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Original Research

The Impact of a Landfill Site in Żółwin – Wypaleniska on the Microbiological Quality of the Air

M. Małecka-Adamowicz*, J. Kaczanowska, W. Donderski

Laboratory of Microbiology, Institute of Biology and Environment Protection,
Bydgoszcz University of Kazimierz Wielki, Chodkiewicza 30, 85-064 Bydgoszcz, Poland

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Abstract

This paper presents research on the number of heterotrophic bacteria, mannitol-positive *staphylococci*, *actinomycetes* and mould fungi in the air on the premises of a landfill site in Żółwin-Wypaleniska and in the surrounding area. Researches has revealed that mould fungi were the most numerous, constituting approximately 60-61% of the entire air microflora. Heterotrophic bacteria and actinomycetes were less numerous (accordingly 30% and 8-9% on average). Mannitol-positive *staphylococci* made the least numerous group, making up only 1% of the microbial communities.

The highest number of airborne microorganisms was identified in the central part of the landfill site (Station III). Further from the landfill site, the lower number of microorganisms was detected. *Cocci* were the most numerous (68%), while *bacilli* and rod-shaped morphological forms were less numerous, constituting between 15% and 16% of the entire population of microorganisms.

Keywords: heterotrophic bacteria, *staphylococci*, *actinomycetes*, mould fungi, bioaerosols

Introduction

Being the place of origin of bioaerosols, landfill sites pose a significant microbiological risk for the environment [1]. Bioaerosols are small drops of fluid or tiny particles of solid matter which contain fungal spores, bacteria, viruses and pollen [2]. Krzysztofik [3] points out that bioaerosols may not only deteriorate the hygienic conditions of the air but also prove highly infectious for humans, animals and plants, food products, chemical agents and building materials.

According to Michałkiewicz [4] microflora in the air around landfill sites may contain saprophytic bacteria, haemolysing *staphylococci,* endospores of aerobic and anaerobic bacteria and numerous species of fungi. The fungi detected in the air may possibly form pathogenic flora responsible for numerous infections in humans, e.g. lung mycosis caused by strains of the genus *Aspergillus* and lungs geotrichosis caused by the genus *Geotrichum.* Fungal spores present in the air, when inhaled in bronchial tubes and lungs, may lead directly to numerous allergies, including asthma. It seems reasonable to mention that mycoses of the respiratory system are principally triggered by saprophytes, the abundance of which is recorded in municipal solid waste sites and sewage landfills. Nevertheless, they become pathogenic only after invading a weak organism [5].

The aim of the present study was to estimate the number of heterotrophic bacteria, mannitol-positive *staphylococci*, *actinomycetes* and mould fungi in the air on the premises of a landfill site in Żółwin-Wypaleniska and in the surrounding area.

Material and Methods

The microbiological air tests were conducted on the landfill site Żółwin–Wypaleniska in the village of Żółwin

*Corresponding author; e-mail: marmal@ukw.edu.pl

located 4 km to the south of the outskirts of the town of Bydgoszcz.

On the premises of the landfill site and in the surrounding area eight measuring stations were arranged with the main directions of the winds taken into consideration (Fig.1). Stations I, II, III, IV and V were located within the limits of the landfill, while stations VI, VII and VIII were located outside, respectively 100, 200 and 300 m from the landfill. Station III was situated right in the centre of the landfill.

Sampling

The investigation was pursued in a seasonal cycle using two methods of air sampling, namely the sedimentation method according to Polish Norm [6] and the impaction method based on the MAS – 100 air sampler by MERCK.

In the sedimentation method, open Petri dishes containing agar nutrient media were exposed at the measuring stations for 30 minutes, 150 cm above the ground level.

In the impaction method 100 litres of air was filtered through the sampler's chamber containing a Petri dish filled with a suitable nutrient medium. The microflora in the air stream sucked in by the air sampler deposited on the surface of the medium.

On each station the air sampling based on the above mentioned methods was conducted in three parallel repeats.

Both methods required transporting the air samples to the laboratory, where they were inserted in a thermostat and incubated for a specific time at an appropriate temperature. After the incubation the number of growing colonies was counted and the result was recalculated per 1 m3 of the air (CFU/ m3). The sedimentation method was based on Omeliański's formula [3]:

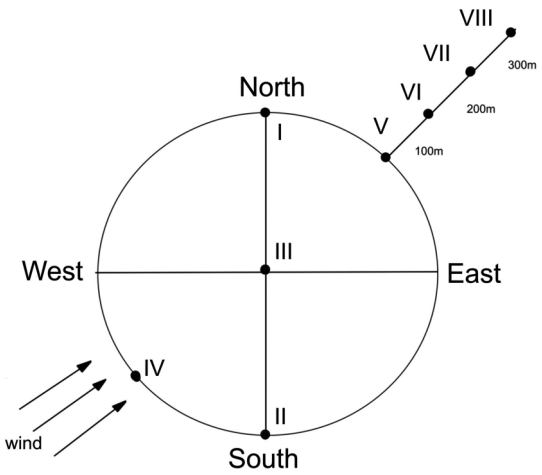

Fig. 1. The location of stations.

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

where: A – total number of bacteria per 1m^3 (CFU/m^3),
a – average number of bacteria on a dish,
r – radius of a Petri dish in cm,
πr^2 – area of a Petri dish,
t – exposure time of a dish (in minutes).

The impaction method involved using a measuring table by Feller [7], enclosed to the Manual of the MAS – 100 Air Sampler by MERCK.

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

where: Pr – most probable number of microorganisms in the tested air volume,
N – number of holes in the perforated base of the sampler (400),
r – number of single units (CFU) making colonies.

Microbiological Research

The microbiological research at the municipal landfill site in Żółwin–Wypaleniska investigated the following:

1) the total number and the morphology of heterotrophic bacteria,
2) the number of mannitol-positive bacteria of the genus S*taphylococcus spp*.,
3) the number of *actinomycetes*,
4) the number of mould fungi.

The total count of heterotrophic bacteria was determined using TSA agar medium. The bacteria were incubated at 37°C for 48 hours, then the number of growing colonies was counted (CFU) and the results were calculated per one cubic metre of air.

With the purpose of determining the morphology of heterotrophic bacteria, single random bacterial colonies were split off and seated on agar slants TSA medium, then incubated at 37°C for 48 hours. The next steps involved preparing microscopic samples and colouring them using Gram's method. The study was heavily concentrated on detecting *cocci, bacilli* and rod-shaped forms.

The presence of mannitol-positive *staphylococci* was detected in accordance with the Polish Norm [8] using Chapman's nutrient medium. The bacterial cultures were incubated at 37°C for 48 hours, then the colonies of mannitol-positive bacteria were counted. Light, yellow zones around a developed colony indicated a positive result.

The number of actinomycetes was determined using Pochon's nutrient medium. Their incubation was conducted at 26°C for 5 days, then the number of colonies were counted (CFU) and the results were calculated per 1 m^3 of air.

The total number of mould fungi was determined using Sabouraud's nutrient medium. Their incubation was conducted at 26°C for 5 days, and then colonies were counted.

On the basis of the final results the level of air contamination on the landfill site in Żółwin–Wypaleniska and in the surrounding area was evaluated in accordance with the Polish Norm [8, 9].

Results

The findings of the survey into the number of different groups of airborne microorganisms on the landfill site Żółwin-Wypaleniska and in the surrounding area are presented in Tables 1 and 2, and in Figs. 2, 4 and 5. There is strong evidence that both applied methods brought about similar results, which demonstrates that the highest number of heterotrophic bacteria was detected at Station III located in the centre of the landfill. The number determined by the sedimentation method was 3580.0 CFU/m^3, while the number determined by the impaction method was 3362.5 CFU/m^3 (Table 1).

In relation to Polish Norms [8, 9], Station III was presumed to be heavily contaminated by bacteria (Table 2). The further from the landfill site, the lower number of airborne bacteria was detected. Stations VII and VIII, located 200 and 300 m away from the centre, were considered uncontaminated. At Station VII the number of heterotrophic bacteria evaluated by the sedimentation method was 803.0 CFU/m^3 on average and evaluated by the impaction method was 712.5 CFU/m^3, while at Station VIII the number of heterotrophic bacteria evaluated by the sedimentation method was 567.8 CFU/m^3 and evaluated by the impaction method was 580.0 CFU/m^3 (Table 1).

The analysis of different seasons reveals that the highest number of heterotrophic bacteria in the air of the investigated landfill site was recorded during summer and autumn, while the lowest–during winter (Fig. 2). *Cocci*, which constituted 68% of the bacterial microflora of the air, were a dominant morphological form of heterotrophic bacteria. The numbers of *bacilli* and rod-shaped bacteria were comparable–each group constituted approximately 15-16% of the total number of the bacteria (Fig. 3).

The measurements of the air contamination by bacteria of the genus *Staphylococcus* on the landfill site in Żółwin-Wypaleniska and in the surrounding area determined that the highest numbers had been recorded at Station III (in the centre of the site): 107.0 CFU/m^3 by the sedimentation method and 98.8 CFU/m^3 by the impaction method. The lowest numbers were recorded at Station VIII (situated 300 m away from the site): 19.5 CFU/m^3 by the sedimentation method and 10.0 CFU/m^3 by the impaction method (Table 1). During summer and autumn the number of *staphylococci* in the air increased, while during winter the number decreased significantly (Fig. 2).

Table 1. The number of investigated groups of microorganisms per 1m^{3}of air on the premises of the landfill site in Żółwin-Wypaleniska and the surrounding area.

Research site	Heterotrophic bacteria		*Staphylococci*		*Actinomycetes*		Mould fungi	
	A	B	A	B	A	B	A	B
I.	(1222.3)	(1172.5)	(45.5)	(50)	(360.3)	(340)	(3111.3)	(3197.5)
	523-1834	560-1570	0-104	0-100	26-655	0-630	262-5109	290-5530
II.	(653)	(1250)	(26)	(18)	(270.2)	(265.8)	(3120.5)	(3580.3)
	480-1380	570-2230	0-52	0-35	26-533	30-610	220-5100	241-5742
III.	(3580)	(3362.5)	(107)	(98.8)	(511)	(482.5)	(3373.3)	(3747.5)
	2619-4192	2320-3860	52-186	30-180	131-917	100-870	393-5240	450-5860
IV.	(689.3)	(1480)	(26)	(17.5)	(294.8)	(272.5)	(3386)	(3762.5)
	253-1309	610-2940	0-52	0-40	52-472	30-450	183-4899	200-5010
V.	(1545)	(1612.5)	(26)	(18.5)	(451.8)	(432.5)	(3000)	(3227.5)
	785-2279	780-2770	0-78	0-42	52-786	80-770	210-4454	300-4640
VI.	(1344.3)	(1365)	(26)	(17.8)	(445.3)	(457.5)	(2770.3)	(2912.5)
	261-2253	220-2750	26-26	0-30	0-707	0-680	52-5056	120-5010
VII.	(803]	(712.5)	(26)	(25)	(393)	(295.8)	(2333.5)	(2491.3)
	786-1100	500-980	0-52	0-60	26-655	30-710	0-4200	35-4550
VIII.	(567.8)	(580)	(19.5)	(10)	(353.8)	(357.5)	(2056.5)	(2010)
	131-1048	200-1150	0-26	0-20	26-524	30-510	26-4192	50-4200

A – sedimentation method, B – impaction method, () – average values in brackets

Table 2. The level of air contamination by microorganisms at different research stations on the landfill site in Żółwin-Wypaleniska and in the surrounding area according to Polish Norm (PN-89/Z-04111/02 i PN-89/Z-04111/03).

Research site	Number of microorganisms in 1m^3 air					
	Heterotrophic bacteria		Actinomycetes		Mould fungi	
	A	B	A	B	A	B
I.	1222.3	1172.5	360.3	340.0	3111.3	3197.5
	**	**	***	***	*	*
II.	653.0	1250.0	270.2	265.8	3120.5	3580.3
	*	**	***	***	*	*
III.	3580.0	3362.5	511.0	482.5	3373.3	3747.5
	***	***	***	***	*	*
IV.	689.3	1480.0	294.8	272.5	3386.0	3762.5
	*	**	***	***	*	*
V.	1545.0	1612.5	451.8	432.5	2770.3	2912.5
	**	**	***	***	*	*
VI.	1344.3	1365.0	445.3	457.5	3000.0	3227.5
	**	**	***	***	*	*
VII.	803.0	712.5	393.0	295.8	2333.5	2491.3
	*	*	***	***	*	*
VIII.	567.8	580.0	353.8	357.5	2056.5	2010.0
	*	*	***	***	*	*
Polish Norm of air:						
not pollution *	< 1 000		10		3 000 – 5 000	
medium pollution **	1 000 – 3 000		10 – 100		5 000 – 10 000	
heavily pollution ***	> 3 000		> 100		> 10 000	

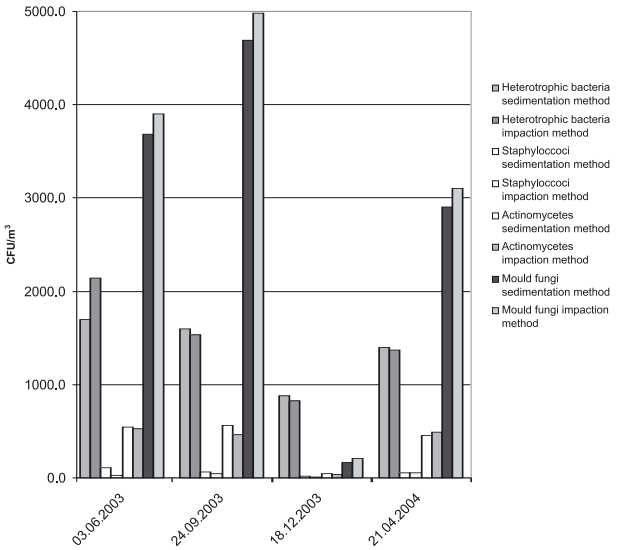

Fig. 2. An average number of microorganisms per 1m^3 of air on the premises of the landfill site in Żółwin-Wypaleniska and in the surrounding area depending on the season.

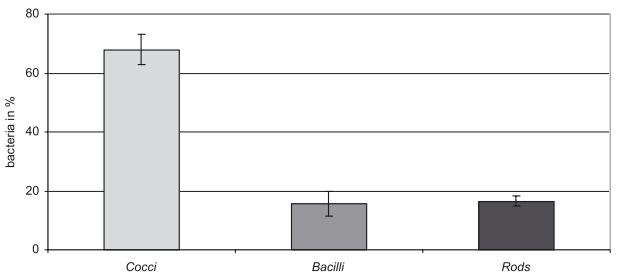

Fig. 3. Percentage fraction of morphological forms of bacteria on the premises of the landfill site in Żółwin-Wypaleniska.

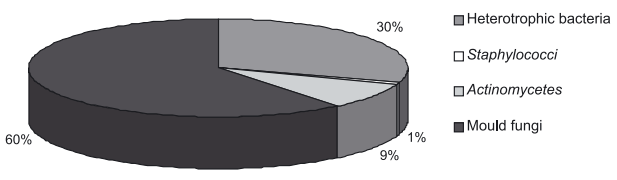

Fig. 4. Percentage fraction of microorganisms in the air on the landfill site in Żółwin-Wypaleniska and in the surrounding area (determined by the sedimentation method).

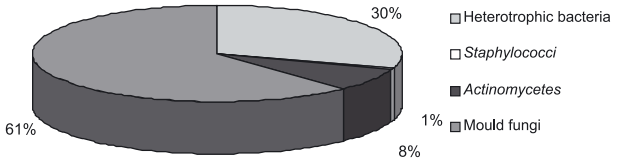

Fig. 5. Percentage fraction of microorganisms in the air at the landfill site in Żółwin-Wypaleniska and in the surrounding area (determined by the impaction method).

The measurements of air contamination by *actinomycetes* on the landfill site and in the surrounding area determined that the highest numbers had been recorded in June, September and April, while in December a tenfold drop was observed (Fig 2). All measuring stations were severely contaminated by *actinomycetes* (Table 2). However, they were the most numerous in the centre of the site at Station III (511.0 CFU/m^3 by the sedimentation method and 482.5 CFU/m^3 by the impaction method). The number of *actinomycetes* diminished to the north and north-east of the site. The minimum numbers of *actinomycetes* (270.2 CFU/m^3 by the sedimentation method and 265.8 CFU/m^3 by the impaction method) were detected at Station II situated in the southern part of the site (Tables 1 and 2) but still it was considered highly contaminated according to the Polish Norm.

Similarly to other microrganisms, mould fungi prevailed in the air of the studied area in autumn, summer and spring, whereas in winter the lowest number was recorded (Fig. 2).

The evaluation based on the Polish Norm revealed that none of the researched measuring stations was significantly contaminated by fungal microflora (Table 2). The highest number of fungi was recorded at Stations III, IV, I, II and V (approximately between 3000-3800 CFU/m^3). At Stations VI, VII and VIII located 100, 200 and 300 m from the site the number of fungi in the air did not exceed 3000 CFU/m^3 (Tables 1 and 2).

Mould fungi prevailed and constituted around 60-61% of the entire airborne microflora of the studied area. Less numerous heterotrophic bacteria constituted about 30% of all airborne microbial communities (Figs. 4 and 5), the least numerous, *actinomycetes* and *staphylococci*, constituted, respectively, between 8 and 9%, and around 1% (Figs. 4 and 5) of the airborne microflora on the landfill site in Żółwin-Wypaleniska.

Discussion

Municipal landfill sites, particularly unmanaged ones, are responsible for the release of chemical and microbiological contaminants into surface and underground waters as well as into the atmosphere. In addition to being a source of dust, odorants and noise emission, they influence the exploitation and management of the neighbouring land, affect the topographic profile of the area and unfavourably transform the landscape [10]. Moreover, municipal waste poses a serious microbiological threat to the environment as it contains bacteria, *actinomycetes*, fungi and viruses, including pathogenic types [1].

In the final analysis one should not overlook the fact that the results of air tests are only of temporary value, i.e. they are credible at the moment of obtaining samples. Physical and chemical properties of the air may cause sudden, considerable changes of its contamination level in a specific spot. Thus the results presented in this paper give no more than an approximate idea of the number of microorganisms; they primarily help to define their number as low or high [11].

From the results of the tests conducted on the landfill site in Żółwin-Wypaleniska near Bydgoszcz by the sedimentation and impaction methods it is possible to conclude that the air on Station III (located in the centre of the site) was generally highly contaminated by both heterotrophic bacteria and *actinomycetes*.

Activities connected with dumping and storing waste as well as the vehicles which transport waste to the landfill site influenced the results to a great extent, though their impact was significantly lower in places located further from the site.

Stations located on the periphery of the landfill site (Stations I, II, IV, V) were considerably less contaminated by microorganisms than the remaining ones, though the least contaminated were Stations VI, VII and VIII located furthest from the site. High microorganism content is reduced by 90-99% in the 200 m distance from the emission source [12].

Weather conditions of a particular area considerably affect the number of microorganisms in the air [13-15]. On the landfill site in Żółwin-Wypaleniska in winter months (characterised by weather conditions unfavourable for microbial development) the number of airborne microbes was significantly lower than in summer and autumn months (characterised by weather conditions favourable for microbial growth and spread).

The same conclusions were drawn by members of the Microbiological Section of the Scientific Society of Student Biologists at Nicolai Copernicus University in Toruń [16]. Their microbiological research conducted in 2000 on the area adjoining the Municipal Landfill Site in Toruń revealed that the lowest number of microorganisms had been recorded during winter, while any significant increase had been typically caused by dealing with dumping and storing waste, which agrees with the conclusion of the present paper as well.

The thesis that air contamination is dependent on weather conditions is confirmed in the research conducted on the premises of a municipal landfill site in Pomnik [17], where the highest counts of microorganisms were recorded in autumn. The fact was caused by local strong winds as well as by arranging the measuring stations along an access road, which, combined with 'dry day' conditions and high-speed air currents, caused raising microorganisms along with dust (moved by cars' wheels) into the air. All these factors contributed greatly to a considerable in-

crease in the number of bacteria and mould fungi in the air [17].

The air tests carried out in the surroundings of the landfill site in Opatowo [17] demonstrated an extremely high level of air contamination. Elevated microbial air contamination was detected around all research stations, including the one situated outside the landfill site.

Regardless of the season, the contamination by haemolysing *staphylococci* vastly exceeded accepted standards. The highest number of these microbes was recorded within the landfill boundary.

The same results were obtained on the premises of the landfill site in Żółwin-Wypaleniska near Bydgoszcz. However, a very low level of air contamination by mannitol-positive bacteria during winter may have been connected with these bacterias' high sensitivity to unfavourable weather conditions.

Elevated levels of mannitol-positive and mannitol-negative *staphylococci* as well as heavy air contamination by *actinomycetes* were also noticed in the surroundings of the landfill site Sobuczyn-Młynek [17].

On the premises of the landfill site in Żółwin-Wypaleniska at all measuring stations the air contamination by actinomycetes exceeded the accepted standards, while the contamination by mould fungi was low enough to presume the air to be uncontaminated. Mould fungi were dominant in the summer-autumn period, while their number were significantly lower in winter on the landfill site in Żółwin-Wypaleniska. A similar phenomenon was observed by Donderski [18] and Bugajny [19] in the air of the cities of Toruń and Poznań.

As can be seen from the results of the investigation into the morphology of the airborne bacteria on the landfill site in Żółwin–Wypaleniska, Gram-negative bacteria, being poorly adapted to living outside their natural environment, formed a small percentage (approx. 16%) of the entire microbial world. In contrast, Gram-positive bacteria: *cocci* and *bacilli* forms prevailed and constituted accordingly around 68% and 15% of the total number of all microbes. Pawlik [20] and Donderski [18] obtained similar results while examining the air in the Municipal Sewage Treatment Plant in Toruń and in the air of the Old Town in Toruń, where they recorded more than 80-90% of Gram-positive bacteria in the airborne bacterial microflora.

Both methods of air testing: sedimentation and impaction, which were used in this research gave similar results. The method of sampling proved to be critical for the recovery of microorganisms from aerosol. After Kruczalak and Olańczuk–Neyman [21] when the sedimentation method was used, the number of detected microorganisms was by an order of magnitude higher than in the case of impaction method.

Landfill sites may cause an increase in the number of microorganisms in the air, which consequently leads to the deterioration of the sanitary conditions in the nearby area. Therefore, it is vitally important to monitor microbial air quality near potential sources of microbial aerosols' emission, i.e. sewage treatment plants and municipal landfill sites.

Conclusions

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

1. In the microflora of the air around the landfill site Żółwin-Wypaleniska located near the town of Bydgoszcz and in the surrounding area mould fungi were the most numerous followed by heterotrophic bacteria, whereas mannitol-positive *staphylococci* and *actinomycetes* were the least numerous.
2. The highest number of microorganisms was recorded in the central part of the landfill site (at Station III), though the number diminished when the distance from the landfill site increased (Stations VI, VII, VIII).
3. Airborne microorganisms were the least abundant during winter and the most abundant during spring and summer.
4. At all the research stations the level of air contamination by *actinomycetes* exceeded permissible standards. At the same time the air of the studied area was assumed to be uncontaminated by mould fungi. The number of heterotrophic bacteria at different measuring stations varied, thus the contamination by these microorganisms reached various levels. Station III was presumed to be highly contaminated, while Stations VII and VIII were presumed to be uncontaminated. At the remaining stations intermediate levels of microbial air contamination were recorded.
5. *Cocci* were a prevailing morphological form of bacteria detected in the air, while *bacilli* and rod-shaped forms were less numerous.

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