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ASSESSMENT OF POSSIBILITIES OF SPREADING OF BIOAEROSOL FROM DIFFERENT TECHNOLOGICAL OBJECTS IN SMALL SEWAGE TREATMENT PLANTS

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Abstract
The Covid-19 pandemia increased the attention of the world community to air biocontamination. Sewage treatment plants (STPs) generate a bioaerosol during different technological operations. Research aimed to estimate the range of bioaerosol emission from different technological objects of 5 small STPs. Such knowledge is very important for risk assessment, monitoring programs and pollution limitation. The sedimentation method was used for the detection of mesophiles, psychrophiles, *Escherichia coli*, pigmented bacteria, *Streptococcus faecalis*, *Pseudomonas fluorescens*, and mold fungi. The highest level of psychrophiles and mold fungi (> 1000 cfu/m^3) was detected in points located near activated sludge chambers, sludge thickening tanks, and secondary clarifiers. The mesophiles (>500 cfu/m^3) and *E. coli* aren't a normal component of air microflora, but were detected in all measurement points, especially near a pomp station (inflow), grit, activated sludge, sludge thickening chambers. At the points located at the leeward, the number of microorganisms was higher than in the windward. The research results indicate the necessity of constant monitoring of the STP impact on the air quality.

Keywords: **Bacteria; bioaerosol; *E. coli;* Mesophiles; Microbiological air quality; Mold fungi; *P. fluorescens*; Psychrophiles; Sewage treatment plant; *S. faecalis*.**

1. INTRODUCTION

Municipal objects such as sewage treatment plants, necessary for sustainable water management, can have an adverse impact on human health and decrease the quality of the environment. In municipal sewage, the concentration of microorganisms is very high. A particular risk to human health poses a large number of pathogenic viruses, bacteria, fungi, protists, and parasitic organisms contained in them. The strength of this adverse impact depends on the type of the sewage, the size and object location, used technologies, the kind, and the number of emission sources, terrain conditions, plant cover, weather conditions, etc. The most onerous are odorous and bioaerosols emissions [1, 2].

Bioaerosols are the airborne live and dead particles of microbial, plant or animal origin, which can be attached to vapor water or solid particles. Their composition is various, changeable, and depends on a huge amount of factors. These air contaminations contain bacterial cells, fungal spores, viruses, endotoxins, mycotoxins, pollens, etc. The size of bioaerosol particles ranges from 0.01 μm (viruses) to several hundred μm (e.g. seeds) [3, 4]. The microbiological quality of air directly impacts human health, safety, and the quality of the environment. The origin and composition of bioaerosols determine their health effects, including spreading infectious diseases, causing allergic reactions or irritating the respiratory tract [1–3, 5]. This problem has been particularly publicized recently in the face of the ongoing Covid-19 pandemic, which pointed out the need for regular air quality monitoring. It is especially important in the case of such objects as sewage treatment plants, where bioaerosols are constantly generated during numerous technological processes. These specific airborne microbiological contaminants contain various pathogens mostly viruses (including the Sars-Cov2 virus), bacteria and fungi [3, 6–9]. It was proved, that sewage treatment plants are also emitters of antibiotic resistance genes [7, 10].

The purpose of the research was an assessment of the impact of 5 small STPs on air quality. The study was focused on an assessment of the impact of different technological objects on microbiological air quality, the range of spread of the generated bioaerosol and the estimation of threats to human health. The aim of the study was also an assessment of the possibility of usage of the pigmented bacteria (their share in total bacteria number) as a suitable indicator of changes in air quality in the case of small sewage treatment plants.

2. MATERIALS AND METHODS

2.1. Samples collection

Studies were conducted at 5 small STPs which are located out of the industrial and highly agglomerated areas in the south of Poland (upper and down Silesia). The average capacity of studied treatment plants ranged between 1200-9500 $m^3 \cdot d^{-1}$. All objects had a turbine system of aeration and opened activated sludge chambers.

Samples were collected at various distances from 8 different technological objects. The list of them is presented in Table 1.

Table 1.
The list of used abbreviations of monitored technological objects and background

Abbreviation	Technological object
TS	transfer stations
G	grits
PC	primary clarifiers (open)
ASC	activated sludge chambers (open with turbin system of aeration)
SC	secondary clarifiers (open)
OFC	open fermentation chambers
STT	sludge thickening tanks
SP	sedimentary plots
WW	windward
LW	leeward

The background was determined depending on the wind direction, on the windward (WW) side in relation to the location of the treatment plant. These points were located at 2–3 m from the STP's external border. The opposite point was located in a straight line from the WW according to the wind direction, on the leeward (LW). The lines connected the WW and LW were conducted in a way that crossed the location of ASCs, as potentially greatest emitters. The LW point was also located about 2–3 m from the external border of the STP's. The sampling distances [m] from technological objects were signed as number index at their name abbreviation (e.g. ST10, ST15, etc.). In all studied STPs the SC were located near the ASC.

The sedimentation method was used for the detection of bacteria and fungi in the air. Samples were collected at the end of summer (August/September), always between 3 and 5 p.m., three times for each STP. The dates of sampling were determined by weather conditions. Samples were always taken at least 3 days after rainfall, on slightly cloudy and almost windless days, at air temperature between 21°C and 24°C.

2.2. Microbiological analysis

In Poland, because of the lack of actually applicable standards, the assessment of microbiological air contamination is based mainly on previous Polish Standards (PN-89 / Z-04111 / 01–03: 1989) [11–13]. Accordingly to these standards the presence of mesophiles (total bacteria number), *Pseudomonas fluorescens* and mold fungi were under detection in this study. Additionally, *Escherichia coli* and *Streptococcus faecalis* were used as indicators of faecal contamination of air due to their high concentration in municipal sewage and bioaerosols generated from them. The number of psychrophiles and pigmented bacteria as typical outdoor air microflora were also determined. Estimation of their share in the sum of the number of psychrophiles and mesophiles additionally helped to evaluate the impact of STPs on air quality.

Each sample was taken by sedimentation method using 3 different sampling times (5, 15, 30 min). All tests were performed in triplicate for each sampling time. For microbiological analysis, different growth media and conditions were used (Tab. 2).

Table 2.
Microbiological air analysis – growth media and culture conditions

Detected microorganisms	Growth media	Incubation temperature [°C]	Duration of incubation [h]
Mesophiles	nutrient agar (BTL)	37	24-48
Psychrophiles	nutrient agar (BTL)	26	48-96
pigmented bacteria (counted on psychrophiles plates)	nutrient agar (BTL)	26	48-96
Escherichia coli	selective agar Endo (BTL)	37	24-48
Pseudomonas fluorescens	selective *Pseudomonas* Agar Base with supl. (Oxoid),	35	24-48
Streptococcus faecalis	SF agar (Difco)	45	24-48
mold fungi	Czapek agar (BTL)	26	72-96

The diagnosis of microorganisms was carried out on the basis of macro- and microscopic observations, performing morphological and physiological examinations.

Accordingly to the standards PN-89 Z04111/02 [12] and PN-89 Z04111/03 [13] bacterial and fungal concentrations in air were calculated by means of Omeliański formula with Gogoberidze modification (1) and was expressed as the number of colony-forming units (cfu) in 1 m3 of the air.

$$X = (a \cdot 10^4)/\pi r^2 \cdot 0.2 \cdot t \qquad (1)$$

where:

X – number of bacteria or fungi (cfu) in 1 m^3 of air;

a – average number of bacteria or fungi detected on surface of Petri dishes;

r – radius of Petri dish [cm];

t – exposure time [min];

0.2 – time exposure conversion coefficient.

3. RESULTS AND DISCUSSION

Determination of microbiological air quality is based on the presence of specific groups of microorganisms and endotoxins. The degree of microbiological air pollution is determined by the detected number of microorganisms (cfu) of each group in 1 m^3 of air.

The mesophiles were detected at all measurements points. The presence of mesophilic bacteria in air microflora is pointing to their probable human or animal origin [14–16]. Their number didn't exceed the value recommended for clear air (<1000 cfu/m^3) but was significantly higher in comparison with background (WW) at almost all sampling points (especially at points located near the transfer station (TS), grit (G), primary clarifiers (PC), activated sludge chambers (ASC) and sludge thickening tanks (STT)) (Fig. 1, Tab. 3). In some cases (PC10, PC40, SP5–SP25) independently of the increased distance from the technological object, the number of mesophiles was constant or increased, which was probably due to the interaction/synergistic impact of the neighboring facilities (eg. near location of the SCs and ASCs). The numbers of mesophilic bacteria in cfu/m^3, as an average value from all STPs with SD, in all sampling points were presented in Fig. 1.

At all sampling points, the number of psychrophiles was higher than at the background (WW). This was observed especially near the TS, G, ASC, STT, SP. The most numerous psychrophiles microflora (> 1000 cfu/m^3) were detected at points located at ASC10 and STT10 (Fig. 2, Tab. 3). The number of bacteria detected at STT10 and SP20-25 was higher than in points located nearer to these objects (STT5 and SP5). It was probably due to the impact of the neighboring facilities on air quality (as in the case of mesophiles).

The pigmented bacteria are commonly found in the air, and they have a significant contribution in airborne microflora. Pigmentation, mainly carotenoids, protects bacteria cells from UV irradiation and allows them to survive at low temperatures [2, 17]. During the long-lasting sunny weather, their share within the airborne microflora tends to increase due to the protective role of bacteria carotenoids. The number of this group of bacteria was counted at plates within the psychrophiles. Their percentage share within the sum of the number of psychrophiles and mesophiles was calculated and shown in Fig. 3. Their range min-max in CFU/m^3 in given sampling points is shown in Tab. 3. A decrease in the percentage contribution of pigmented bacteria relative to the background pointed out the increased share of

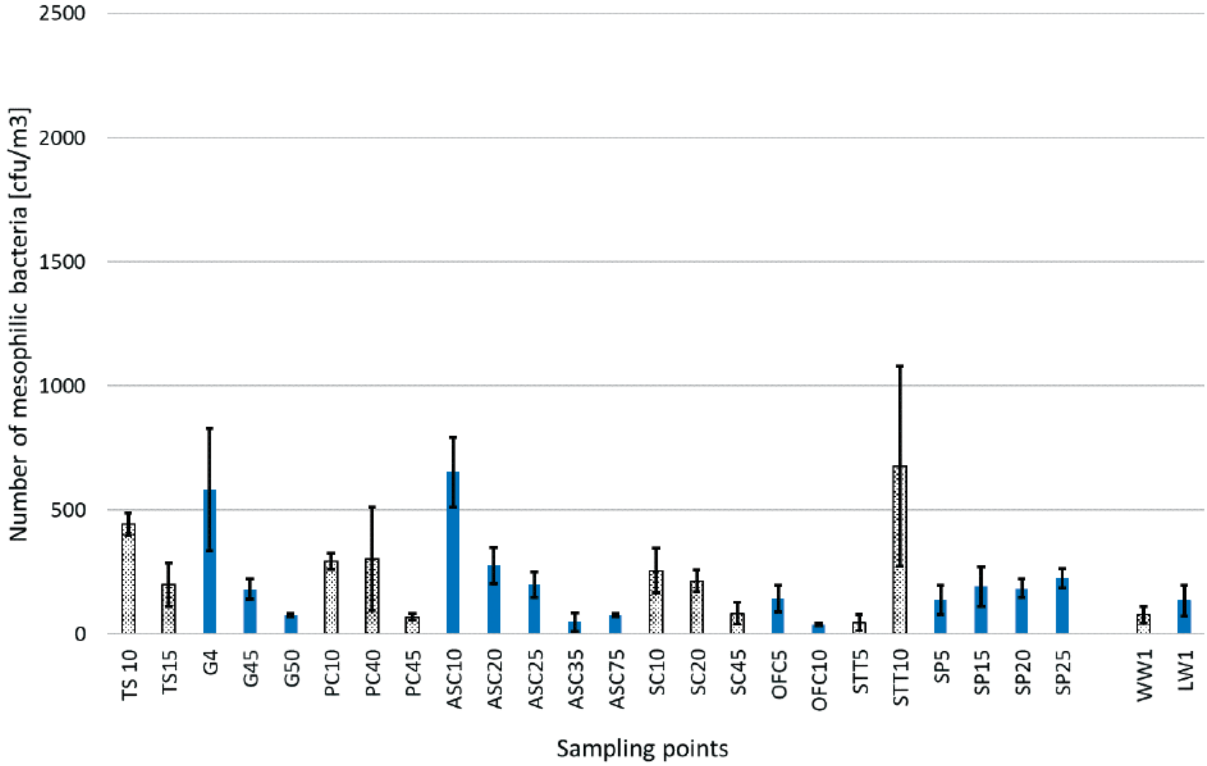

Figure 1.
The number of mesophilic bacteria [cfu/m^3] in different sampling points (average value from all STPs with SD)

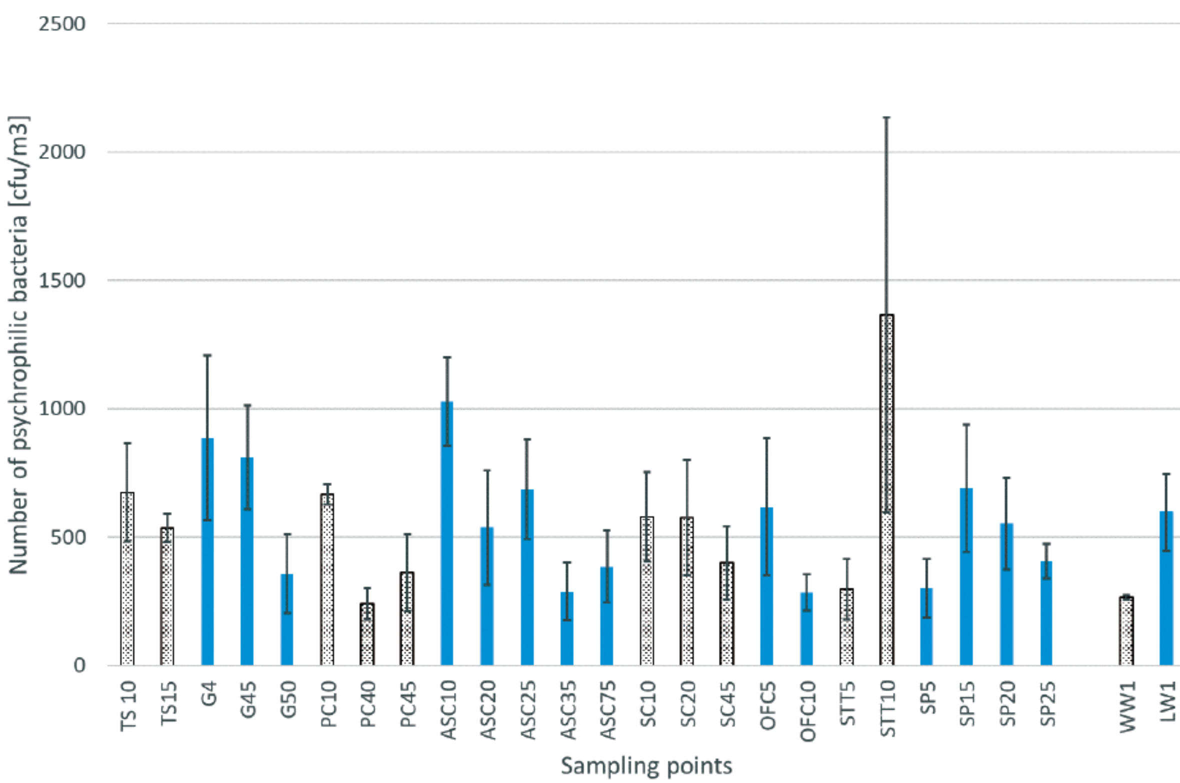

Figure 2.
The number of psychrophilic bacteria [cfu/m^3] in different sampling points (average value from 5 STPs with SD)

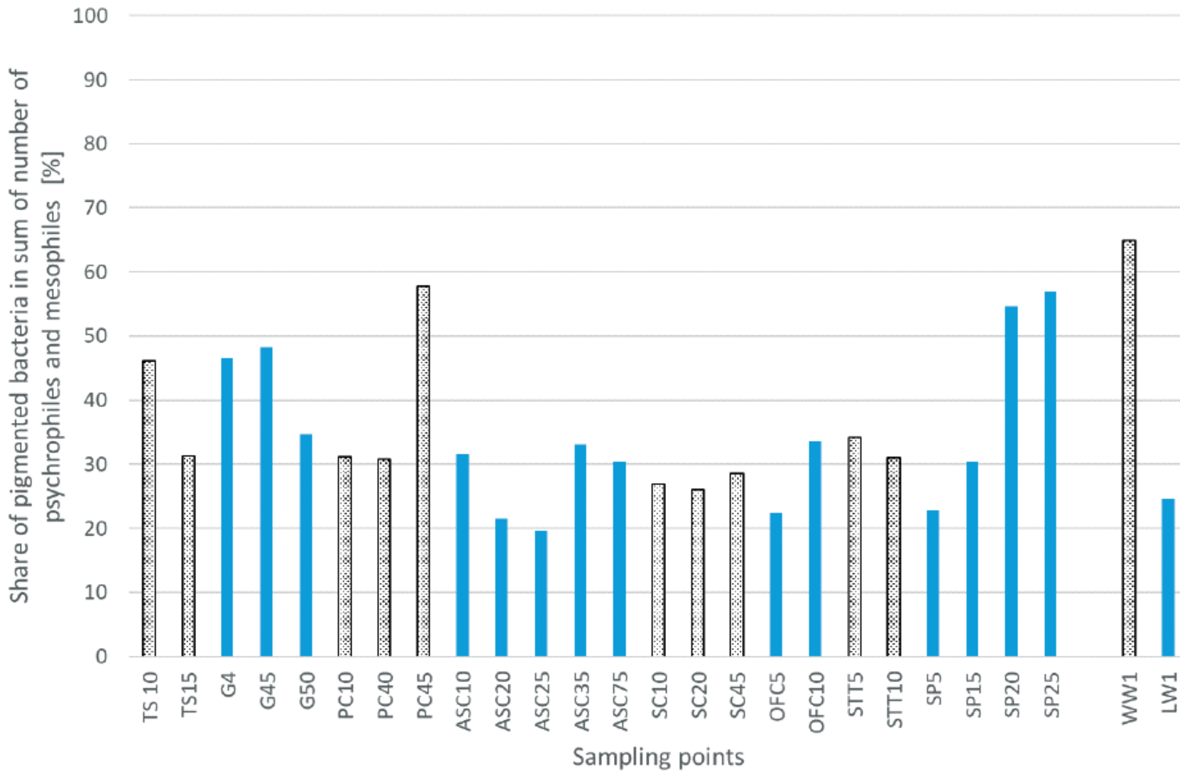

Figure 3.
Contribution of pigmented bacteria [%] in the sum of mesophilic and psychrophilic bacteria in different sampling points (average value from 5 STPs)

bacteria emitted from different technological objects. These relations were observed especially in such points as ASC, SC, OFC, STT, SP, which were the greatest emitters of mesophiles and psychrophiles (Fig. 1–2, Tab. 3). In the background (WW) the share of pigmented bacteria was 65% (while at LW it was 26%) and this value was not exceeded at any point. The highest percentage share of them was observed at points PC45 (58%), SP20 (55%), and SP25 (57%) (Fig. 3).

Escherichia coli is one of the most important indicators of faecal contamination of air. It is particularly used for monitoring the wastewater treatment plants' operation and health threats [18–21]. *Pseudomonas fluorescens* is an indicator of the bioaerosol of surface waters [22, 23]. Both *E. coli* and *Ps. fluorescens* were detected at such points as TS10, G4, PC10, ASC10-35, SC10-45, STT10, and SP25 (Fig. 4, Tab. 3). The highest number of these bacteria was detected at ASC10-25, SC10, and G4. The presence of *E. coli* in aerosol pointed out the potential of occurrence and spreading of other faecal pathogens present in municipal sewage and increasing health threats for STP's workers, especially in the surroundings of mentioned points. The number of detected *Ps. fluorescens* (> 50 cfu/m^3) indicated the medium level of air contamination (Fig. 4, Tab. 3). It should be noted that at WW (background) and LW neither *E. coli* nor *Ps. fluorescens* were detected. *Streptococcus faecalis* was detected only near the point ASC10 (6 CFU/m^3).

The number of mold fungi (Fig. 5, Tab. 3) exceed the value recommended for clean air only in points located near primary clarifiers (PC10) and sedimentary plots (SP5) and was above 5000 cfu/m^3. The air in these points was classified as negatively affecting human health. At the rest of the measurement points the number of mold fungi didn't exceed the value of 3000 cfu/m^3 and the air was classified as not polluted by fungi.

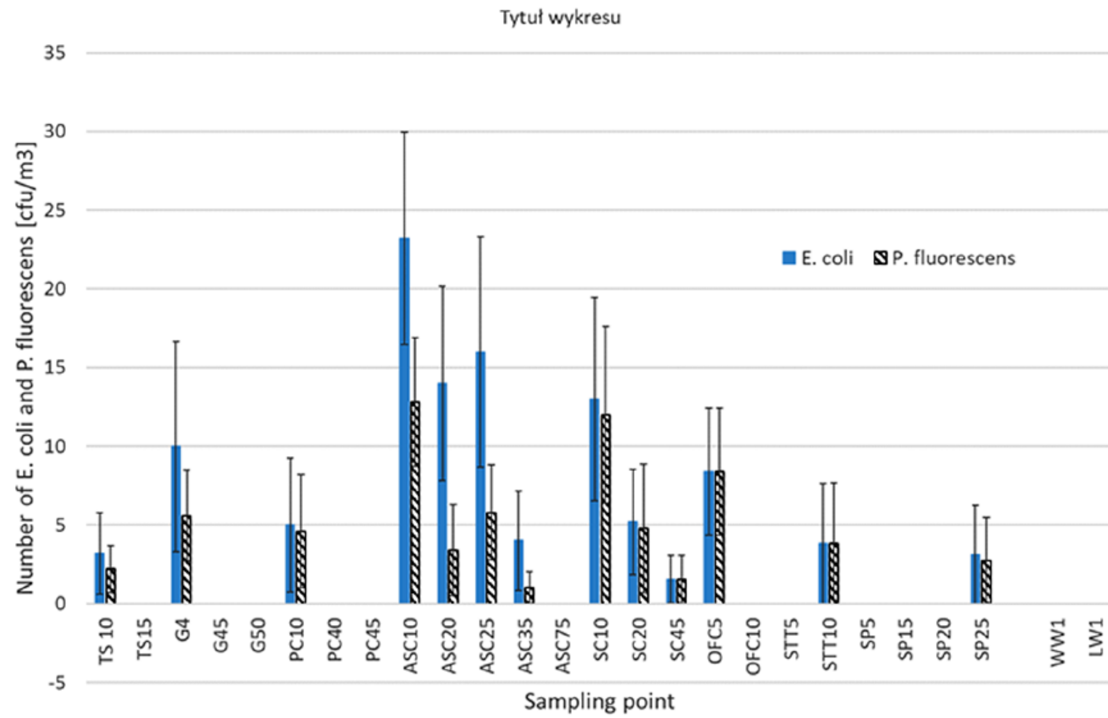

Figure 4.
The number of *Escherichia coli* and *Pseudomonas fluorescens* [cfu/m^3] in different sampling points (average value from 5 STPs with SD)value from 5 STPs)

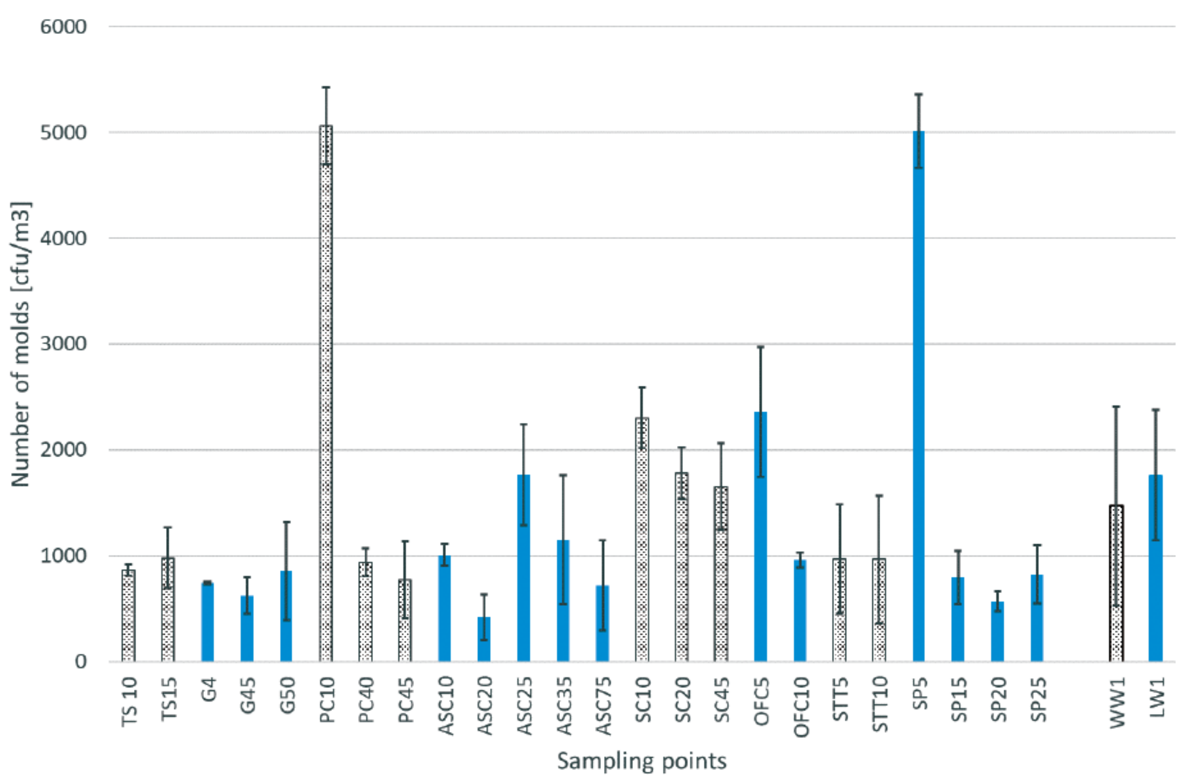

Figure 5.
The number of mold fungi [cfu/m^3] in different sampling points (average value from 5 STPs with SD)SD)value from 5 STPs)

Table 3.
The number of different groups of bacteria (range min-max in CFU/m³). M – mesophiles; P – psychrophiles, PB – pigmented bacteria; E – *E. coli*; Ps – *P. fluorescens*; F – mold fungi (the highest microorganisms number are marked as grey cells)

Sampling point	Group of microorganism					
	M	P	PB	E	Ps	F
TS 10	393-505	428-882	445-577	0-7	0-4	806-907
TS15	66-302	459-604	223-236	0	0	486-1181
G4	229-865	446-1323	347-812	0-20	0-9	727-786
G45	118-222	530-1017	172-797	0	0	446-826
G50	66-83	144-511	39-289	0	0	275-1284
PC10	255-340	626-711	226-379	0-11	0-6	4468-5459
PC40	26-511	157-301	92-249	0	0	786-1088
PC45	59-88	164-492	59-364	0	0	275-1284
ASC10	498-806	842-1296	341-629	6-22	7-16	917-1127
ASC20	197-368	276-722	118-223	0-12	0-6	197-682
ASC25	149-265	382-828	170-181	11-42	11-12	961-2166
ASC35	13-92	105-393	26-249	0-4	0-2	459-1808
ASC75	66-83	144-511	39-289	0	0	275-1284
SC10	119-361	316-733	175-293	6-19	6-8	2002-2663
SC20	149-265	382-828	149-265	0-8	0	1561-2166
SC45	66-141	214-572	92-183	0-4	0	1023-1982
OFC5	85-212	159-828	85-212	4-12	0-4	1465-2930
OFC10	32-43	181-361	96-127	0	0	892-1051
STT5	13-92	105-393	26-249	0	0	459-1808
STT10	182-1127	223-2188	118-996	0-8	0	262-1638
SP5	74-191	149-417	64-117	0	0	4768-5579
SP15	131-276	394-983	131-353	0	0	381-1037
SP20	197-223	321-747	249-524	0	0	472-708
SP25	170-262	328-485	282-445	0-7	0	550-1212
WW	32-117	255-276	195-227	0	0	606-1280
LW	83-196	414-828	106-210	0	0	1019-2497

The highest number of detected bacteria and mold fungi was estimated at points that were located near to the monitored objects. Le et al. (2021) reported that with the increase in distance from the emission source (0 m, 50 m, 100 m), the concentration of bioaerosols decreased significantly. In our studies, it was confirmed in most cases. As moving away from technological devices the number of microorganisms were decreasing, however, not always. In some cases, a higher number of bacteria and fungi was detected at points located in the longer distance from considered objects, which was probably connected with overlapping the zones of influence of different kinds of them (e.g. the number of psychrophiles at STT10, ASC 25, SP15).

The obtained results confirm the observation of others authors that the largest emitters of bioaerosols at STPs are activated sludge chambers (ASC), grit (G), secondary clarifiers (SC), and sludge thickening tanks (STT) [10, 20, 24-26]. At points located near these objects the concentration of all studied groups of microorganisms was higher than in the background (bacteria < 500 cfu/m³, fungi <1500 cfu/m³). Li et al. (2016) detected the highest level of culturable bacteria (up to 1697 cfu/m³) and fungi (up to 930 cfu/m³) in air samples collected near the sludge thickening chambers (STT).

The comparison of quality and quantity composition of bioaerosol from given STPs' areas with suitably determined backgrounds allowed us to estimate the impact of the different technological objects on air quality. At the points located at leeward (LW) the number of microorganisms numbers in all of the considered groups (mesophiles, psychrophiles, and mold fungi) were higher than at windward (WW) (Fig. 1–2 and Fig. 5). Additionally, the percentage of pigmented bacteria was lower at LW, than at WW sampling points. This pointed out the adverse impact of STPs on microbiological air quality. It should be emphasized, that at these sampling points (WW and LW) no *E. coli, P. fluorescens*, and *S. faecalis* were detected. (Fig. 3–4, Tab. 3).

Our studies were conducted during the summer period only. Regular monitoring of STPs' air quality would be useful for a complete picture of health and environmental threats. Most of the technological objects into STPs negatively impact either the STPs' air quality or the outside surrounding (air quality at leeward) pose the risk for human health. Spreeded bioaerosols also may take a part in the SARS-CoV-2 distribution. The presence of the SARS-CoV-2 virus genome was proved in untreated wastewater samples [6, 8–9, 27].

The STPs' management systems have to reduce such health risks through mitigation strategies. The establishment of the obligatory cycle of monitoring of microbiological air quality at STPs allows to evaluate the impact of different technological devices on air quality, identify the potential sources of biocontamination, the efficiency of the implemented sanitation programs, and determination the areas at a high risk of contamination and the workers' threats [27].

4. CONCLUSIONS

Obtained results showed that all studied technological objects at 5 different STPs were the emitters of bioaerosols. The increase of the number of microorganisms at the area of STPs as well as at the leeward (LW) in comparison with background (WW) was observed. In most cases, the number of microorganisms decreased when the distance from the objects increased. At the points with the highest number of mesophiles (grits (G), activated sludge chambers (ASC), and secondary clarifiers (SC)) the highest number of *E. coli*, the indicator of the faecal air contamination, was detected. The number of mesophiles, which was much higher than at background, did not exceed the value recommended for clean air at any point, in opposite to psychrophiles which exceed this value at points located near the ASC (activated sludge chambers) and STT (sludge thickening tanks). In the neighborhood of the most efficient emitters of bacteria, the lower percentage of the pigmented ones was observed. The highest number of mold fungi was observed near the activated sludge chambers (ASC), secondary clarifiers (SC), open fermentation chambers (OFC), leeward (LW), and in points located near the primary clarifiers (PC) and sludge plots (SP). These values exceed the level recommended for clean air and reached the level of medium air contamination. The research results indicate the necessity of constant monitoring of the municipal wastewater treatment plants impact on the air quality. Such monitoring should be performed regularly with the frequency of once per quarter of year. To obtain the real state of threats caused by such objects, the monitored parameters, apart from the total number of mesophiles, psychrophiles and mold fungi always should include at least determination of the number of *E. coli* and *P. fluorescens*. Determination of other pathogens expected in municipal sewage should be considered for purposes of this monitoring. This should be done especially during pandemic. A properly planned monitoring of microbiological air quality will be helpful in developing a suitable program of protection of the workers and environmental safety.

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