green and phenol red acc. to Kauffmann (37 °C, 24 h),
– *Escherichia coli* and other bacteria of the family *Enterobacteriaceae* on ENDO agar with fuchsin and lactose (37 °C, 24 h),
– bacteria of the genus *Enterococcus* on agar with kanamycine, esculine and azide (37 °C, 24 h).

All the air measurements for the studied groups of microorganisms were made in four replications. The qualitative diagnostics of bacteria was conducted based on additional physiological and biochemical analyses. In the case of *E. coli* tests from the biochemical series IMViC were used, for *Salmonella* sp. A serological test with the polyvalent serum Hm. The final identification of faecal streptococci type – D was made using the serological Phadebact-test. Identification and determining the species composition of fungi were made based on mycological guidebook according to Gilman [18].

Concentration of microorganisms detected in the studied air was expressed as the number of colony-making units – cfu, per 1 m^3 of atmospheric air (cfu $\cdot$ m^{-3}). The results were worked out using a conversion table according to Feller for the air monitoring system MAS-100. The evaluation of the atmospheric air pollution level was performed according to the recommendation given in the Polish Standards: PN-89/Z-04111/02 and PN-89/Z-04111/03 [19, 20].

Results and discussion

The results of the study concerning the number of microorganisms and the degree of the atmospheric air pollution were presented in Tables 1–5.

Based on the obtained results, it was stated that microbiological air pollution occurred most frequently at the waste sorting station. The total number of bacteria at that stand in the period from April to November exceeded the values for strongly polluted air. Only in December their number fell, and the studied air was considered as moderately polluted. However, the highest value for the total number of bacteria was obtained at the place of waste disposal (54200 cfu · m^{-3}). At that point strong air pollution with total number of bacteria and with *P. fluorescens* remained in spring and summer months, whereas substantially fewer of them were isolated in autumn (Tables 1, 5).

The concentration of actinomycetes also remained on a very high level at the sorting station and strong air pollution occurred at as many as six times, and moderate pollution at the others. The maximal value of actinomycetes there amounted to 1984 cfu · m^{-3}. The air in the active sector of the landfill was strongly or moderately polluted with actinomycetes in all the research period. Indicator bacteria *Pseudomonas fluorescens* most frequently polluted the air at stand 1. The highest value, which amounted to 670 cfu · m^{-3}, was recorded in August at stand 2. Definitely fewer bacteria and actinomycetes was found at the point 200 m away from the facility. In the case of *P. fluorescens* a maximum 18 cfu was detected, whereas there were 84 cfu actinomycetes, and the total number of bacteria did not exceed 1095 cfu · m^{-3} (Table 1, 5). The obtained results for bacteria and actinomycetes indicate that they were not transmitted by air from the stands at which their very high concentration was observed to the area beyond the waste landfill limits.

Table 1

Concentration of selected groups of microorganisms in atmospheric air on the premises of Municipal Waste Disposal Complex and the surrounding area

Stand measurement	Term research [month]								
	IV	V	VI	VII	VIII	IX	X	XI	XII
	Total bacteria [cfu · m^{-3}]								
1*	15200	19373	12880	10673	24360	8775	10845	5028	1780
2	20287	54200	3340	6100	15200	1470	100	78	81
3	300	300	1095	660	138	135	330	330	133
Pseudomonas fluorescens [cfu · m^{-3}]									
1	62	180	430	174	110	73	105	25	34
2	204	87	70	134	670	15	10	2	10
3	7	10	12	18	0	17	2	0	2
Actinomycetes [cfu · m^{-3}]									
1	770	1633	1984	330	25	82	253	35	560
2	312	60	140	210	485	11	10	27	27
3	18	84	53	40	5	64	80	10	4

* Stand 1 – the waste sorting station, 2 – the active sector of the landfill site, 3 – 200m beyond the facility.

Each waste landfill site, even properly designed and operated, constitutes a source of pollution of the surrounding environment [20, 21]. The results of studies by local and foreign authors show that the atmospheric environment in the area of waste disposal is characterized by the occurrence of high concentrations of bacterial aerosol [6, 7, 23–25].

The highest concentration of bioaerosols in the area of the monitored the District Complex of Municipal Waste Disposal was recorded at the place where wastes were sorted and at the place of their deposition. Intensive car traffic, constant moving of vast heaps of impurities, increase the pollution of atmospheric air with microorganisms. Kocwa-Haluch et al [26] reported that irrespective of the time of the year, the total number of bacteria and fungi reaches the maximal values over the active landfill. Also the study carried out by Butarewic and Kowaluk-Krupa [27] at the municipal waste landfill site at Augustow indicated that the facility, in spite of properly managed operation, was a source of microorganism emission, both saprophytic ones and pathogenic towards the surrounding environment.

The high number of bacteria of the family *Enterobacteriaceae* and of faecal streptococci in the studied air turned out a potential health hazard to the workers of the monitored waste landfill site (Tables 2 and 3).

Table 2

The number of bacteria of the family *Enterobacteriaceae* in atmospheric air on the premises of Municipal Waste Disposal Complex and the surrounding area

Stand measurement	Term research [month]								
	IV	V	VI	VII	VIII	IX	X	XI	XII
	Escherichia coli [cfu · m $^{-3}$]								
1*	4	70	0	25	8	80	119	19	69
2	130	20	20	65	100	25	33	2	5
3	6	0	4	4	0	0	0	0	0
Salmonella sp. [cfu · m $^{-3}$]									
1	190	23	285	50	48	113	45	71	36
2	180	33	0	1	160	120	26	0	5
3	8	0	4	4	0	6	0	2	0
Other bacteria of the family *Enterobacteriaceae* [cfu · m^{-3}]									
1	18	1900	67	36	82	75	116	56	53
2	1740	30	54	78	155	22	45	0	8
3	10	20	12	3	0	6	0	0	2

* For a description, see Table 1.

Strong air pollution at the sorting station remained throughout the research period, whereas at the landfill in some months they were not found or there were substantially less of them than at stand 1. The highest value of *Enterococcus* sp. was obtained in May at the cap of the landfill, where 2800 cfu · m^{-3} was recorded. The number of bacteria of

the family *Enterobacteriaceae* also stayed on a high level, in the case of *Escherichia coli* the maximal value amounted to 130, and in *Salmonella* sp. – 285 cfu · m^{-3}. High concentrations of faecal streptococci and bacteria of the family *Enterobacteriaceae* can affect deterioration in the sanitary state of the air and may pose a potential environmental hazard. However, it must be stressed that their number in the point appointed 200 m beyond the facility definitely decreased and did not exceed 20 cfu · m^{-3}. Based on those results, it may be concluded that a considerable emission of pathogenic bacteria from the source of their generating did not occur.

Table 3

The number of fecal streptococci in atmospheric air on the premises of Municipal Waste Disposal Complex and in surrounding area

Stand measurement	Term research [month]								
	IV	V	VI	VII	VIII	IX	X	XI	XII
1*	250	800	840	640	1250	1105	330	648	145
2	340	2800	12	20	160	245	115	2	2
3	2	0	0	6	0	4	0	0	4

* For a description, see Table 1.

Many authors [3, 14, 23, 28] indicate that the number of microorganisms getting to the air considerably decreases along with a distance from the emission source. This refers both to the total number of microorganisms and of bacteria not typical of the air, deriving from wastes. Both for bacteria of the family *Enterobacteriaceae* and faecal streptococci there are no threshold values determining the permissible content of those bacteria in the air. However, even their low concentration in the air may pose a potential hazard to the natural environment, as well as people and animal health.

Microorganisms making their way from the stored wastes into the air are subjected to numerous factors unfavourable to their development. Part of them die during several seconds, mainly due to drying, high temperatures, as well as because of the exceeding solar radiation. Only those microorganisms which are the most resistant and to the highest degree adopted to the unfavourable living conditions, retain their vitality long in the air [21, 29].

Fungi constitute an important fraction of bioaerosol. The vast majority of hyphal fungi and considerably less of yeast-like fungi were detected in the studied air. The obtained results show that also at the sorting station the air was most polluted with fungal bioaerosol, and the number of spores reached up to 53433 cfu · m^{-3}, whereas beyond the facility their amount definitely decreased and did not exceed the permissible level for the unpolluted air (Tables 4, 5). The determined concentration of fungi at the cap of the landfill indicated the moderately clean air, and the obtained values did not exceed 4000 cfu · m^{-3}. The species composition of air borne fungi is of great importance. The genera predominated among determined fungi included *Aspergillus* (*A. niger*), *Penicillium* (*P. notatum*), *Rhizopus* (*R. nigricans, R. oryzae*) as well as *Sclerotinia* (*S. sclrotorium*), whereas in smaller amounts were isolated *Epicoccum nigrum, Arthrinium phaespermum.* Moreover, potentially pathogenic species were identified,

such as: *Aspergillus fumigatus Cladosporium herbarium*, *Alternaria alternata* Their presence in the air at the studied stands both at the facility and beyond the landfill can pose a health hazard to the workers and the residents of the surrounding areas, since those fungi excrete mycotoxins and induce allergic reactions.

Table 4

Concentration of moulds and yeast-like fungi in atmospheric air on the premises of Municipal Waste Disposal Complex and in surrounding area

Stand measurement	Fungi [cfu · m^{-3}]	Term research [month]								
		IV	V	VI	VII	VIII	IX	X	XI	XII
1*	moulds	1924	3848	9175	3600	5900	31310	53433	8901	15460
	yeast-like	0	0	50	20	1400	0	0	0	0
2	moulds	427	854	690	600	3800	750	2070	65	879
	yeast-like	287	0	50	40	50	0	0	0	0
3	moulds	505	1010	360	4605	600	295	70	81	48
	yeast-like	40	0	10	50	0	0	0	0	0

* For a description, see Table 1.

Table 5

Degree of microbial air contamination at research stands on the premises and in the vicinity of Municipal Waste Disposal Complex from April to December 2008, acc. to recommendations of PN [19, 20]

Month	Total bacteria			*Pseudomonas fluorescens*			Actinomycetes			Fungi		
	Stand measurement											
	1	2	3	1	2	3	1	2	3	1	2	3
IV												
V												
VI												
VII												
VIII												
IX												
X												
XI												
XII												

heavily pollution medium pollution not pollution

Fungi are the predominating group in the air and they account for about 70 % of all microorganisms. More than 40000 species of fungi have been isolated so far [30]. Their common occurrence is determined by production of very numerous spores and modest nutritional and environmental demands [31–34]. Environmental pollution and threat connected with the presence of fungi in the air results from the fact that they can cause: allergies, asthma, broncho- and pulmonary mycoses and general infections. For

example, *Aspergillus fumigatus* is responsible for more than 90 % pulmonary mycosis in people. Small sizes of conidia (2–3 μ) allow them to spread throughout the respiratory system [37]; whereas toxinogenic species of the genera *Aspergillus*, *Fusarium*, *Penicillium* etc may induce cytotoxic, neurotoxic, teratogenic and cancerogenic actions towards other organisms [9, 35–38].

Microclimatic conditions have a substantial effect on the number of microorganisms in the atmospheric air, which is confirmed with the studies by other authors [24, 31, 39]. The time of the year, climate and varied meteorological conditions have a decisive effect on the spread of pollutions in the atmosphere and the range of their effect on the natural environment and people [2, 5, 14]. Those relationships were noticeable in the author's study conducted both on the premises and beyond the facility. The numbers of microorganisms in the air, depends first of all on the temperature. The results of microbiological analyses proved that the largest pollution with bacteria occurred in the spring and summer period, whereas with fungi in the autumn months. Atmospheric air has limited abilities to self-cleaning, thus it is necessary to control its microbiological cleanliness, in order to reduce processes of its exceeding pollution.

An increase in the waste mass at landfill sites has a considerable effect on generating pollutions emitted to the atmosphere [40]. Many scientific studies and reports indicate the connection between the concentration of sub- and micronic particles making air pollutions and the state of health and mortality of populations exposed to inhaling this type of air [41–43].

Conclusions

1. The high level of the total numbers of bacteria, *Pseudomonas fluorescens* and actinomycetes was observed in atmospheric air at the determined stands at Municipal Waste Disposal Complex in Zolwin-Wypaleniska near Bydgoszcz, based on which the studied air was considered as strongly polluted in the spring and summer period.

2. Sorting station and the active sector of the landfill site turned out to be also serious emission sources of bacteria of the family *Enterobacteriaceae* (such as *Escherichi coli* and *Salmonella* sp.) as well as faecal streptococci.

3. Determined amounts of fungal aerosol indicates that it did not pose a micro-biological threat and the studied air was described as moderately clean throughout the research period; only on the premises of the sorting station it was polluted at several times. However, among the isolated fungi some potentially pathogenic species were detected, such as: *Aspergillus fumigatus Cladosporium herbarium*, *Alternaria alternata*.

4. High concentration of potentially pathogenic bacteria and the presence of toxinogenic fungi in the air at the waste landfill site, proves a potential a health hazard to persons working as well as temporarily staying at that facility. Therefore, a proper protection should be created for workers exposed to the action of airborne biohazards.

5. Based on the low concentration of bacteria and fungi determined at the point 200 m away from the facility, it should be assumed that the studied air most often was not polluted there, and thus there was no bioaerosol transmission from the sources of emission, that is from the waste landfill and the sorting station, to the nearby areas.

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BIOAEROZOLE SKŁADOWISKA ODPADÓW KOMUNALNYCH JAKO ŹRÓDŁO MIKROBIOLOGICZNEGO ZANIECZYSZCZENIE POWIETRZA I ZAGROŻENIA ZDROWOTNEGO

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Abstrakt: Celem pracy było oznaczenie składu bioaerozolu bakteryjnego i grzybowego na terenie Zakładu Unieszkodliwiania Odpadów Komunalnych w Żółwinie-Wypaleniska koło Bydgoszczy oraz ocena stopnia

mikrobiologicznego zanieczyszczenia powietrza na wyznaczonych stanowiskach. Koncentrację mikroorganizmów w powietrzu określano na terenie sortowni odpadów i w sektorze czynnym składowiska oraz w punkcie położonym 200 m poza obiektem. Stwierdzono, że liczba bakterii ogółem w powietrzu kształtowała się od 10^2 do 10^4 jtk · m^{-3} i doszło wielokrotnie do silnego zanieczyszczenia powietrza na terenie zakładu. Wysoki poziom koncentracji promieniowców i bakterii wskaźnikowych *Pseudomonas fluorescens* przyczynił się także do silnego lub średniego zanieczyszczenie powietrza na 1 i 2 stanowisku badawczym. Potencjalnie chorobotwórcze bakterie z rodziny *Enterobacteriaceae* (m.in. pałeczki *Salmonella* sp. i *Escherichia coli*) oraz paciorkowce kałowe w największych ilościach występowały również na stanowisku 1 i 2, a ich liczba dochodziła do 10^3 jtk · m^{-3}. Uzyskane wartości wskazują, że zarówno sortownia, jak i sektor czynny składowiska odpadów były poważnymi emitorami niebezpiecznego aerozolu bakteryjnego, zatem stanowić mogą zagrożenie zdrowotne dla pracowników przebywających na tych stanowiskach pracy. Oznaczona ilości aerozolu grzybowego świadczy, że nie stanowił on zagrożenia mikrobiologicznego, a badane powietrze określono jako przeciętnie czyste w całym okresie badawczym. Jedynie na terenie sortowni w kilku terminach doszło do jego skażenia. Wśród wyizolowanych grzybów dominowały gatunki z rodzaju *Aspergillus* i *Penicillium* oraz *Sclerotinia sclerotorum*. Jednakże wykryto w badanym powietrzu również gatunki potencjalnie chorobotwórcze, takie jak: *Aspergillus fumigatus Cladosporium herbarium*, *Alternaria alternata* i in. Ich obecność może wpływać na zdrowie ludzi (pracowników zakładu i mieszkańców przyległych terenów) oraz ryzyko skażenia środowiska, bowiem gatunki te znane są z produkcji mykotoksyn oraz wywoływania reakcji alergicznych. Na podstawie zarejestrowanego stężenia bakterii i grzybów w punkcie oddalonym 200 m od zakładu, należy przyjąć, że badane powietrze było niezanieczyszczone i nie doszło do przenoszenia bioaerozolu ze źródeł emisji na okoliczne tereny.

Słowa kluczowe: aerozol bakteryjny, aerozol grzybowy, promieniowce, bioaerozol, składowisko odpadów komunalnych