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QUALITATIVE ASSESSMENT OF THE BIOAEROSOL IN THE SURROUNDINGS OF MAŚLICE LANDFILL IN WROCŁAW

A microbiological assessment of the ambient air was carried out in the surroundings of a municipal landfill Maślice in Wrocław. The species present in the samples collected by the sedimentation method were identified. Mycological studies revealed in the air the presence of a number of fungal species potentially pathogenic, toxigenic and allergenic, the latter dominating. In the bacteriological studies, there were found potentially pathogenic streptococci, staphylococci and the *Proteus* bacteria. Based on the bacteria identification a zone of impact of the bioaerosol on the surroundings was determined. The presence of microfungi was detected up to a distance of 500 m from the landfill. The range of transport of bacterial cells was 1000 m. An extension of standard microbiological analysis of the ambient air to include some chosen analyses of fungi and bacteria was recommended.

1. INTRODUCTION

Pollutants' emission in the form of biological aerosols is a relatively weakly identified element of hazards generated by landfills. Correct assessment of the noxiousness associated with the imission requires not only a knowledge of the concentration of microorganisms forming the bioaerosol, but also a recognition of its species composition. That is because the same number of microorganisms may have various adverse health effects, depending on the proportion of pathogenic, toxigenic and allergenic species. The determination only of the cell count in a unit of air volume does not reflect the real hazard associated with the bioaerosol investigated. Moreover, qualitative studies may be helpful in determining a zone of impact of the emitter on its surroundings. The border of this zone may be established based on the range of species typical of the source of emission [1]. The assessment of microbiological pollution of the ambient air recommended by the Polish Standards [2]–[4] does not give a suffi-

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cient recognition of its qualitative composition. It concerns particularly fungi which, according to the standard, should be detected only quantitatively. Similarly, the analysis of bacteria, while including the determination of certain groups of these microorganisms, not always allows a true assessment of the sanitary state of the air investigated. In the standard, for example, the detection of streptococci is not taken into account, and the analysis of potentially pathogenic mesophilic bacteria is limited to their total count. Although it is recommended to check whether *Pseudomonas fluorescens* and actinomycetes are present or not in the air, these bacteria allow us only to state if the microorganisms found in the air come from aquatic and soil environments represented by *P. fluorescens* and actinomycetes, respectively. The presence of pathogenic bacteria in the air is indicated only by the count of haemolytic staphylococci in addition to the total count of mesophilic bacteria. This scope of qualitative studies is not sufficient for a reliable assessment of the sanitary state of the ambient air. The basis of that assessment can only be a careful identification of species.

The study was performed on the only municipal solid waste disposal site in Wrocław currently in operation (2000–2005), located in the north-western part of the city, in the Maślice district. The landfill has been exploited since 1966. It has been receiving approx. 240 000 Mg of solid waste per year. The site has the area of 9.1 hectares and the height of approx. 30 m [5]. Microbiological studies of the ambient air in the vicinity of this landfill have been undertaken at intervals since 1988, however they were restricted to standard analysis carried out according to the Polish Standard [6], [7]. The work presented in this paper was the first attempt to identify in detail the species of microorganisms present in the ambient air in the surroundings of the Maślice landfill.

This work was started with the aim of assessing the sanitary state of the ambient air in the vicinity of the municipal landfill Maślice in Wrocław and of determining the range of its impact on the neighbourhood on the basis of the species composition of the bioaerosol.

2. MATERIALS AND METHODS

2.1. MYCOLOGICAL STUDIES

Samples were collected in March 2000 by the sedimentation in trail method according to the Polish Standard [2]–[4]. The samples were collected from 10 locations on the leeward side of the landfill, 100 m apart, and 2 control locations on the windward side. At each location 6 plates were used for the examination of fungi: 3 with Czapek medium and 3 with agar broth. The plates were incubated at 26 °C for 7 days. Fungi were identified on the basis of the morphology of colonies and microscopic examinations of micro-cultures and slides dyed with cotton blue with addition of lactophenol [5].

2.2. BACTERIOLOGICAL STUDIES

Qualitative analysis of bacteria present in the ambient air was carried out on the agar medium with ram blood. The tests were conducted in March 2000. Diagnostic studies of isolated bacteria were done with the ID 32 GN and API 20E tests of Biomerieux using a computer system. Identification of bacteria was carried out in collaboration with the Medical Academy of Wrocław.

3. RESULTS AND DISCUSSION

3.1. QUALITATIVE ANALYSIS OF FUNGI

Fungi identified in the ambient air are presented in figures 1–12. The diagrams show the percentage of each species in the microflora of the ambient air at different distances from the Maślice landfill. At the location No. 1, the closest to the base of the landfill (100 m), the fungi were characterized by the most diversified species composition (figure1). At this point 9 strains were identified, among which 8 was not found on the windward side (control locations No. 11 and 12, figures 11 and 12).

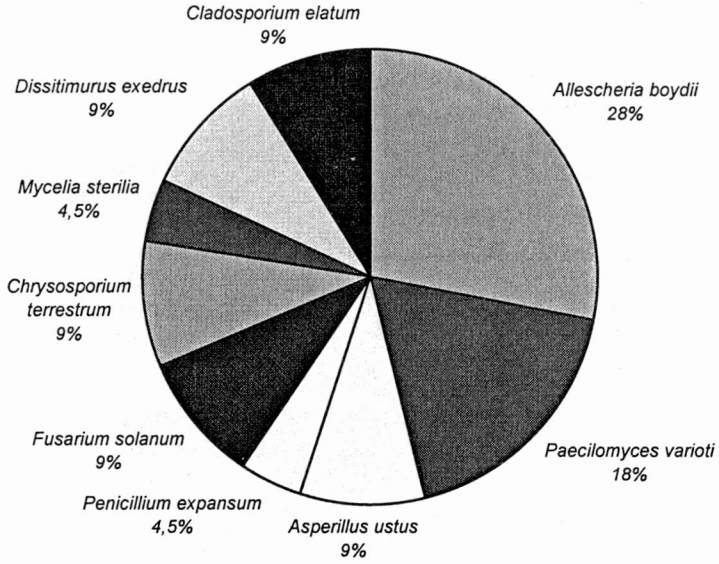


Fig. 1. Percentages of fungal species in the ambient air 100 m from the landfill base

Some of them, e.g. *Paecilomyces varioti* and *Cladosporium elatum* (phot. 1, 2), dominated also in the microflora at locations No. 2 and 3 (figures 2 and 3). From among the fungi found at the location No. 1, the widest range had *Cladosporium elatum* and *Penicillium expansum* found 400 m away from the landfill (figure 4). Beginning from the location

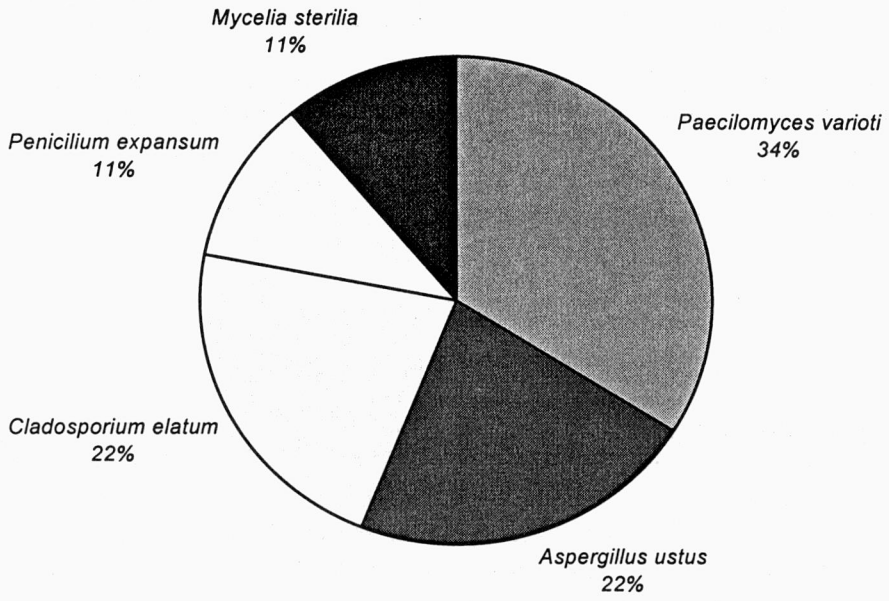


Fig. 2. Percentages of fungal species in the ambient air 200 m from the landfill base

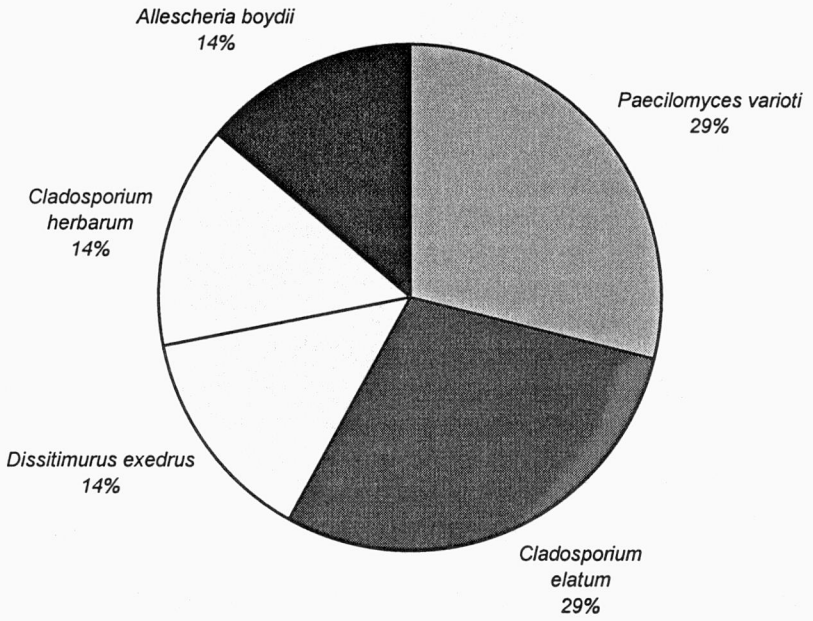


Fig. 3. Percentages of fungal species in the ambient air 300 m from the landfill base

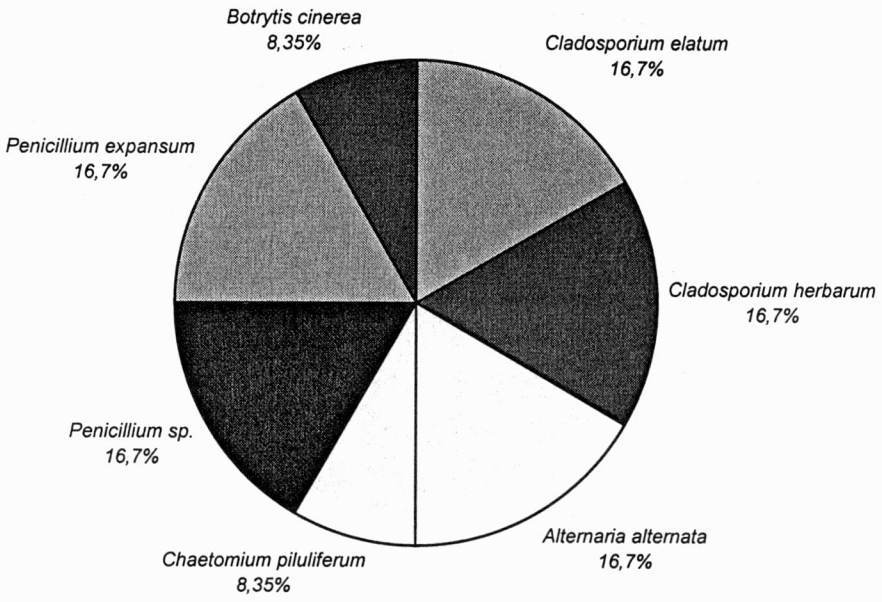


Fig. 4. Percentages of fungal species in the ambient air 400 m from the landfill base

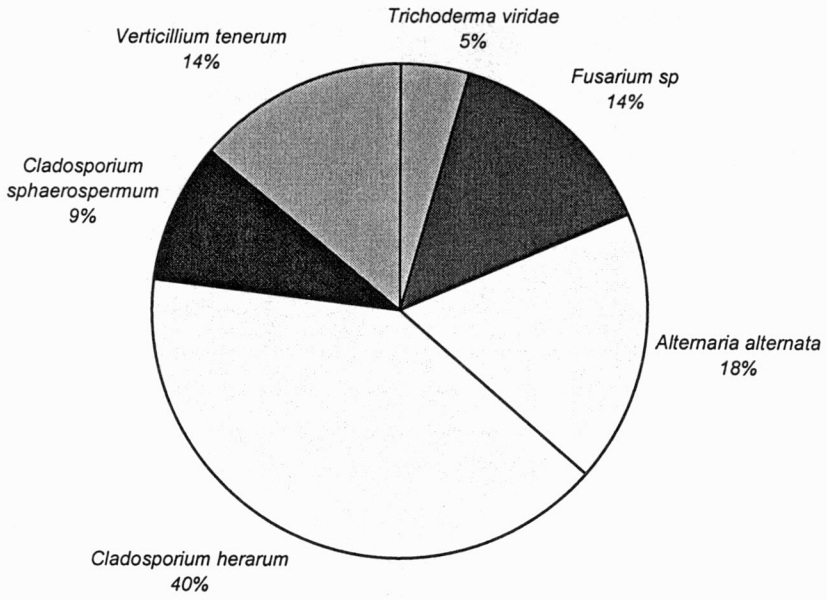


Fig. 5. Percentages of fungal species in the ambient air 500 m from the landfill base

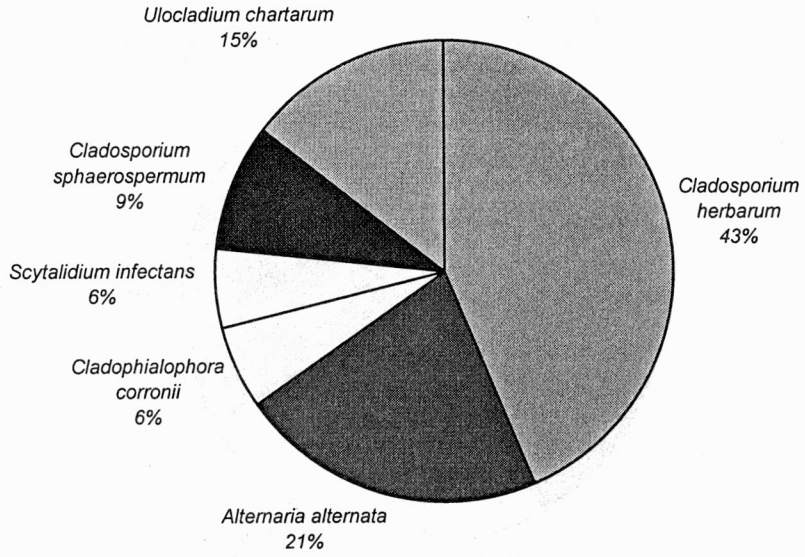


Fig. 6. Percentages of fungal species in the ambient air 600 m from the landfill base

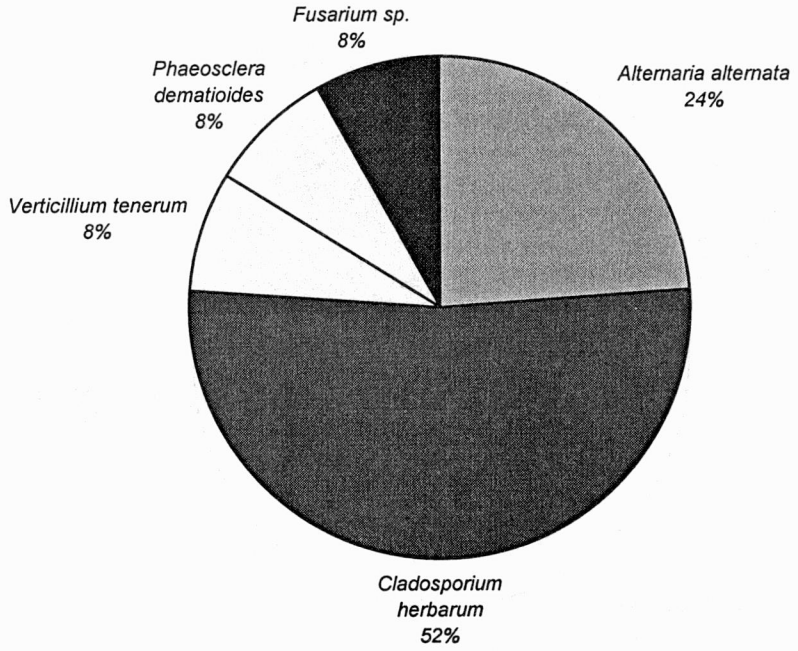


Fig. 7. Percentages of fungal species in the ambient air 700 m from the landfill base

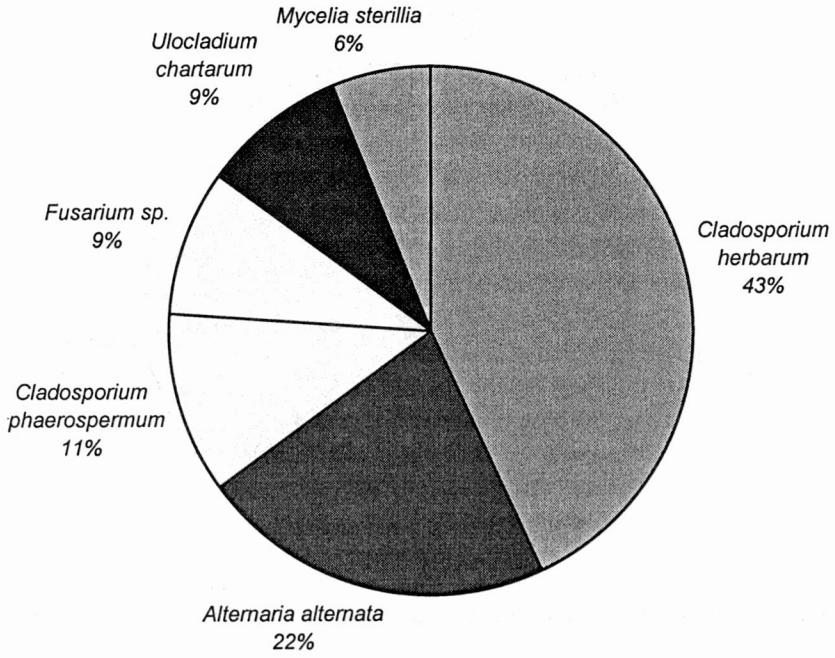


Fig. 8. Percentages of fungal species in the ambient air 800 m from the landfill base

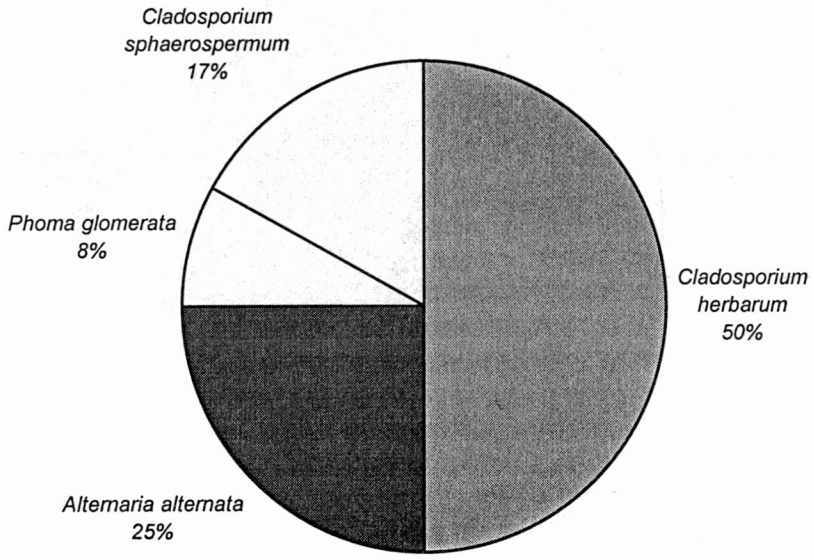


Fig. 9. Percentages of fungal species in the ambient air 900 m from the landfill base

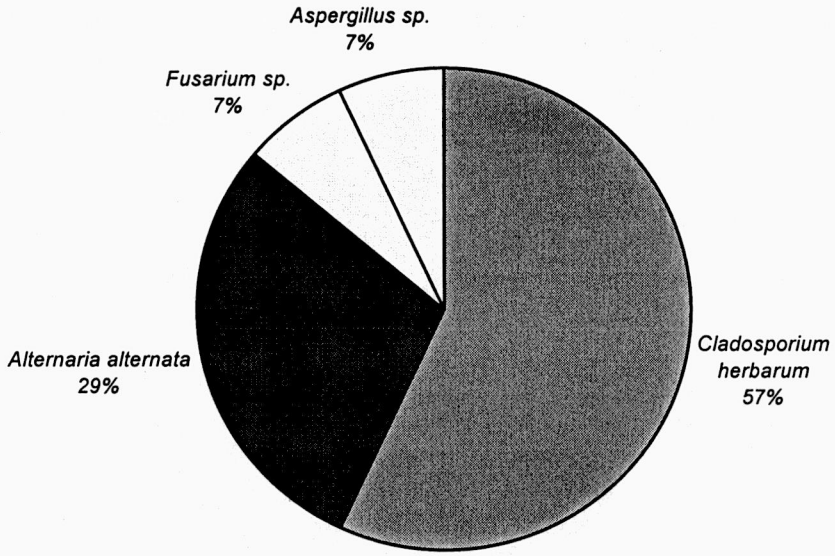


Fig. 10. Percentages of fungal species in the ambient air 1000 m from the landfill base

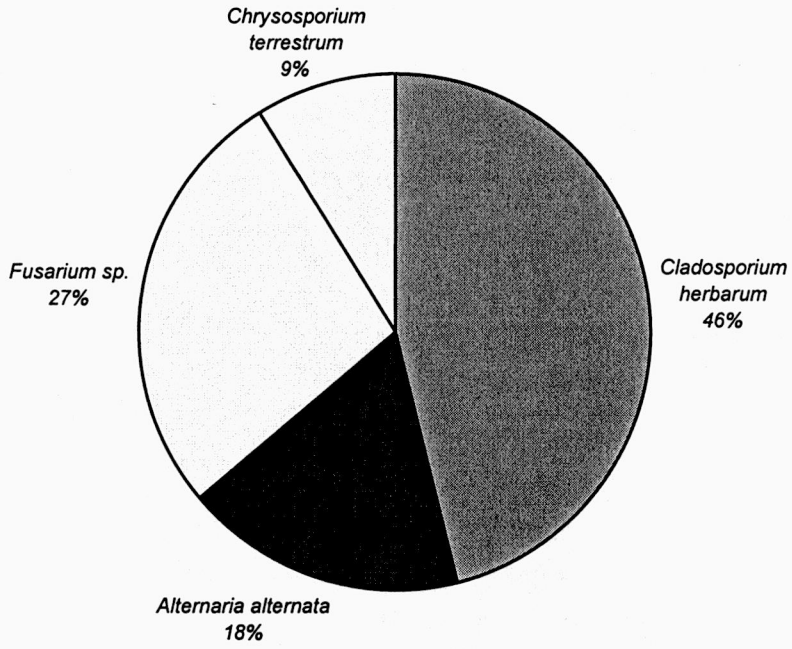


Fig. 11. Percentages of fungal species in the ambient air 100 m from the landfill base on the windward side (control 1)

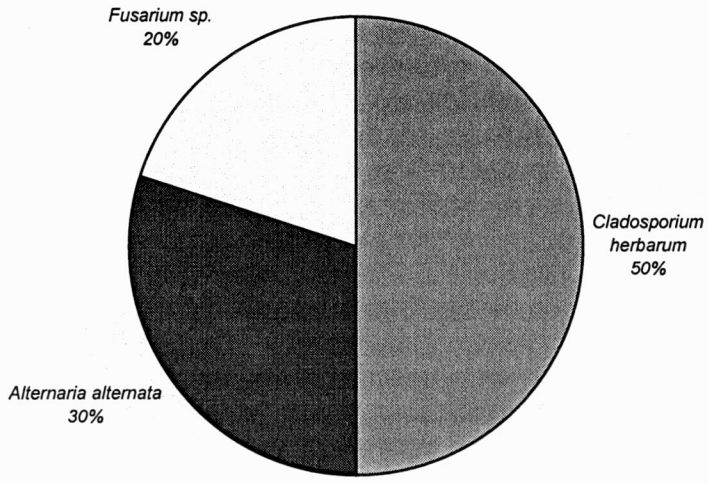
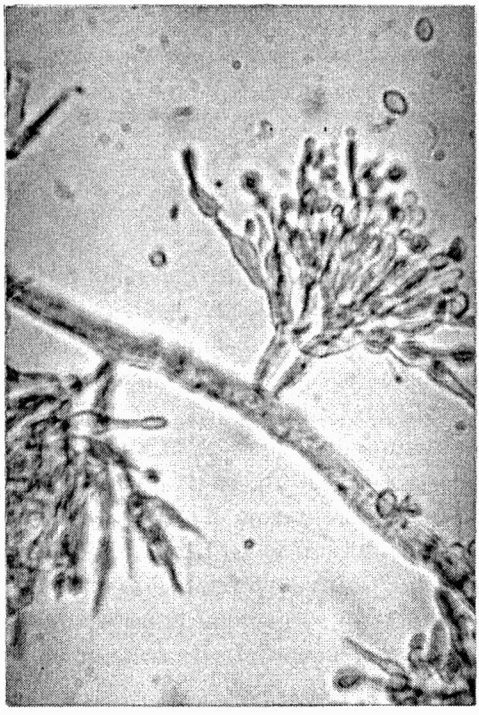
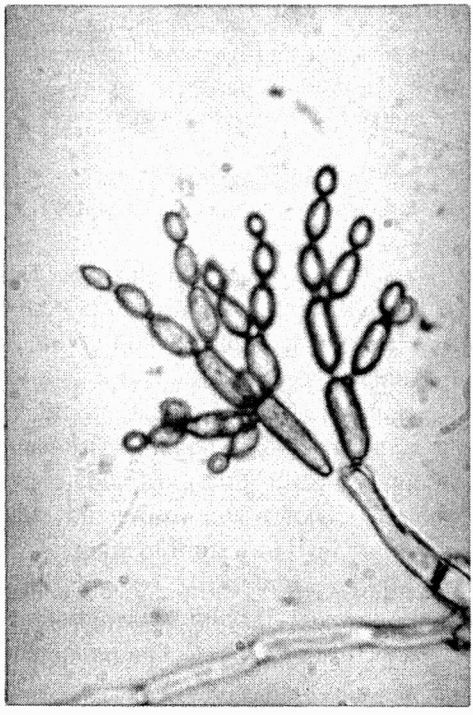


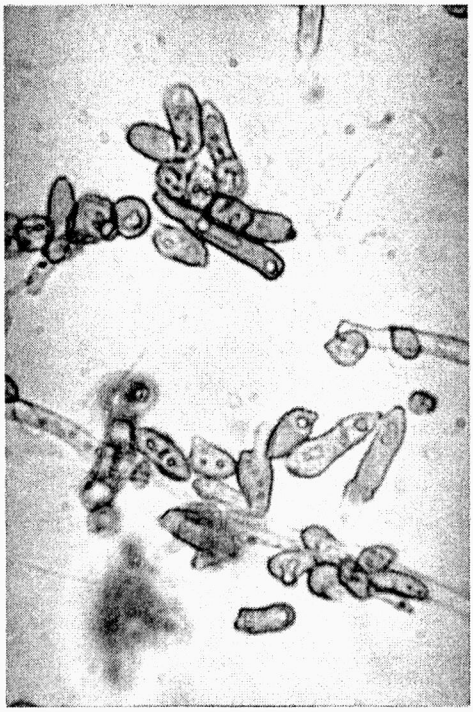
Fig. 12. Percentages of fungal species in the ambient air 200 m from the landfill base on the windward side (control 2)



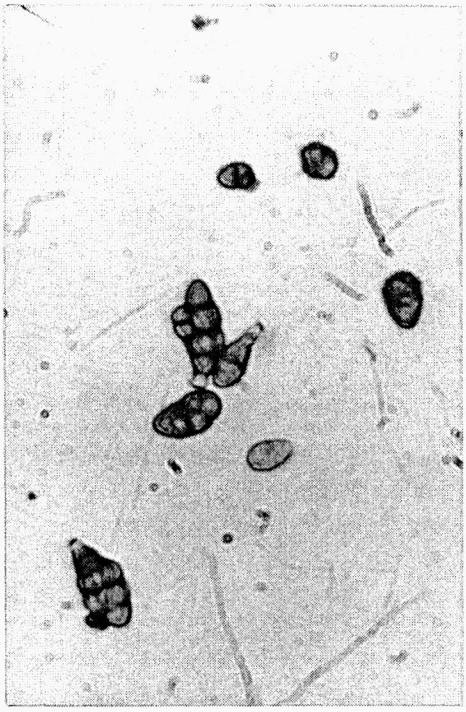
Phot. 1. *Paecilomyces varioti*



Phot. 2. *Cladosporium elatum*



Phot. 3. *Cladosporium herbarum*



Phot. 4. *Alternaria alternata*

No. 5 (500 m) the mycoaerosol composition was dominated by the species characteristic of the windward side (figures 5, 11 and 12). These were mainly ubiquitous saprophytes: *Cladosporium herbarum, Alternaria alternata* (phot. 3, 4) and *Fusarium* sp. accompanied by less numerous species. At the locations most remote from the landfill (900 and 1000 m) a small variety of species was observed (4 species), with *Cladosporium herbarum* dominating, similarly as at the control location (figures 9 and 10).

Although fungi are generally accepted as indicators of an environmental impact of landfills [1], [6], not many studies include detailed identification of the species. Some authors recommended thermophilic fungi as indicators of an impact of landfills on the ambient air [7] and on the quality of the indoor air in buildings located in the neighbourhood [8]. The results obtained by these authors cannot be compared with those presented in this paper because of different methods of sample collection and cultivation. The above-mentioned authors restricted the species identification to the strains growing at 37 °C. However, there are a number of species of potentially harmful fungi preferring much lower temperatures, e.g. dermatophytes and some fungi causing allergies and producing toxins. Therefore the incubation at 37 °C inhibits growth of many potentially pathogenic fungi. In our studies, the identification was done for strains incubated at 26 °C, the temperature suitable for the majority of fungi,

including many pathogenic strains. According to some authors a good solution that allows growth of fungi differing in thermal optima would be the incubation of two lots of plates at different temperatures (26 °C and 37 °C) or the initial incubation at 37 °C for 24 hours followed by 3 days at 26 °C [9] or, as suggested by DUTKIEWICZ et al. [10], the incubation for 4 days at 30 °C and for the next 4 days at room temperature (22 °C).

The identified fungi included species potentially pathogenic (*Allescheria boydii, Cladosporium elatum, Paecilomyces varioti, Penicillium expansum, Fusarium solani, Scytalidium infectans, Dissitimurus exedrus, Verticillium* sp.), toxigenic (*Penicillium expansum, Aspergillus ustus, Fusarium solani, Trichoderma viridae*), allergenic (*Chaetomium* sp., *Cladosporium herbarum, Alternaria alternata*) and less dangerous saprophytes (*Cladophialospora* sp., *Ulocladium chartarum, Phoma glomerata, Botrytis cinerea, Cladosporium sphaerospermum*). From the above list of organisms a particular attention should be paid to *Allescheria boydii*, a geophilic ascomycete causing mycosis of skin and lungs [10]–[12], and *Paecilomyces varioti* causing the so-called paecilomycosis [13]. The only species identified by us and by the above-mentioned researchers identifying thermophilic fungi [8] was *Alternaria alternata*, a mildew fungus tolerating high temperature and causing allergic rhinitis and bronhial asthma [10].

Table 1

Microbial contamination of the ambient air in the surroundings of the Maślice landfill on March 31st, 2000

Sampling point	Distance from the landfill	Presence of microorganisms (in CFU/m^3)							
		Mesophiles	Actinomycetes	*Pseudomonas fluorescens*	Staphylococci				Fungi
					Haemolysis		Fermentation of mannitol		
					beta	alfa	+	–	
1	100	160	0	26	0	26	52	0	610
2	200	80	26	0	26	0	26	0	470
3	300	80	0	0	26	26	26	0	470
4	400	80	80	0	0	0	0	0	240
5	500	160	0	0	0	26	80	0	470
6	600	80	0	0	26	0	26	0	1260
7	700	80	0	0	26	26	0	0	710
8	800	160	0	0	26	0	0	0	470
9	900	240	52	0	26	26	0	0	550
10	1000	400	26	0	26	26	0	0	470
11 C	100	40	0	0	0	0	0	0	240
12 C	200	80	0	0	0	0	0	0	390

The count of the strains listed and the total count of microfungi in the ambient air were very low (table 1). The concentration of bioaerosol was extremely low

and therefore the presence of potentially hazardous species in the air under investigation was considered safe for human health. However, due to the possibility of large fluctuations in the concentration of microorganisms in time, for a complex assessment of the noxiousness of the imission in the vicinity of the landfill it is necessary to recognize if the pathogenic species found are constantly present in the microflora and what is the range of their concentrations in different seasons and under different meteorological conditions. This will be a subject of the authors' further studies.

Based on the identification studies of fungi an attempt was made to delimit a zone of impact of the landfill on the environment. Species found at the location No. 1 at the base of the landfill on the leeward side can be considered typical of the microflora of the emitter studied since they did not occur on the windward side (except *Chrysosporium terrestrum*) (figures 1, 11 and 13). Some of these species occur as far as at the location No. 5 and farther they disappear (figures 5–10). The disappearance of species beyond the location No. 5 allowed us to establish the border of the zone of impact of the fungal microaerosol at 500 m from the landfill base. Quantitative investigation showed that the total count of fungi gradually decreased from the concentration of 610 CFU/m^3 at the landfill base to the background level (240 CFU/m^3) at the location No. 4 (table 1). As a matter of fact, at further locations (particularly No. 6 and 7) a significant increase in the total count of fungi was noted (table 1), however, since the indicator species disappeared, the local increase in the concentration of microfungi could be regarded as a result of the influence of the secondary emission sources along the line of sample collection (allotments). Therefore it can be acknowledged that the results of quantitative studies are correlated with the results of the qualitative analysis and confirm the existence of the border of the zone of impact of the landfill mycoaerosol at a distance of approx. 400–500 m.

Since mycological identification is time-consuming (particularly in the case of moulds), it is necessary to restrict qualitative studies to the detection of indicator species on standard and differentially-selective media. The analyses recommended by the authors should include:

- identification of the genus of 3 types of the most numerous colonies and of the species of *Aspergillus* colonies growing on agar broth at 26 °C for 5 days [14],
- detection of dermatophytes and *Candida albicans* on the Dermatophyte Test Medium (DTM) enriched with cycloheximide after incubation at 26 °C for 14 days [11],
- determination of the total count of fungi (distinguishing moulds and yeasts) on the Sabouraud medium at 26 °C and 37 °C after 5 days of incubation.

3.2. QUALITATIVE ANALYSIS OF BACTERIA

Results of the analysis are given in table 2. Diagnostic studies revealed small diversity of genera and species of bacteria present in the ambient air in the surroundings

Table 2

Genera and species of bacteria found in the ambient air in the surroundings of the Maślice landfill (the bacteria that were also found on the windward side of the landfill were marked by bold letters)

Sampling point	Distance from the landfill [m]	Genera and species
1	100	***Micrococcus* sp.** *Streptococcus* *Staphylococcus saprophyticus*
2	200	***Bacillus* sp., *Micrococcus* sp.** *Staphylococcus saprophyticus*
3	300	***Bacillus* sp., *Micrococcus* sp.** *Streptococcus* *Staphylococcus haemolyticus* *Staphylococcus epidermidis*
4	400	***Bacillus* sp., *Micrococcus* sp.** *Streptococcus* *Pseudomonas fluorescens*
5	500	***Micrococcus* sp.** *Streptococcus* *Staphylococcus epidermidis* ***Bacillus* sp.**
6	600	***Bacillus* sp., *Micrococcus* sp.** *Streptococcus bovis I* *Staphylococcus epidermidis*
7	700	*Micrococcus* sp., *Bacillus* sp. ***Staphylococcus epidermidis*** *Streptococcus* *Proteus mirabilis*
8	800	***Bacillus* sp.** *Staphylococcus epidermidis* ***Micrococcus* sp.** *Staphylococcus haemolyticus*
9	900	***Bacillus* sp., *Micrococcus* sp.** *Enterococcus faecalis* *Streptococcus* *Staphylococcus epidermidis* *Staphylococcus haemolyticus*
10	1000	***Bacillus* sp.** ***Micrococcus* sp.** *Enterococcus faecalis* *Staphylococcus epidermidis*
11	Control (windward side)	*Micrococcus* sp. *Staphylococcus epidermidis*
12	Control (windward side)	*Bacillus* sp., *Corynebacterium* sp. *Flavobacterium* sp.

of the landfill. They mainly represented the following genera: *Micrococcus, Streptococcus, Staphylococcus* and *Bacillus*. The representatives of three of them (*Micrococcus, Staphylococcus* and *Bacillus*) were found also in the samples collected on the windward side, where the emission from the landfill had no influence on the qualitative and quantitative composition of bacteria. The organisms hazardous to health present in the air investigated included the following potentially pathogenic species: *Staphylococcus haemolyticus*, *Enterococcus faecalis*, *Streptococcus bovis I*, and *Proteus mirabilis*. The presence of this kind of bacteria proves that the standard microbiological analysis should also include determination of the count of bacteria from the *Enterococcus faecalis* species, *Streptococcus* genus and from the *Entreobacteriaceae* family. It would facilitate a more accurate assessment of hazards associated with the emission of microorganisms from municipal structures such as solid waste disposal sites. Due to as small number of the bacteria mentioned above the air under investigation is safe for human health (table 1).

Bacteria typical of the emitter (not present in the background) were found even at a distance of 1000 m from the landfill (*Enterococcus faecalis*). In the quantitative investigations, the increased concentrations of mesophilic bacteria and actinomycetes, in comparison with the background, were also found (table 2). This indicated that the range of bacteria was at least twice as large as that of fungi, which could be explained by a faster sedimentation of heavier fungal cells. Therefore it is necessary to locate additional sampling points at a distance greater than 1000 m from the emitter. However, it must be emphasized that the range of transport of cells in the air depends on many factors, such as: the size of emission of microorganisms, wind velocity, season, and also the kind of waste stored and the way of landfill management [1]. Therefore, the preliminary border of the impact zone delineated in this work holds true for the conditions under which the samples were collected and it will be verified in further studies.

4. CONCLUSIONS

1. Microbiological analysis of the ambient air should be extended in such a way as to include qualitative studies. This applies particularly to fungi, the analysis of which is usually restricted to the determination of total cell count in m^3.

2. Standard determination of the count of fungi in the ambient air should be supplemented with the determination of the count of dermatophytes and with the identification of at least three most often occurring types of colonies.

3. The analysis of the count of bacteria should be supplemented with the determination of the count of bacteria representing the *Streptococcus* genus and the *Enterobacteriaceae* family.

4. In the ambient air in the surroundings of the Maślice landfill, the species of fungi and bacteria hazardous to human health, including pathogenic, toxigenic and allergenic strains, were found.

5. A preliminary zone of an impact of the landfill bioaerosol was established. The range of bacteria (1000 m) was found twice as large as that of fungi (500 m).

6. An adequate assessment of the risk associated with the emission of harmful microorganisms and final delineation of the zone of impact of the landfill in its neighbourhood require further studies which would allow us to determine the range of concentrations (imission) and the stability of the composition of the bioaerosol in different seasons of the year and changing meteorological conditions.

ACKNOWLEDGEMENT

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BADANIA JAKOŚCIOWE BIOAEROZOLU W OTOCZENIU SKŁADOWISKA ODPADÓW „MAŚLICE"

Wykonano mikrobiologiczne badania powietrza wokół wysypiska odpadów komunalnych „Maślice" we Wrocławiu. Próby pobrane metodą sedymentacyjną poddano identyfikacji gatunkowej. Badania mikologiczne wykazały, że w badanym powietrzu znajduje się wiele gatunków grzybów potencjalnie chorobotwórczych, toksykogennych i alergiogennych; dominują te ostatnie. Stwierdzono też występowanie warunkowo patogennych paciorkowców, gronkowców oraz bakterii *Proteus*. Na podstawie badań identyfikacyjnych wyznaczono strefę oddziaływania bioaerozolu na otoczenie. Zaproponowano rozszerzenie standardowej analizy mikrobiologicznej powietrza o wybrane badania grzybów i bakterii.