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Original Research

Influence of Water Treatment Plant on Microbiological Composition of Air Bioaerosol

B. Breza-Boruta, Z. Paluszak*

Department of Microbiology, University of Technology and Life Sciences, ul. Bernardynska 6, 85-029 Bydgoszcz, Poland

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Abstract

Microbiological research of bioaerosols was carried out at the Municipal Sewage Treatment Plant in Toruń. The concentration of selected bacteria, fungi and actinomycetes in the atmospheric air was estimated in the vicinity of sand catchers, aeration chambers and maturing compost piles, as well as 100 m beyond the treatment plant. It was found that the air at the test stands showed different degrees of microbiological pollution. The largest bioaerosol emission sources were the sand catcher and the maturing compost storage facility. The total number of bacteria and fungi amounted to a maximum of 10^4 CFU m^{-3} and of actinomycetes – 10^3 CFU m^{-3}. The bacteria of the genus *Pseudomonas* (fluorescent subgroup) occurred at all the stands throughout the study except December. The number of *Escherichia coli* and bacteria of the genera *Enterococcus* and *Salmonella* remained at the very low level of about 10^1CFU m^{-3}, and of all these bacteria only fecal streptococci D-type were isolated at stand 4 (beyond the treatment plant).

On the basis of the research carried out and the results obtained at the control stand one may conclude that the test facility does not pose a hazard in respect of the tested bacteria emission.

Keywords: bioaerosol, bacteria, actinomycetes, fungi, wastewater treatment plant

Introduction

Microbiological pollution emitted from wastewater treatment plants are spread in the form of bioaerosol containing viruses, bacteria, actinomycetes and fungi [1, 2]. The source of bioaerosol is sewage and sewage sludge, where pathogenic microorganisms can be found. The microorganisms find suitable conditions for growth or survival in sewage sludge, hence their survival time is relatively long and it ranges from several to several dozen days [3, 4]. According to Teltsch et al. [5] microorganisms can be raised from aerated sewage into air only when their concentration in sewage exceeds 10^3 cells in 1 cm^3. It should be emphasized that in atmospheric air they are in considerably less favourable conditions [6]. Predominant among bacteria detected in bioaerosols emitted by municipal facilities are Gram-positive bacteria such as streptococci and staphylococci, and Gram-negative bacilli [7-9].

Contaminated air can be a source of infection for human beings. Depending on the content of bioaerosol's it can induce the occurrence of simple irritations and ailments, allergic reactions, infections and serious infectious diseases and toxic reactions [10-12]. The highest threat is posed by components of bioaerosol which, transferred by dust or drops in the air, enter an organism through skin, mucous membranes and even stings of blood-sucking arthropods, and more rarely – with food [13, 14].

Monitoring research of atmospheric air are essential for maintaining the appropriate state of the environment in terms of people and animals' health. Microbiological

*Corresponding author; e-mail: paluszak@utp.edu.pl

cleanness control of air around the facilities which are arduous for the environment remains in sufficiently developed by the Polish legislature.

The lack of generally established guidelines regarding bioaerosol assessment is not limited exclusively to Polish legislation. Treshold values for air microflora and substances of microbial origin also seem to be insufficient on a global scale [15].

The aim of this study was to estimate the content of bacterial and fungal aerosols emitted on the premises of the Municipal Wastewater Treatment Plant in Toruń and to assess the degree of air microbiological contamination in the summer-autumn period.

Material and Methods

The study was carried out at the Municipal Wastewater Treatment Plant in Toruń. WWTP "Centralna" is located on the Vistula River in the western part of Toruń. The town lies in Kujawsko-Pomorskie province in northern Poland. The plant covers an area of 9.5 ha.

Research sites were selected on the basis of a previous on-site visit in three measurement points considered to be potential sources of microbiological pollution, that is at the sand catcher (point 1), the aeration chambers (point 2) and around composting piles (point 3). The control site was situated at a distance of 100 m from the wastewater treatment plant (point 4). Its location depended on the wind direction in the area on the day of research. The scheme of the treatment plant and the site location are presented in Fig. 1.

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 ground level. Through the head of the apparatus a strictly determined air volume was sucked onto a Petri dish with agar medium, according to the season of the year and atmospheric conditions (from 10 l to 1000 l depending to the isolated group of bacteria). The research was conducted monthly in the period from May to December 2004 (except for actinomycetes, which were determined from June to December).

The following groups of microorganisms were determined at suitable selective media:

- total bacteria number on Standard I nutrient agar (incubation at 37°C – 72 h)
- total number of mildew fungi on wort agar acidified with 10% citric acid to pH 3.5-4 (incubation at 26°C – 72 h)
- bacteria of the genus *Pseudomonas* (fluorescent subgroup) on the King B medium (incubation at 26°C – 48h)
- Actinomycetes on the Pochon medium (incubation 26°C – 7 days)
- *Escherichia coli* on agar ENDO with fuchsine and lactose (incubation at 37°C – 24 h)
- Fecal streptococci D-type on agar with kanamycine, esculine and azide (incubation at 37°C, 24h). The final identification of enterococci involved using the serological Phadebact-test
- Bacteria of the genus *Salmonella* on agar BPL with brilliant green and phenol red according to Kauffmann (37°C, 24h). Each colony was sieved and then tested serologically using the serum Hm.

Each air measurement for the tested groups of microorganisms was made in four replications. Meteorological conditions during the sample collection were presented in Table 1 (data comes from the Meteorological Station in Toruń).

On the basis of the microorganism number obtained, the means were calculated from the colony-making units. The number of positive holes for the MAS-100 air monitoring system was worked out using a conversion table

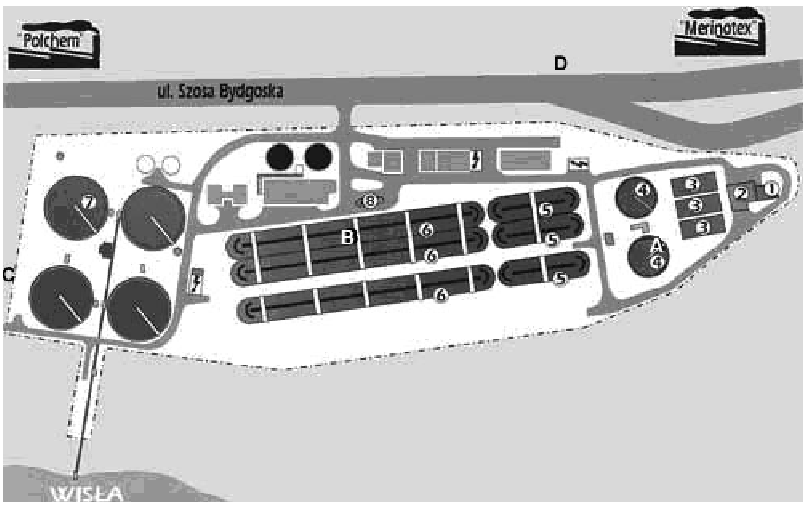

Fig. 1. Scheme of the Waste Water Plant „Centralna" in Toruń and location of measuring points.
1 – raw sewage pumping station; 2 – mechanical screens building; 3 – sand catches; 4 – preliminary sedimentation tanks; 5 – dephosphatation chambers; 6 – nitrification and denitrification chambers; 7 – secondary settlement tanks; 8 – mechanical sewage drainage building; A – stand 1; B – stand 2; C – stand 3; D – stand 4

Table 1. Meteorological conditions during air sampling (according to the Toruń Meteorological Station).

Sampling date	Pressure (hPa)	Mean temperature (ºC)	Humidity (%)	Wind direction	Wind velocity (m/s)
13.05.04.	1004	10.8	27.1	NNE	4
14.05.04.	1008.7	10.4	53.3	W	2
14.06.04.	1011.2	17.8	57.7	W	1
13.07.04.	996.7	15.9	69.5	NW	0.2
14.07.04.	1002.3	15.7	65.1	W	5
11.08.04.	1008.1	24.3	55.2	E	4
12.08.04.	1003.2	25.2	46.8	N	1.2
06.09.04.	1019.4	18.9	66.5	WSW	3
07.09.04.	1017.4	19.3	67.6	W	0.5
07.10.04.	1006.9	14.9	75.8	SW	2
08.10.04.	1004.7	12.4	70.9	SSW	3
06.11.04.	1001.7	7.4	74.9	WSW	5
07.11.04.	1004.6	7.1	76.6	NE	3
03.12.04.	1004.6	4.3	79.7	WSW	3
04.12.04.	1015.2	4.5	79.5	WSW	4

according to Feller, and the obtained colony number was counted over 1 m^3 of the atmospheric air.

The evaluation of the atmospheric air pollution level was performed according to the recommendation given in the Polish Standards: PN-89/Z-04111/02, PN-89/Z-04111/03 [16, 17].

Results

The number of the microorganisms in the atmospheric air at the studied WWTP in Toruń varied depending on location and time of sampling. The results showing the number of the microorganisms and the level of air pollution were put together in Tables 2–8.

The total number of bacteria in the air at the treatment plant tested ranged from 10^1 to 10^4 $CFUm^{-3}$ (Table 2). The highest average number of bacteria emission was observed in the area of composting piles made of sewage sludge. May to October at this site saw strong air pollution and the total number of bacteria amounted to 27,100 $CFUm^{-3}$. Additionally, the number of bacteria admitted permissible by Polish Standards was exceeded in the vicinity of aeration chambers in June and September, as well as beyond the facility (at the control point) in May and June.

In the case of mildew fungi the highest concentration of spores, 21,800 $CFUm^{-3}$, also was reported in the air in the area of the composting piles. Only in the vicinity of the sand catcher air pollution with fungi was it strong, amounting to 13,950 $CFUm^{-3}$. The least number of fungi were isolated at a point situated 100 m beyond the treatment plant. Throughout the study the number of fungi there ranged from 113 $CFUm^{-3}$ to 4667 $CFUm^{-3}$. Considerably higher values of spore concentration occurred in summer months from June to September (Table 3).

Of the indicatory bacteria tested, the most frequently isolated were actinomycetes, whose number reached 1040 $CFUm^{-3}$. At the treatment plant and beyond it the number of actinomycetes exceeded the treshold values recommended by the Polish Standards. The largest source of actinomycete emissions proved to be heaped compost piles, where 10-fold excess of the standards was noted in September. Strong air pollution occurred as follows: at the aeration chambers (to 380 $CFUm^{-3}$), at the sand catcher (to 172 $CFUm^{-3}$) and beyond the treatment plant (to 139 $CFUm^{-3}$). The high concentration of actinomycetes at the control point, situated 100 m from the treatment plant, remained for almost all the months except November, when less then 10 $CFUm^{-3}$ were isolated. Analyzing the growth dynamics of *Actinomycetes* population a conclusion should be drawn that their largest amounts occurred during the summer (June–September) and the smallest in November and December (Table 4).

The bacteria of *Pseudomonas* genus (fluorescent subgroup) caused medium air pollution at all measurement points throughout the research, except December, when their presence beyond the treatment plant was not reported (Table 5). The sand catcher proved to be the largest source of bacterial emission and their number during the research ranged from 9 to 44 $CFUm^{-3}$. A high emission

Table 2. The level of atmospheric air contamination with total number of bacteria on the premises and in the vicinity of the WWTP from May to December 2004.

Stand	Concentration [CFU m $^{-3}$]							
	May	June	July	August	September	October	November	December
I*	1150**	1730	1300	2867	523	752	1175	145
II	233	6600	650	1867	3503	520	45	41
III	5615	27100	13100	16600	19450	3600	400	84
IV	3603	3050	1700	2213	253	395	85	127

* Stand I – the sand catcher, II – the aeration chambers, III – around composting piles, IV – at a distance of 100 m from the WWTP
** – scale description acc. to recommendations of PN-89 Z-04111/02
>3000 CFU m $^{-3}$ – strongly contaminated air
1000-3000 CFU m $^{-3}$ – moderately contaminated air
<1000 CFU m $^{-3}$ – uncontaminated air

Table 3. The level of atmospheric air contamination with total number of fungi on the premises and in the vicinity of the WWTP from May to December 2004.

Stand	Concentration [CFU m $^{-3}$]							
	May	June	July	August	September	October	November	December
I*	748**	5600	4100	13950	3450	4070	1720	348
II	345	5180	4300	7600	2550	3650	1140	240
III	1280	7300	5400	8650	21800	4450	660	242
IV	1616	4667	1700	1400	2000	1600	495	113

* for a description, see Table 2
** – scale description acc. to recommendations of PN-89 Z-04111/03
>10000 CFU m $^{-3}$ – polluted air, posing a hazard for human environment
5000-10,000 CFU m $^{-3}$ – polluted air, with a potential negative effect on human environment,
3000-5000 CFU m $^{-3}$ – approximately clean atmospheric air

Table 4. The level of atmospheric air contamination with actinomycetes on the premises and in the vicinity of the WWTP from June to December 2004.

Stand	Concentration [CFU m $^{-3}$]						
	June	July	August	September	October	November	December
I*	145**	35	172	50	28	12	11
II	81	194	40	380	13	3.3	4
III	185	60	285	1040	94	17	8
IV	139	107	32	29	84	6.8	34

* for a description, see Table 2
** – scale description acc. to recommendations of PN-89 Z-04111/02
>100 CFU m $^{-3}$ – strongly contaminated air
10-100 CFU m $^{-3}$ – moderately contaminated air
<10 CFU m $^{-3}$ – uncontaminated air

Table 5. The level of atmospheric air contamination with the bacteria of *Pseudomonas* genus (fluorescent) on the premises and in the vicinity of the WWTP from May to December 2004.

Stand	Concentration [CFU m $^{-3}$]							
	May	June	July	August	September	October	November	December
I*	33**	15	17	23	44	36	15	9
II	6.1	8.6	6.7	8.1	7.1	4	5	2
III	17	27	12	22	10	7.2	2.3	2
IV	25	17	7.5	5	9.9	11	3.2	not detected

* for a description, see Table 2
** – scale description acc. to recommendations of PN-89 Z-04111/02
>50 CFU m $^{-3}$ – strongly contaminated air
≤50 CFU m $^{-3}$ – moderately contaminated air
not detected – uncontaminated air

Table 6. The number of *E. coli* in atmospheric air at the stands tested from May to December 2004.

	Concentration [CFU m $^{-3}$]							
Stand	May	June	July	August	September	October	November	December
I*	13	4	10	16	62	40	20	14
II	n.d. **	n.d.	n.d.	5	n.d.	1.7	17	n.d.
III	n.d.	2	n.d.	n.d.	n.d.	n.d.	n.d.	6
IV	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

* for a description, see Table 2
** n.d.- not detected

of the bacteria was also noted in the air taken from the area of the compost piles (2-27 CFUm^{-3}). The concetration of fluorescent *Pseudomonas* detected in the air at the aeration chambers did not exceed 8.6 CFUm^{-3}. Beyond the treatment plant a considerable number of bacteria was isolated in May and June, while in December they were not detected in the air.

Escherichia coli and *Salmonella* spp. as well as D-type fecal streptococci were determined in the largest numbers in the air collected at the sand catcher, although their concentration was low. *E. coli* predominated with a number of 62 CFUm^{-3} (Table 6). In the air at the aeration chambers *E. coli* emissions reached a maximum of 17 CFUm^{-3}. However, in the air at the composting piles their presence was reported only in two months of study, namely June and December. Fecal streptococci and *Salmonella* were isolated in similar numbers, up to 20 CFUm^{-3} and up to 18 CFUm^{-3}, respectively (Tables 7 and 8). The bacteria of the genera *Salmonella* and *Enterococcus* were found in the air collected in the vicinity of the piles in considerably larger numbers in comparison with the aeration chambers. Definitely the smallest amount of D-type fecal streptococci, *Salmonella* spp. and *E. coli* occurred in the air beyond the treatment plant. The presence of enterococci was reported here only in the period from May to July, and in the other months they were not detected. *E. coli* and *Salmonella* sp. were not isolated in the air samples collected at a distance of 100 m from the treatment plant throughout the research period.

The results obtained indicate that the diversification in the number of the bacteria and fungi tested in the air depends not only on the place of sampling, but also on the season of the year. A larger level of microorganisms was observed in warmer months, that is from May to September, and then their number decreased gradually. Only the bacteria of the *E. coli* were isolated in larger numbers from September to November.

Discussion

The present study proves that the wastewater plant contributed in the highest degree to the total emission of bacteria and actinomycetes. The level of bacterial and microfungal bioaerosol concentration was dependent mainly on the location of a measurement point. Significantly the largest source of emission of the total number of bacteria, actinomycetes and fungi proved to be the area of the

Table 7. The number of *Enterococcus* spp. in atmospheric air at the stands tested from May to December 2004.

Stand	Concentration [CFU m $^{-3}$]							
	May	June	July	August	September	October	November	December
I*	10	20	6	2	10	20	n.d.	n.d.
II	6	2	n.d.**	n.d.	1.7	1.7	6.7	n.d.
III	10	19	8	14	8	n.d.	n.d.	2
IV	6	4	4	n.d.	n.d.	n.d.	n.d.	n.d.

* for a description, see Table 2
** n.d.- not detected

Table 8. The number of S*almonella* spp. in atmospheric air at the stands tested from May to December 2004.

	Concentration [CFU m $^{-3}$]							
Stand	May	June	July	August	September	October	November	December
I*	18	2	4	2	16	n.d.**	2	4
II	n.d.	n.d.	1.7	n.d.	n.d.	1.7	n.d.	n.d.
III	n.d.	6	n.d.	4	6	n.d.	n.d.	n.d.
IV	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

* for a description, see Table 2
** n.d.- not detected

composting piles made from sewage sludge. The number of actinomycetes in September exceeded the permissible standards by 104%. A high level of the indicatory bacteria of the genus *Pseudomonas*, which contaminated the air on the premises of the wastewater plant and beyond the area in all the dates tested except December, is worthy of note. The species was considerably most numerous in the air emitted from the sand catchers.

According to Piekarska and Traczewska [6] accepting of the total number of microorganisms as an indicator of air pollution generally eliminates a possibility of finding the health hazard. Thus particular attention should be paid to specific microorganisms, occurring only in the given area, since owing to their presence a range of public utilities' impact on air quality can be precisely determined. A decrease in number of specific bacteria in the air proceeds much faster than a fall in the total number of bacteria. This phenomenon is justified by the specific bacteria larger susceptibility to ambient environment conditions in comparison with saprophytic microflora normally occurring in the air [4, 18].

Both for bacteria of the family *Enterobacteriaceae* and of the genus *Enterococcus* no standards exist to establish the permissible content of these groups of bacteria in the air [6, 16]. The analyses conducted at German composting plants showed a substantially higher level of *E. coli* than in the present study. Böhm [19] reports that at a composting plant the number of *E. coli* amounted even to 2400 CFUm^{-3} of the air.

The experiments conducted by Ossowska-Cypryk [18] in the vicinity of the municipal sewage treatment plant indicated that air microflora qualitative composition is closely connected with the type and number of microorganisms present in waste. The microorganisms come out with sewage drops to ambient air, particularly from aeration chambers.

Paluszak et al. [20] in the study on pathogen presence on subsequent stages of waste treatment process at the Municipal Waste Water Plant in Toruń reported the highest bacteria concentration in the first stage of the purification. Also the largest emission of *P. fluorescens, E. coli, Enterococcus* sp. and *Salmonella* sp. was detected in the air at this site.

At the present study throughout the experiment a high concentration of actinomycetes was noted in the air at the composting piles. Heavy air pollution with actinomycetes at this site resulted from favourable conditions for their mass proliferation in composted biomass.

Being a material rich in organic compounds, compost stimulates the growth of actinomycetes, which can use as many as 50 various sources of carbon [21, 22].

According to Fernando and Fedorak [1] the number of bacteria in the air emitted from aeration chambers and the other sites decreases along with a distance from the source of emission. They are driven by autochtonic microflora, pigment bacteria and mildew fungi. Also Bauer [23] and Brandi [3] point out that the number of bacteria in the air undergoes considerable reduction along with a distance of the emission source.

The results obtained by Ossowska-Cypryk [18] indicate that the number of microorganisms penetrating into the air decreases remarkably along with the distance from an emission source. The author found that the total number of bacteria decreased from 4716 CFUm^{-3} of the air (with a distance of 5 m) to 1886 CFUm^{-3} of the air (with a distance of 150 m). Only the microorganisms which are the most resistant and the best adapted to unfavourable living conditions maintain the longest vitality [7, 24].

On the basis of the previous studies, the conclusion should be drawn that the area adjacent to aeration chambers are the most exposed to the action of biological aerosols [1, 23, 25]. In personal studies such tendencies were not observed, since the highest emission of the tested microorganisms was not observed at aeration chambers. The most serious source of air pollution proved to be the sand catchers and the place of maturing compost storage.

The extent of the wastewater plant influence as well as the number and sort of microorganisms emitted depend also on meteorological conditions and season of the year. The research proved that most microbiological air pollution with bacteria occurred in summer months and in early autumn. In the study by Petrycka et al. [25] the highest air contamination was also reported in the summer period. Łebkowska [8] and Pillai [9] claim that the high ambient temperature can contribute to the rise in the emission of potentially pathogenic microorganisms.

There is no close references to the number of microorganisms in the air in connection with the epidemiological results of air pollution in the literature. The increased number of microorganisms in the air do not have to cause an increased risk for people staying in a given area. On the contrary, in some cases people spending much time in microbiologically contaminated environments become resistant [26].

Conclusion

1. The highest emission of microbiological air pollution at the Municipal Sewage Treatment Plant in Toruń was found by the sand catcher and at the maturing compost storage facility. Strong air contamination with total population of bacteria (9-fold exceeding acceptable limits), actinomycetes (10-fold) and fungi (2-fold) occurred at those stands.
2. The indicator bacteria of *Pseudomonas* genus (fluorescent subgroup) occurred at all the stands throughout the study except December. The number of the bacteria isolated indicates that the air was moderately polluted with these bacteria.
3. The number of *Escherichia coli, Salmonella* sp. and *Enterococcus* sp. in the air at the treatment plant remained at a very low level of about 10^1 CFU m^{-3}, and of all those bacteria only fecal streptococci D-type were isolated at stand 4 (beyond the treatment plant).
4. The results obtained at the stand located 100 m beyond the sewage treatment plant indicate that the tested facility does not pose a risk in respect of the emission of the tested bacteria, which are considered as potentially pathogenic, and that the local people are not threatened.

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