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Assessment of microbial contamination of atmospheric air in a selected wastewater treatment plant

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Abstract: The aim of the research was to determine the microbiological quality of atmospheric air in the Tuchów Sewage Treatment Plant, based on the presence of mesophilic bacteria, α- and β-hemolytic bacteria, actinomycetes and fungi. Bioaerosol measurements were made at four points (raw sewage inlet, aeration chamber, purified sewage outlet and 150 m from the treatment plant, at the background point) in the period from January to December 2018. Bioaerosol samples were collected using Andersen's 6-stage cascade impactor. The tested atmospheric air was characterized by a qualitatively and quantitatively diverse microflora. The highest amounts of all the studied groups of microorganisms were found at the raw sewage inlet, and in the case of actinomycetes, also twice in the place of biological purification. However, there were analyzes in which a higher concentration of microorganisms was observed outside the treatment plant at the control point constituting the background. This applies to bacteria and fungi. The largest source of emission of microorganisms to the atmosphere was the mechanical part of the sewage treatment plant (raw sewage inlet). The tested treatment plant may therefore contribute to the deterioration of the quality of the atmospheric air.

Introduction

The functioning of a sewage treatment plant is associated with the occurrence of many nuisances, such as noise, odors and pollution. One of the most important threats is the formation of biological aerosols containing numerous microorganisms (Michałkiewicz et al. 2011).

Bioaerosol formed in municipal facilities, including sewage treatment plants, can be a source of pathogens, allergens and toxins (Bawiec et al. 2016, Lee et al. 2016, Miaśkiewicz-Pęska and Szyłak-Szydłowski 2015). Pathogenic microorganisms threaten not only the life and health of humans and animals, but can also lead to contamination of agricultural and urban areas, as well as waters (Adamus-Białek et al. 2015, Przybulewska and Czupryniak 2006, Vantarakis et al. 2016).

Wastewater treatment plants, in addition to the positive role associated with the protection of the natural environment, pose some danger to workers and people permanently present in the facility or in its immediate environment (Nowojewski and Mniszek 2006). This especially applies to people with weakened immune systems, who are exposed to various diseases after entering a large amount of microorganisms in the body (Karwowska et al. 2013, Sánchez-Monedero et al. 2008).

The workers of sewage treatment plants often suffer from digestive system disorders, among others, stomach pain, vomiting, diarrhea, nausea, loss of appetite and stomach ulcers. These symptoms may be associated with the presence of *Helicobacter pylori* in the body, which causes gastritis (Thorn et al. 2002). It is believed that these bacteria may be the cause of gastric cancer (Ahire and Bhalerao 2017). Other symptoms of malaise often occur, such as: headache, muscle and joint pain, increased body temperature, excessive sweating, and tiredness. Workers in wastewater treatment plants may be more likely to suffer from cancer, especially kidney and brain cancer (Thorn et al. 2002, Tiwari 2008).

A high risk to health is also associated with exposure to endotoxins produced by gram–negative bacteria. These substances are harmful to humans and animals, even if they are found in dead bacteria. Endotoxins produced in wastewater treatment plants, composting plants and agricultural industry facilities are also present in the environment. Their concentration depends on climatic and meteorological conditions, season of the year, and technologies used. In sewage treatment plants, the largest amounts of endotoxins occur in the place of sewage sludge storage (Mackiewicz et al. 2015).

It should be noted that the threat is caused not only by the presence of pathogenic microorganisms or toxins of microbial origin in the air, but also the excessive amount of saprophytic bacteria, especially if their composition is little differentiated and dominates organisms of one species. The threat to health and the environment resulting from the emission of bioaerosol makes it necessary to control the microbiological contamination of air in the sewage treatment plant and in its immediate vicinity (Bauer et al. 2002, Korzeniewska 2011, Li et al. 2013).

Considering this state of affairs, the aim of the conducted research was to determine the microbiological quality of atmospheric air in the Sewage Treatment Plant in Tuchów, located in southern Poland, based on the presence of mesophilic bacteria, α- and β-hemolytic bacteria, actinomycetes and fungi.

Sewage Treatment Plant in Tuchów is a small, modern facility equipped with the latest technologies of wastewater treatment. A functioning technological line allows for receiving sewage from areas equipped with a sewage system. Thanks to the expansion of the sewerage network, these areas are becoming more and more attractive to current residents, people planning to settle here and for investors. This has an impact on the intensive development of the commune and increases its economic and tourist values. The extension of the sewerage network allows for connecting more than 10,000 inhabitants. The technological facilities of the sewage treatment plant are located above the level of the great flood from 2010. Thanks to these assumptions, the risk of a major accident of the sewage treatment plant due to flooding has been minimized in relation to the previous state. Currently, breaking the flood embankment does not threaten to flood the equipment involved in the cleaning process.

Small wastewater treatment plants, as low-priority municipal facilities, are often overlooked in the research of threats that may pose to people and the surrounding environment. In connection with the above, in the conducted tests it was checked how large a source of emission of selected microorganisms to the atmospheric air can be a small sewage treatment plant.

Material and methods

The research was carried out in the mechanical-biological Wastewater Treatment Plant in Tuchów (Poland), operating on the basis of the activated sludge method. This sewage treatment plant is located on the Biała River in the south-western part of Tuchów, in the Małopolska province, the Tarnów district. The mechanical part of the treatment plant after extension and modernization completed in December 2013 consists of a main pumping station with a basket grate, storage tank, separation chamber in front of sand catcher, sand catcher, separation chamber for biological treatment strips and drainage point with a tank of transported sewage. The biological part consists of biological reactors with division into 4 chambers: predenitrification, denitrification, dephosphatation and nitrification, separation chamber before secondary settling tanks, secondary settling tanks, excess and recirculated sludge pumping station, pumping station of floating fractions, blowers station, measuring chamber for purified wastewater and technological water pumping station. The sewage is aerated in a fine bubble system. The average daily sewage flow is about 3380 m^3. The sewage treatment plant receives domestic sewage and rainwater from the city of Tuchów and other towns belonging to the Tuchów community.

The bioaerosol assessment was carried out from January to December 2018. Air samples were collected using Andersen's 6-stage cascade impactor at four points: raw sewage inlet (point 1), aeration chamber (point 2), purified sewage outlet (point 3) and a point located 150 m from treatment plant (point 4), this point was the background (Fig. 1).

The impactor sucked in air for 3.5–7 minutes on a 9 cm Petri dish containing a specific solid medium intended for the isolation of selected microorganisms. The volume of air intake was 100 and 200 dm^3. In the case of fungi, 100 dm^3 of air was collected for each sample, for the remaining microorganisms 200 dm^3 each. The device's inlet was always on the windward side. The total fraction of bioaerosol was determined under rainless conditions at a distance of 1 m from the designated research points, from a height of 1.3 m above ground level, i.e., from the human breathing zone. The following microbiological media were used for culturing microorganisms:

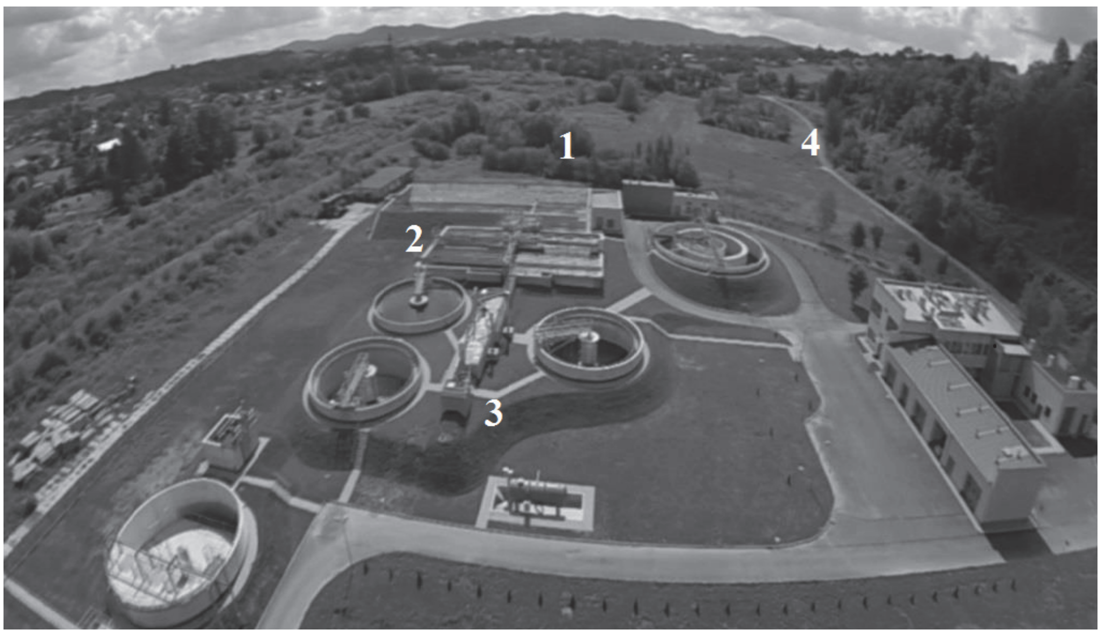

Fig. 1. Location of research points (Dorzecze Białej Spółka Komunalna Sp. z o.o. (2015))

- Tryptic soy agar (TSA LAB – AGARTM, Biocorp, Poland) with 5% addition of sheep blood, in order to determine the total number of bacteria, α- and β-hemolytic bacteria,
- Gauze's medium for isolation of actinomycetes,
- Malt agar (Malt Extract Agar, Biocorp, Poland) for the isolation of mildew fungi.

The TSA plates were incubated for 1 day at 37°C, then 3 days at 22°C and another 3 days at 4°C. The Gauze's plates were incubated at 28°C for 7 days, while MEA plates were incubated for 4 days at 30°C, then 4 days at 22°C. After the incubation period, quantitative analysis of the grown colonies of microorganisms was performed. The bioaerosol concentration was calculated as the number of colony forming units per cubic meter of the air tested (CFU·m^{-3}), separately for each of the groups of microorganisms studied.

To determine the dependence between the ambient temperature and the amount of isolated microorganisms, the Pearson correlation coefficient was calculated using the normal distribution of the analyzed data. The air temperature was measured using the Elmetron PT 105 digital thermometer at a height of about 1.5 m.

Results

At the Wastewater Treatment Plant in Tuchów, the total number of bacteria in individual research points varied from 0 to 510 CFU·m^{-3} (Fig. 2). The largest amount was found in March 2018 in point 1, at the raw sewage inlet (510 CFU·m^{-3}). At the aeration chamber and the outlet of purified wastewater, the largest amount of bacteria was isolated in September 2018 (respectively 495 CFU·m^{-3} and 310 CFU·m^{-3}). At the point constituting the background (point 4), the most of bacteria were found in December 2018 (200 CFU·m^{-3}).

α-hemolytic bacteria were present in almost all air samples tested (Fig. 3). Their numbers ranged from 0–35 CFU·m^{-3}. The largest amount was found in June 2018 at the raw sewage inlet (35 CFU·m^{-3}), near the aeration chamber (25 CFU·m^{-3}) and at the background point (25 CFU·m^{-3}). At the purified sewage outlet (point 3), the most α-hemolytic bacteria were isolated in August and October 2018 (15 CFU·m^{-3}).

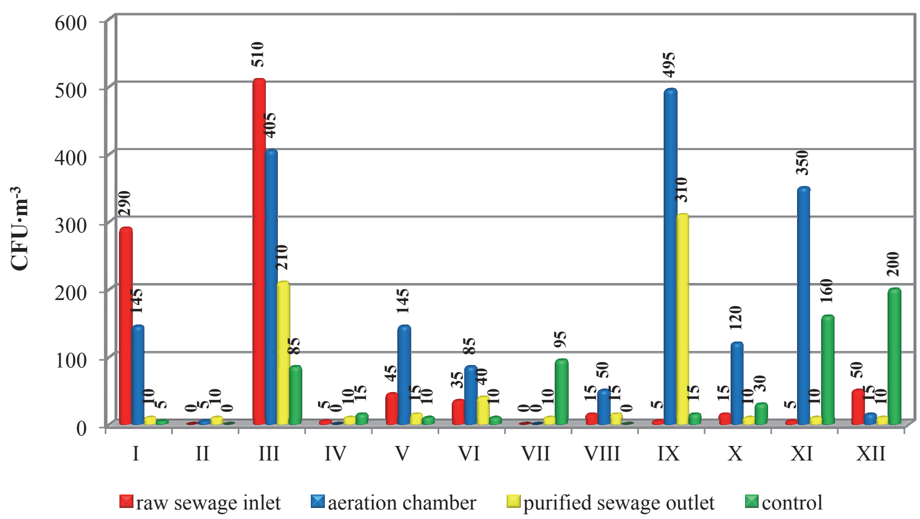

Fig. 2. The number of bacteria in the examined atmospheric air (CFU·m^{-3})

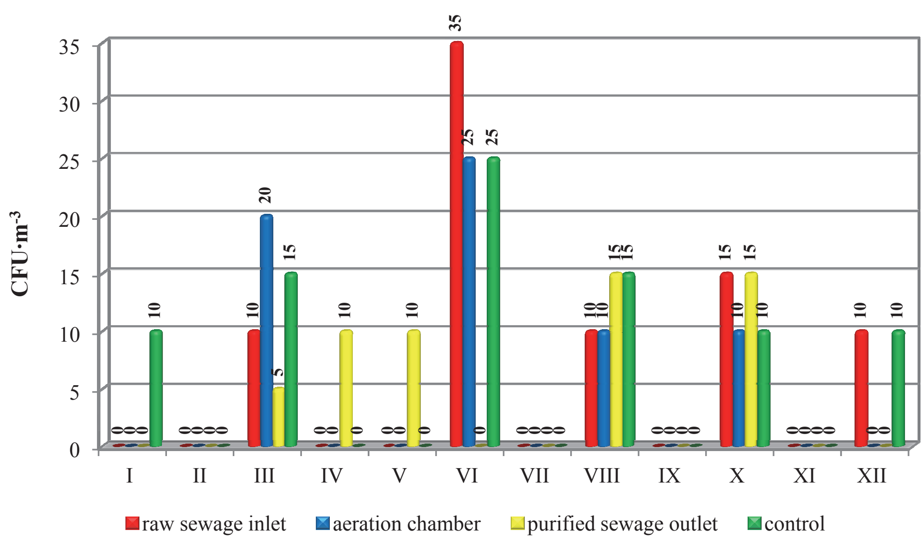

Fig. 3. The number of α–hemolytic bacteria in the examined atmospheric air (CFU·m^{-3})

The amount of β-hemolytic bacteria was at the level of 0–25 CFU·m^{-3}. During 48 analyzes, their presence was observed in 12 of them (Fig. 4). Most often, these microorganisms occurred at point 1 (five times, in the amount from 10 to 25 CFU·m^{-3}), in the largest amount in March and June 2018. Near the aeration chamber (point 2) the number of β-hemolytic bacteria ranged from 0 to 20 CFU·m^{-3}, and at the purified sewage outlet (point 3) from 0 to 10 CFU·m^{-3}. At the point of the background (point 4), these bacteria were not isolated.

The number of actinomycetes in the studied atmospheric air was small, they were isolated only six times, in the amount from 5–25 CFU·m^{-3} (Fig. 5). The largest amount (25 CFU·m^{-3}) was found in October 2018 at the raw sewage inlet (point 1). At the background point (point 4), actinomycetes were not observed in any analysis.

The number of fungi at selected research points varied widely and ranged from 0 to 1165 CFU·m^{-3} (Fig. 6). The highest concentration of mildew fungi was found in March 2018 at the raw sewage inlet (point 1). Near the aeration chamber (point 2) the abundance of these microorganisms ranged from 0 to 985 CFU·m^{-3}, at the purified sewage outlet (point 3) from 15 to 835 CFU·m^{-3}, and at the point of the background from 25 to 810 CFU·m^{-3}.

Discussion

The highest concentration of bioaerosol in the sewage treatment plant is observed near aeration chambers, sand catchers, settling tanks and biological deposits (Li et al. 2013). Penetration of microorganisms into the air takes place mainly as a result of aeration and mixing sewage. The formation of a biological aerosol depends on the concentration of microorganisms in sewage, so in order that microbial cells can reach the air, the number must exceed 10^3·cm^{-3} of wastewater (Bauer et al. 2002, Breza-Boruta and Paluszak 2007, El-Sayed et al. 2015, Kowalski et al. 2017, Polus and Mucha 2019).

In the studies of Filipkowska et al. (2008) and Korzeniewska et al. (2008) no significant differences were found in the number of fungi and bacteria, depending on the location of research points. However, slightly higher amounts

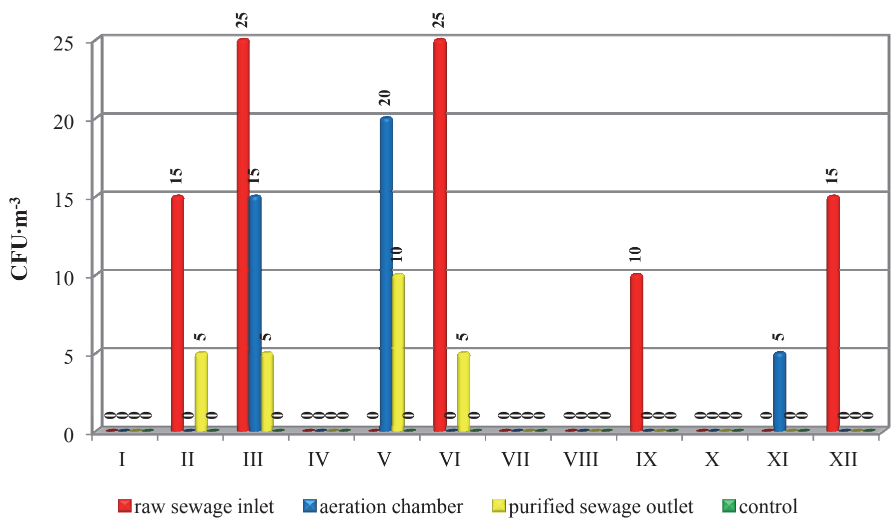

Fig. 4. The number of β-hemolytic bacteria in the examined atmospheric air (CFU·m^{-3})

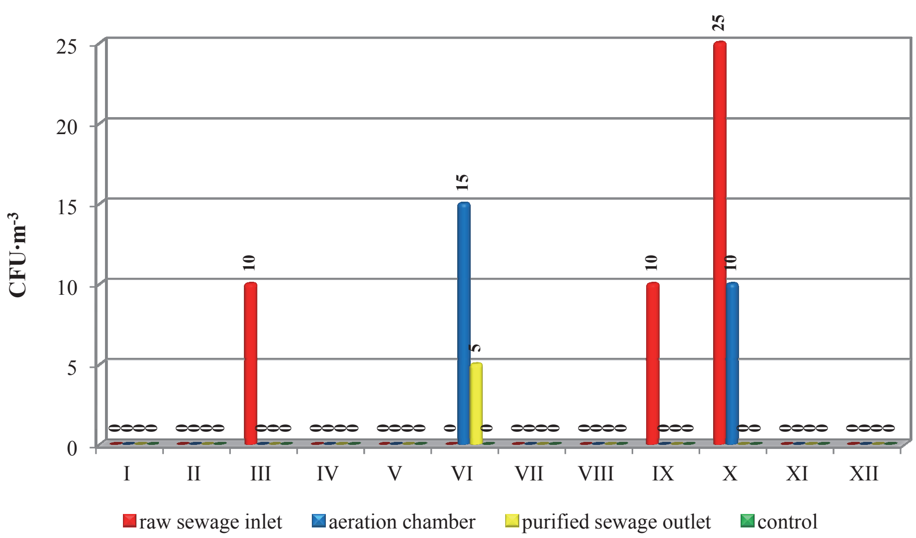

Fig. 5. The number of actinomycetes in the examined atmospheric air (CFU·m^{-3})

of these microorganisms were found in the sewage treatment plant than outside its borders. In the research conducted at the Wastewater Treatment Plant in Tuchów, the highest amount of all tested groups of microorganisms were found at the raw sewage inlet (Fig. 2, 3, 4, 5, 6), and in the case of actinomycetes, also twice in the place of biological treatment (Fig. 5). However, there were analyses in which a higher concentration of microorganisms was observed outside the treatment plant at the control point constituting the background. This applies to bacteria and fungi (Fig. 2, 6). The small size of the Sewage Treatment Plant in Tuchów suggests that the individual research points in its area were located close to each other, which could result in the mixing of pollutants from different sources.

It is widely recognized that weather conditions such as temperature, humidity, insolation, wind speed, rainfall and snow cover have a large influence on the number of microorganisms in the air (Guo et al. 2014, Korzeniewska et al. 2008, Seetha et al. 2013). Breza-Boruta and Paluszak (2007) found that the concentration of microorganisms in the air depends mainly on temperature. In the research conducted at the municipal waste landfill, the highest number of bacteria were isolated in the spring and summer season and the fungi in the autumn season. Niazi et al. (2015) in research conducted in the sewage treatment plant in Tehran showed a significant dependence between the environmental parameters and the concentration of bacteria and fungi in the air. Humidity had the strongest effect on the occurrence of fungi and the air temperature on the occurrence of bacteria. In the case of bacteria, the effect of wind speed and UV index was also found. In studies by Michałkiewicz et al. (2011) the highest concentration of bacteria and fungi was observed in the summer, when the temperature was comparable to spring, but the humidity was higher. Fewer microorganisms occurred at other seasons of the year. Korzeniewska et al. (2008) in studies carried out in the area of the hydrophyte sewage treatment plant and in its vicinity also showed a dependence between the number of microorganisms in the air and the season of the year. The largest amount of bacteria were isolated in summer and the lowest in winter. The exception was the most numerous actinomycetes occurring in spring. In the tests conducted at the Wastewater Treatment Plant in Tuchów, the prevailing ambient air temperature was recorded during sampling. The results obtained with Pearson's correlation coefficient suggest that there was no dependence between the temperature and the amount of bacteria, β-hemolytic bacteria and fungi in the examined atmospheric air. In the case of α-hemolytic bacteria and actinomycetes, this dependence was weak (Table 1).

Among all microorganisms isolated from atmospheric air in the Wastewater Treatment Plant in Tuchów, 25% were found in the analysis performed in winter, 15% in autumn, 21% in spring, and 39% in summer, which suggests a small dependence of their occurrence on the season and temperature. The amount of bacteria in the winter and spring was at a similar level, the least bacteria were observed in the autumn, the most in the summer. Actinomycetes were most abundant in autumn, while fungi in summer.

In addition to climatic factors, the genetic determinants, composition and size of bioaerosol molecules as well as the number of microorganisms and the physiological state have an influence on the survival of microorganisms in the air (Hung et al. 2010, Kvanli et al. 2008, Malakootian et al. 2013). Individual cells have a much lower ability to survive in the atmosphere than the microorganisms included in the biological aerosol. Greater survival in the air is characterized by bacterial and fungal spores, microorganisms that form mucus capsules (*Klebsiella* sp.) and pigments that protect against ultraviolet radiation (*Micrococcus* sp.). Usually gram-negative bacteria are less resistant to adverse conditions than gram-positive bacteria (Kruczalak and Olańczuk-Neyman 2004).

In research conducted by Budzińska et al. (2011) the highest amount of mesophilic bacteria was isolated in the spring and summer period at grids point (2020 CFU·m^{-3}) and in the autumn and winter period at the SBR reactor (1562 CFU·m^{-3}). In own studies, a relatively high concentration of bacteria was also found at the aeration chamber, however, their number was more than three times lower (495 CFU·m^{-3}) (Fig. 2). Large

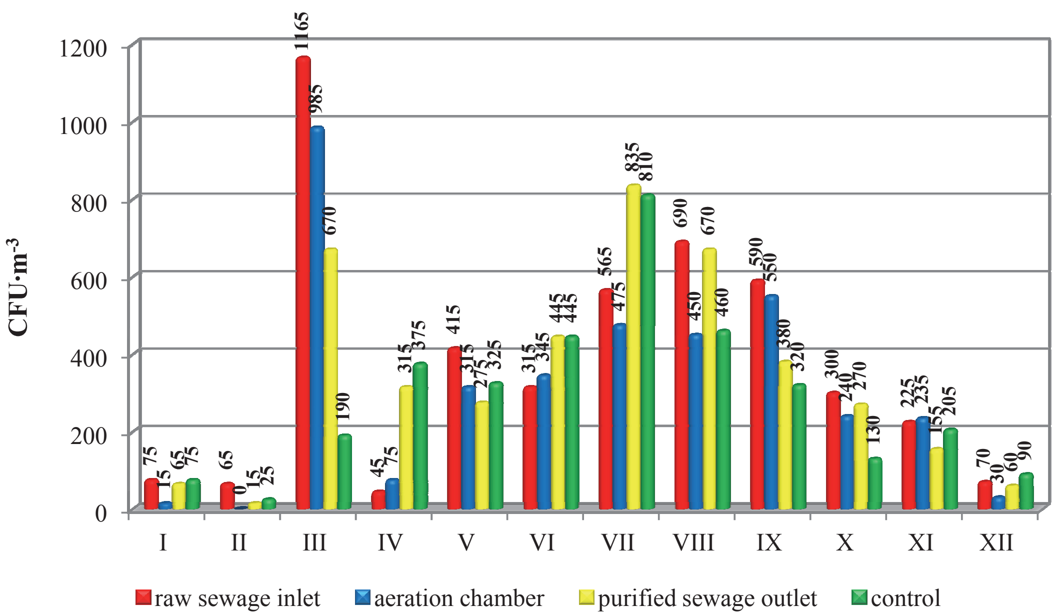

Fig. 6. The number of fungi in the examined atmospheric air (CFU·m^{-3})

amount of bacteria, up to 13,000 CFU·m^{-3}, were isolated by Breza-Boruta and Paluszak (2007) at the sand catcher during the summer. At the points located near the aeration chamber, composting plant and outside the sewage treatment plant, the amount of bacteria was much smaller and amounted to a few hundred CFU·m^{-3}. Bacterial counts exceeding 3000 CFU·m^{-3} were also observed at the aeration chambers and at a distance of 100 m from the treatment plant (Korzeniewska 2011). In the Wastewater Treatment Plant in Tuchów, such high amounts were not found in any of analysis. The largest amount of bacteria (510 CFU·m^{-3}) occurred at the raw sewage inlet. At the control point the number of bacteria was up to 200 CFU·m^{-3} (Fig. 2).

In research conducted by Wlazło et al. (2001), among the bacteria isolated from the air, about 35% were gram-negative bacteria, mainly from the *Enterobacteriaceae* family. The most frequent species were: *Enterobacter agglomerans*, *Acinetobacter calcoaceticus*, *Pseudomonas putida*, *Yersinia enterocolitica*, *Citrobacter freundii*. From the gram-positive bacteria, the following species were present: *Micrococcus*, *Staphylococcus*, *Bacillus*. In the presented studies, α-hemolytic bacteria were most often present in air samples collected in spring months. The highest amounts were observed at the raw sewage inlet (Fig. 3).

Research carried out in many wastewater treatment plants indicates that the largest group of actinomycetes occurs at the composting and sewage sludge storage sites (Breza-Boruta and Paluszak 2007, Budzińska et al. 2011, Budzińska et al. 2013). In the Wastewater Treatment Plant in Tuchów, the actinomycetes were the group of microorganisms that occurred in the air in the smallest quantities (maximum 25 CFU·m^{-3}). They were isolated mainly in spring and autumn (Fig. 5). They were present three times in the first test point (raw sewage inlet), twice in the second (aeration chamber) and only once in the third (purified sewage outlet). At the control point, no actinomycetes were found. In the studies of Budzińska et al. (2013) the number of actinomycetes was higher and amounted to 42 CFU·m^{-3}. In the studies of Breza-Boruta and Paluszak (2007), the concentration of these microorganisms reached even 1040 CFU·m^{-3}. Kołwzan et al. (2012) found that actinomycetes isolated from the air came from sources other than the sewage treatment plant, because there were few of them in the sewage and in the air they were at the level of average pollution. Korzeniewska et al. (2008) found that actinomycetes should not be considered as indicators of pollution when assessing the range of impact of these objects on the atmospheric air, because they are constantly present in the air near the sewage treatment plant. This has not been confirmed in the presented studies conducted in the Tuchów Sewage Treatment Plant, in which this group of microorganisms was detected in less than 13% of the samples, and were not found outside the treatment plant.

Of all the microorganisms in the air, about 70% are fungi, so they are the largest group of microorganisms in the air. This is due to their small requirements in relation to the environment and food, as well as the production of large amounts of spores (Cabral 2010, Breza-Boruta and Paluszak 2007, Małecka--Adamowicz et al. 2017). In own research, fungi accounted for 78% of all the microorganisms isolated in the entire study period. According to Breza-Boruta and Paluszak (2007), the concentration of fungi in the air of the treatment plant is very

Table 1. Dependence between temperature and the number of microorganisms (CFU·m^{-3}) in the examined atmospheric air

The date of sampling	Temperature °C	The number of colony forming units (CFU) in m^3 of the tested air				
		Bacteria	α-hemolytic bacteria	β-hemolytic bacteria	Actinomycetes	Fungi
I 2018	-10	450	10	0	0	230
II 2018	-3	15	0	20	0	105
III 2018	11	1210	50	45	10	3010
IV 2018	-1	30	10	0	0	810
V 2018	21	215	10	30	0	1330
VI 2018	29	170	85	30	20	1550
VII 2018	30	105	0	0	0	2685
VIII 2018	28	80	50	0	0	2270
IX 2018	19	825	0	10	10	1840
X 2018	16	175	50	0	35	940
XI 2018	13	525	0	5	0	820
XII 2018	5	275	20	15	0	250
Average:	13.2	339.58	23.75	12.92	6.25	1320
r:	–	-0.06	0.4	0.1	0.3	0.06

r – Pearson's linear correlation coefficient
<0.2: no linear dependence
0.2–0.4: weak dependence
0.4–0.7: moderate dependence
0.7–0.9: quite strong dependence
>0.9: very strong dependence

often comparable with the concentration in the background air. For this reason, the assessment of air pollution based on this group of microorganisms is not reliable. This is confirmed by studies (Breza-Boruta and Paluszak 2007) in which the highest fungal content (4600 CFU·m^{-3}) was found at the control point located 150 meters from the treatment plant, while in the aeration chambers and in the compost storage area the number of these microorganisms did not exceed 2000 CFU·m^{-3}. Budzińska et al. (2011) also showed that the content of fungi was significant at the control station outside the wastewater treatment plant. At this point, the average number of these microorganisms was higher than at the grit sand catcher and aeration chambers, and comparable to the place of storing sewage sludge. This did not allow confirmation of the impact of the treatment plant on the content of fungi in the air. In the Wastewater Treatment Plant in Tuchów, the number of fungi varied. From 48 research series, in four of them the number of fungi at the control point was higher than at the raw sewage inlet and in seven higher than at the aeration chamber. However, the average number of these microorganisms was similar in all examined points (Fig. 6). Cyprowski et al. (2008) analyzing air samples from four treatment plants showed the highest concentration of fungi near the sewage sludge processing sites and the lowest in the places of biological treatment. In addition, they did not find significant differences between the concentration of fungi in the treatment plant and in the background, but they showed qualitative differences. Research by Filipkowska et al. (2008) suggests that mildew fungi should not be considered as pollutants emitted from wastewater treatment plants because they are commonly occurring microorganisms in the environment. On the other hand, yeasts are characteristic for wastewater and it should be used when assessing the impact of a sewage treatment plant on the sanitary condition of air.

Conclusions

In the Sewage Treatment Plant in Tuchów, diverse bacterial and fungal microflora was found. The highest pollution of atmospheric air with microorganisms occurred at the raw sewage inlet and at the aeration chamber.

The research results indicate that air pollution of microorganisms, including pathogens, occurs in the treatment plant. A bioaerosol formed in this facility can therefore adversely affect human health and the environment.

The conducted research showed a slight variation in the number and quality of microorganisms depending on the location of the research point and the period in which samples were taken. In majority of analyses, the air in the sewage treatment plant was characterized by a higher content of microorganisms than at the control point.

The tested treatment plant may contribute to the deterioration of the quality of the atmospheric air. For this reason, pollution monitoring should be carried out for a longer period of time.

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Ocena skażenia mikrobiologicznego powietrza atmosferycznego w wybranej oczyszczalni ścieków

Streszczenie: Celem prowadzonych badań było określenie jakości mikrobiologicznej powietrza atmosferycznego na terenie Oczyszczalni Ścieków w Tuchowie, na podstawie występowania bakterii mezofilnych, bakterii α- and β-hemolizujących, promieniowców i grzybów. Pomiary bioaerozolu wykonano w czterech punktach (wlot ścieków surowych, komora napowietrzania, wylot ścieków oczyszczonych oraz 150 m od oczyszczalni, w punkcie stanowiącym tło) w okresie od stycznia do grudnia 2018 r. Próbki bioaerozolu pobierano za pomocą 6-stopniowego impaktora kaskadowego Andersena. Badane powietrze atmosferyczne charakteryzowało się zróżnicowaną ilościowo i jakościowo mikroflorą. Najwyższe ilości wszystkich badanych grup mikroorganizmów stwierdzono przy wlocie ścieków surowych, a w przypadku promieniowców również dwukrotnie w miejscu biologicznego oczyszczania. Zdarzały się jednak analizy, w których wyższe stężenie drobnoustrojów obserwowano poza terenem oczyszczalni, w punkcie kontrolnym stanowiącym tło. Dotyczyło to bakterii i grzybów. Największym źródłem emisji mikroorganizmów do atmosfery była mechaniczna część oczyszczalni (wlot ścieków surowych). Badana oczyszczalnia może więc przyczyniać się do pogorszenia jakości powietrza atmosferycznego.