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SMALL WASTEWATER TREATMENT PLANTS AS SOURCES OF BIOAEROSOL EMISSIONS INTO THE ATMOSPHERIC AIR

Wastewater treatment plants are a source of odour and bioaerosol emissions into the atmosphere. Therefore, this study aimed to determine the concentration of selected groups of bacteria and fungi around four small municipal wastewater treatment plants located near Wrocław (SW Poland). The wastewater was treated using the activated sludge method. Bioaerosol samples were collected in triplicate using the aspirometric method onto Petri dishes with appropriate microbiological media. Bioaerosol concentrations around the studied plants and in the vicinity of individual devices were relatively low; in some cases, fecal bacteria and human pathogenic microorganisms were detected in the bioaerosol. The obtained results justify the need for further research on bioaerosols emitted by wastewater treatment plants and systematic monitoring of bioaerosol concentrations in their vicinity.

1. INTRODUCTION

Atmospheric air is not a permanent habitat for microorganisms. This is due to the lack of nutrients and unfavorable physicochemical conditions. However, it provides a pathway for microorganisms, including pathogenic ones, to environments conducive to their development and reproduction. Wastewater treatment plants are important sources of microbiological air pollutants, including viruses, bacteria, and fungi that are pathogenic to humans, as well as worm and insect eggs [1]. Among other reasons, wastewater treatment installations designed to serve no less than 100,000 equivalent inhabitants are classified in Poland as projects that can always significantly affect the environment, while wastewater treatment installations designed to serve no less than 400 equivalent inhabitants are classified as projects that may potentially significantly affect the environment [2]. Monitoring of the presence of biologically harmful factors in the air is also required at least within the plant's premises [3]. In Poland, however, as

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in many other countries, there is no legal or normative act regulating the scope, frequency, or methodology of monitoring the impact of wastewater treatment plants on air quality. Therefore, in many treatment plants, air quality monitoring is conducted sporadically, irregularly, or not at all [4].

Microorganism emissions are another reason, alongside the emissions of malodorous substances, why locating wastewater treatment plants near residential areas is a source of social conflict in Poland and other countries. Locating them away from areas already developed and suitable for future development is difficult due to the high population density in Poland and similar countries. An increase in the incidence of various diseases has been observed among treatment plant workers and people living nearby, but the cause-and-effect relationship has only been elucidated in some cases [5]. This justifies research into the actual impact of wastewater treatment plants on air quality. The COVID-19 pandemic has increased interest in the topic and, consequently, the number of publications on the topic [6].

Bacterial bioaerosol emissions from wastewater treatment plants decreased during the pandemic [7]. This may have been due to the widespread use of disinfectants. However, the share of antibiotic-resistant strains increased [8]. The presence of coronavirus RNA was detected in both treated wastewater and in the air within the wastewater treatment plant. This indicated the possibility of using wastewater as an indicator of the health of human populations and the relatively high epidemic exposure of wastewater treatment plant workers. Coronaviruses die in wastewater within a few days [9], but this time is sufficient to pose a threat to human health.

The goal of this paper was to determine the emissions of selected microorganisms into the ambient air from key components of several small municipal wastewater treatment plants under different atmospheric conditions during the winter and summer seasons, with particular emphasis on Gram-negative bacteria. Subsequent events fully confirmed the validity of this research.

2. MATERIALS AND METHODS

Wastewater treatment plants studied. The study was conducted at four small wastewater treatment plants, designated A-D. They were located near Wrocław (SW Poland). In all of these plants, wastewater is treated using the activated sludge method with fine-bubble aeration. The process lines of these plants varied (Table 1). Bioaerosol samples were collected at the research sites described in Table 2.

Methods. Bioaerosol was sampled using the impact method onto agarified microbiological media in 90 mm diameter Petri dishes. Using a Duo SAS Super 360 air sampler with two 219-hole heads, bioaerosol was collected from 100 dm^3 of air within 1 minute to determine the number of psychro- and mesophilic bacteria.

Table 1

Characteristicsof the examined sewage treatment plants

Feature	Wastewater treatment plant			
	A	B	C	D
Population equivalent	5192	1600	20 216	16 164
Average daily flow, m^3	70	150	2317	1816
Wastewater treated, m^3	municipal 100%			municipal – 81%, industrial[a] – 19%
Selector chamber, m^3	90	lack		
Sand trap, m^3	lack			2×20
Primary settling tank, m^3	lack	lack	lack	420
Pre-denitrification chamber, m^3	lack	one activated sludge chamber, active capacity: 195.5	67	lack
Dephosphatation chamber, m^3	lack		112, 168	alternating chamber[c]: active capacity: 2000 m^3
Denitrification chamber, m^3			343, 112.5, 331	
optional chamber, m^3	Lack		142	
Nitrification number, m^3	514 m^3		1432	2×950 m^3
Secondary settling tank, m^3	30.2	75	610	2×942.5[b]
Aerobic sludge stabilization chamber, m^3	200	75	lack	
Receiver	river	drainage ditch		river
Sediment plots, m^2	lack			2000, emergency use
Sludge dewatering sampling point capacity, m^3/h operation, h/day hall volume, m^3	 1 3–6 367.5	lack	 10–12 ca. 10 6 days/week 143	lack

[a]Metallurgy, dairy, food tank washing.
[b]Currently one in operation.
[c]Dephosphatation, denitrification, denitrification/nitrification.

Table 2

Research sites

Site No.	WWTP	Location of the sampling point
1	A	control site located 2.3 km NW of WWTP
2		technology hall in the sludge dewatering building of WWTP
3		above the denitrification chamber of WWTP
4		above the nitrification chamber of WWTP
5	B	technology hall of WWTP
6		control site in front of the technology hall of WWTP
7	C	technology hall in the sludge dewatering building of WWTP

Table 2

Research sites

Site No.	WWTP	Location of the sampling point
8		above the denitrification chamber of WWTP
9		above the nitrification chamber of WWTP
10		control site on the windward side – near the fence of WWTP
11	D	above the alternating chamber of WWTP
12		above the nitrification chamber of WWTP
13		above the sludge fields of WWTP
14		Technology hall in the sludge dewatering building of WWTP
15		control site on the windward side – at the fence of WWTP

Gram-negative bacteria and molds. Bioaerosol was collected from 200 dm^3 of air within 2 minutes to determine the number of other microorganism groups. The microorganisms were incubated under conditions appropriate for each group (Table 3). After incubation, the number of microorganism colonies growing on the media was counted, taking into account the correction recommended by the sampler manufacturer. All samples were collected in triplicate. The results were given as the number of colony-forming units (CFU) per unit of air volume (1 m^3) – arithmetic mean for three replicates and standard deviation.

Table 3

Methods of incubation of microorganisms

Group of microorganisms	Microbiological medium	Incubation	
		Temperature [°C]	Time [day]
Mesophilic bacteria	nutrient agar	37	1
Psychphilic Bacteria		22	3
Gram-Negative Mesophilic Bacteria	MacConkey	37	1
Gram-Negative Psychrophilic Bacteria		22	3
Coliforms	Endo	37	1
Escherichia coli	Endo	44	1
Salmonella and *Shigella*	SS	37	1
Enterococcus	Slanetz–Bartley	37	1
Pseudomonas fluorescens	King B	26	5
		4	7
Pseudomonas aeruginosa	CN agar for Pseudomonas	37	1
Mannitol-positive staphylococci	Chapman	37	1–2
Mannitol-negative sCtaphylococci			
Molds	Sabourad with chloramphenicol	26	3–5
Yeasts	Sabourad with TTC	26	3–5

Table 4

Criteria for classifying atmospheric air pollution with bacteria according to PN-89/Z04111/02 [10]

Total number of mesophilic bacteria in 1 m^3 of atmospheric air	Number of specific bacteria in 1 dm^3 of atmospheric air				Degree of atmospheric air pollution
	Actinomycetes	*Pseudomonas fluorescens*	Hemolytic staphylococci		
			α	β	
< 1000	<10	0	0	0	unpolluted
1000–3000	10–100	≤ 50	≤ 25	≤ 50	moderately polluted
> 3000	> 100	> 50	> 25	> 50	heavily polluted

Table 5

Criteria for air pollution with fungi according to PN-89/Z-04111/03 [11]

Total number of fungi in 1 m^3 of atmospheric air	Degree of atmospheric air pollution
3000–5000	average clean air, especially in late spring and early autumn
5000–10 000	pollution that may negatively impact the human environment
> 10 000	pollution that threatens the human environment

Table 6

Recommended concentrations of microorganisms in indoor air after [12]

Microbiological factor	Permissible concentration [cfu/m^3]	
	Workrooms with organic dust	Residential and public spaces
Mesophilic bacteria	100 000	5000
Gram-negative bacteria	20 000	200
Thermophilic actinomycetes	20 000	200
Fungi	50 000	5000
Group 3 and 4 threat factors	0	0

Table 7

Proposals for assessing the degree of microbiological contamination of the atmosphere after [13]

Bioaerosol component	Degree of atmospheric air pollution [in cfu/m^3]	
	Acceptable	Unacceptable
Mesophilic bacteria	≤ 5000	> 5000
Gram-negative Bacteria	≤ 200	> 200
Thermophilic Actinomycetes	≤ 200	> 200
Fungi	≤ 5000	> 5000
Group 3 and 4 threat factors	0	> 0

The criteria for selecting the groups of microorganisms included in the study were: the specificity of the research object, recommendations of PN-89/Z-04111/02 [10] (Table 4) and PN-89/Z-04111/03 [11] (Table 5), proposals by Dutkiewicz and Mołocznik from 1993 [12] (Table 6), and proposals of the Expert Team for Biological Factors [13] (Table 7). Particular attention was paid to determining the number of Gram-negative bacteria. Details are provided in the monograph edited by Zwoździak [14].

3. RESULTS AND DISCUSSION

The concentrations of mesophilic and psychrophilic bacteria, as well as Gram-negative psychrophilic and mesophilic bacteria measured above the activated sludge chambers and sludge plots, were relatively low (Tables 8–11). This suggests that these facilities emitted few live bacteria into the air. Bacterial concentrations were also low in the process rooms containing the activated sludge chamber and sludge dewatering sampling points. Representatives of narrower bacterial groups were detected ephemerally at individual study sites. This meant that their concentrations were less than 0.005 cfu/m^3.

The number of mesophilic airborne bacteria at all study sites was less than 1000 cfu/m^3. This corresponded to unpolluted air according to PN-89/Z04111/02, an acceptable level of atmospheric air pollution as proposed by the Expert Team for Biological Factors, and in the case of WWTP Hall B (sampling point 5), the concentration permissible in workrooms with organic dust, and even residential and public spaces. *Pseudomonas fluorescens* was detected at three of the 15 test sites. However, their count was lower than 50 cfu/m^3, corresponding to moderately polluted atmospheric air according to PN-89/Z04111/02. These bacteria were detected only once, and each site was tested twice (winter and summer).

Mold concentrations showed significant variation, both between individual test sites in summer and winter, between sites within the same wastewater treatment plant, and between treatment plants. This indicated a strong dependence of fungal concentrations on current conditions. In most cases, they were lower than 5,000 cfu/m^3, which corresponds to average clean atmospheric air according to PN-89/Z04111/03, acceptable pollution as proposed by the Expert Team for Biological Factors [13], and permissible concentrations in workrooms with organic dust, and even in residential and public spaces according to the recommendations of Dutkiewicz and Mołocznik [12]. Higher values were observed in summer at four sites: 7–9 and 15. This represented 13% of the cases studied (winter and summer combined – 15 sites with two tests each, 30 cases in total).

Significant variation in microorganism concentrations within wastewater treatment plants has also been observed by other authors, and the range of this variation, for example, from tens of cfu/m^3 to several thousand cfu/m^3 for psychro- or mesophilic bacteria or mold fungi, was similar to that found in this study [15–17]. Literature data also indicate significant variation in the concentrations of selected groups of microorganisms isolated from atmospheric air on selective media, such as bacteria from the *Enterobacteriaceae* family, the *Pseudomonas* genus, and staphylococci.

Table 8

Results of microbiological analysis of WWTP A.
Mean concentrations of microorganisms and standard deviations [cfu/m^3]

Group of microorganisms	Season[a]	Sampling point			
		1	2	3	4
Mesophilic bacteria	W	50±24	NT	97±15	53±18
	S	77±25	857±117	127±5	743±170
Psychrophilic bacteria	W	307±54	NT	447±98	740±170
	S	63±12	2243±274	323±69	850±530
Gram-negative mesophilic bacteria	W	3±5	NT	0±0	0±0
	S	7±9	5±1	2±2	0±0
Gram-negative psychrophilic bacteria	W	2±2	NT	2±2	2±2
	S	107±13	35±8	105±45	206±7
Coliforms	W	0±0	NT	3±4	0±0
	S	0±0	0±0	11±4	8±7
Escherichia coli	W	0±0	NT	0±0	0±0
	S	0±0	0±0	3±2	2±2
Salmonella and *Shigella*	W	0±0	NT	0±0	0±0
	S	0±0	0±0	0±0	0±0
Enterococcus	W	0±0	NT	0±0	0±0
	S	0±0	3±5	2±2	2±2
Pseudomonas fluorescens, 26 °C	W	0±0	NT	0±0	0±0
	S	0±0	0±0	0±0	0±0
Pseudomonas fluorescens, 4 °C	W	0±0	NT	0±0	0±0
	S	0±0	0±0	0±0	0±0
Pseudomonas aeruginosa	W	0±0	NT	0±0	0±0
	S	0±0	0±0	0±0	0±0
Mannitol-positive staphylococci	W	0±0	NT	0±0	0±0
	S	0±0	0±0	0±0	0±0
Mannitol-negative staphylococci	W	0±0	NT	13±19	17±14
	S	0±0	0±0	0±0	0±0
Molds	W	160±4	NT	53±24	40±8
	S	4222±1641	1674±76	5865±948	6535±0
Yeasts	W	5±4	NT	7±6	3±2
	S	18±19	5±4	23±21	15±4

[a]S – summer, W – winter, NT – not tested.

In some wastewater treatment plants, such microorganisms are found in higher numbers in the air than found in this study [17, 18], while in others, they are detected at a few study sites [19, 21]. Zabłocka-Godlewska et al. [21] emphasize that *Escherichia* *coli* is not a natural component of the air microbiome, and in the wastewater treatment plant they studied, it was present in the air only near some of its equipment. Møller et al. [22] found exposure to *Escherichia coli* among wastewater treatment plant workers employed only in some positions.

Table 9

Results of microbiological analysis of WWTP B.
Mean concentrations of microorganisms and standard deviations [cfu/m^3]

Group of microorganisms	Season	Sampling point	
		5	6
Mesophilic bacteria	W	133±41	10±7
	S	0±0	0±0
Psychrophilic bacteria	W	243±280	17±17
	S	0±0	0±0
Gram-negative mesophilic bacteria	W	0±0	0±0
	S	0±0	0±0
Gram-negative psychrophilic bacteria	W	0±0	0±0
	S	0±0	0±0
Coliforms	W	0±0	0±0
	S	0±0	0±0
Escherichia coli	W	0±0	0±0
	S	0±0	0±0
Salmonella and *Shigella*	W	0±0	0±0
	S	0±0	0±0
Enterococcus	W	0±0	0±0
	S	0±0	0±0
Pseudomonas fluorescens, 26 °C	W	3±5	0±0
	S	0±0	0±0
Pseudomonas fluorescens, 4 °C	W	0±0	0±0
	S	0±0	0±0
Pseudomonas aeruginosa	W	0±0	0±0
	S	0±0	0±0
Mannitol-positive staphylococci	W	0±0	0±0
	S	0±0	0±0
Mannitol-negative staphylococci	W	0±0	0±0
	S	0±0	0±0
Molds	W	52±9	27±20
	S	0±0	0±0
Yeasts	W	10±7	7±6
	S	0±0	0±0

[a]S – summer, W – winter.

Pseudomonas was among the genera dominant in the airborne bioaerosol at the wastewater treatment plants studied by Zhao et al. [23]. In this study, Gram-negative bacteria were isolated from the air using MacConkey agar, and the results indicated that they constituted a relatively small portion of the psychro- and mesophilic bacteria present in the air at the wastewater treatment plant.

Table 10

Results of microbiological analysis of WWTP C.
Mean concentrations of microorganisms and standard deviations [cfu/m^3]

Group of microorganisms	Season[a]	Sampling point			
		7	8	9	10
Mesophilic bacteria	W	67±35	207±42	23±5	33±17
	S	24±16	20±4	12±1	63±16
Psychrophilic bacteria	W	10 480±3,663	13 070±0	227±52	147±52
	S	40±14	87±12	20±8	4800±4,333
Gram-negative mesophilic bacteria	W	0±0	0±0	0±0	0±0
	S	3±5	0±0	3±5	3±5
Gram-negative psychrophilic bacteria	W	7±4	17±9	3±5	3±5
	S	0±0	0±0	0±0	17±17
Coliforms	W	22±14	3±4	3±4	0±0
	S	0±0	3±4	0±0	14±20
Escherichia coli	W	5±4	0±0	2±2	0±0
	S	0±0	0±0	0±0	0±0
Salmonella and *Shigella*	W	0±0	0±0	0±0	0±0
	S	17±5	0±0	0±0	0±0
Enterococcus	W	5±0	3±5	0±0	0±0
	S	0±0	0±0	0±0	2±2
Pseudomonas fluorescens, 26 °C	W	17±5	3±5	0±0	3±5
	S	0±0	0±0	0±0	0±0
Pseudomonas fluorescens, 4 °C	W	0±0	0±0	0±0	0±0
	S	0±0	0±0	0±0	0±0
Pseudomonas aeruginosa	W	0±0	0±0	0±0	0±0
	S	0±0	0±0	0±0	0±0
Mannitol-positive staphylococci	W	2±2	0±0	0±0	0±0
	S	0±0	0±0	0±0	0±0
Mannitol-negative staphylococci	W	3±5	0±0	0±0	0±0
	S	0±0	0±0	0±0	0±0
Molds	W	25±19	25±4	23±5	32±18
	S	5065±2079	6535±0	6535±0	1563±101
Yeasts	W	2±2	7±2	0±0	0±0
	S	0±0	0±0	2±2	3±2

[a]S – summer, W – winter.

By comparison, mesophilic Gram-negative bacteria growing on eosin-methylene blue agar constituted one-third of the mesophilic bacteria growing on trypticase soy agar above the aeration chamber in the treatment plant studied by Wlazło et al. [24]. However, this result referred to a single bioaerosol sample collected at a single study site. Therefore, it is difficult to assess whether this was a coincidence or whether eosin-methylene blue agar is less selective than MacConkey agar. A comparison of the bacterial and fungal concentrations obtained in this study and in studies conducted at other wastewater treatment plants indicates that molds are a significant component of airborne bioaerosols within the wastewater treatment plant [16–18].

Table 11

Results of microbiological analysis of WWTP D.
Mean concentrations of microorganisms and standard deviations [cfu/m^3]

Group of microorganisms	Season[a]	Sampling point				
		11	12	13	14	15
Mesophilic bacteria	W	27±17	10±8	107±33	70±45	33±17
	S	295±166	43±2	NT	563±446	420±150
Psychrophilic bacteria	W	13±5	60±29	603±183	53±40	153±12
	S	230±35	80±33	NT	220±16	1097±515
Gram-negative mesophilic bacteria	W	0±0	0±0	0±0	0±0	0±0
	S	2±2	3±5	NT	3±5	70±8
Gram-negative psychrophilic bacteria	W	0±0	0±0	0±0	0±0	0±0
	S	52±34	3±5	NT	7±5	83±42
Coliforms	W	3±4	0±0	6±9	0±0	0±0
	S	0±0	0±0	NT	0±0	0±0
Escherichia coli	W	0±0	0±0	2±2	0±0	0±0
	S	0±0	0±0	NT	0±0	0±0
Salmonella and *Shigella*	W	0±0	0±0	0±0	0±0	0±0
	S	0±0	0±0	NT	0±0	0±0
Enterococcus	W	0±0	0±0	0±0	0±0	0±0
	S	2±2	0±0	NT	0±0	0±0
Pseudomonas fluorescens, 26 °C	W	0±0	0±0	13±5	0±0	20±8
	S	0±0	0±0	NT	0±0	0±0
Pseudomonas fluorescens, 4 °C	W	0±0	0±0	0±0	0±0	0±0
	S	0±0	0±0	NT	0±0	0±0
Pseudomonas aeruginosa	W	0±0	0±0	0±0	0±0	0±0
	S	0±0	0±0	NT	0±0	0±0
Mannitol-positive staphylococci	W	0±0	0±0	0±0	0±0	0±0
	S	0±0	0±0	NT	0±0	0±0
Mannitol-negative staphylococci	W	0±0	0±0	0±0	0±0	0±0
	S	0±0	0±0	NT	0±0	0±0
Molds	W	88±12	77±20	107±56	138±19	170±15
	S	4563±1487	1882±457	NT	1870±728	5865±948
Yeasts	W	0±0	2±2	2±2	2±2	8±2
	S	0±0	3±2	NT	5±4	18±5

[a]S – summer, W – winter, NT – not tested.

The results of the air quality study at four small wastewater treatment plants obtained in this study, as well as the results of the authors who conducted similar studies at other treatment plants, indicate the need for systematic monitoring of the concentration and composition of airborne bioaerosols in and around installations designed to collect and treat wastewater, particularly municipal wastewater, and for determining permissible parameters for bioaerosols present in indoor and ambient air, as well as the applicable frequency and methodology for such monitoring. To date, culturing methods for isolating microorganisms from the air have been widely used. These methods allow for the detection of viable microorganisms. This does not fully reflect the health risk posed by airborne bioaerosols to humans and their companion animals. It also contains fragments of dead bacterial cells, including Gram-negative bacteria that produce endotoxins and fungi that produce beta-1,3-glucans. These can cause inflammation and allergic reactions in humans and their companion animals, even though the cells that produced them have already died and are not detectable by culture methods.

5. CONCLUSION

Systematic monitoring of bioaerosol concentration and composition in wastewater treatment plants, including levels of viable indicator microorganisms, endotoxins, and β-1,3-glucans, may facilitate the implementation of more effective preventive measures for wastewater treatment plant workers and for residents of neighborhoods adjacent to the plants [25].

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