JEE Journal of Ecological Engineering

Journal of Ecological Engineering 2022, 23(2), 185–190
https://doi.org/10.12911/22998993/145202
ISSN 2299–8993, License CC-BY 4.0

Received: 2021.11.29
Accepted: 2021.12.20
Published: 2022.01.01

Microbiological Quality of Indoor and Outdoor Air in a Municipal Wastewater Treatment Plant – A Case Study

Amelia Staszowska[1]

[1] Department of Indoor and Outdoor Air Quality, Faculty of Environmental Engineering, Lublin University of Technology, ul. Nadbystrzycka 40B, 20-618 Lublin, Poland

e-mail: a.staszowska@pollub.pl

ABSTRACT

Wastewater treatment plants (WWTPs) have been recognized as a source of odors and microbial pathogens to the outdoor air. The results of many studies revealed that high amounts of microorganisms are not only present in the stream of wastewater or sludges but also in the bioaerosols that are generated during the different stages of the wastewater treatment. Hence, possible migration of biological contaminants into the interiors cannot be excluded. However, there is a knowledge gap in an assessment of the microbiological indoor air quality of the facilities located at WWTPs. The aim of this study was to evaluate the level of outdoor microbial air contamination upon the indoor environment based on the determined outdoor to indoor (O/I) ratios. The sampling of airborne bacteria and fungi was conducted in three replications with the sedimentation and impaction method, during a one-year survey in ten technological and office buildings as well as their vicinity, at the municipal wastewater treatment plant of Lublin (Poland). Moreover, the cleanliness of hand contact surfaces in staff rooms was examined (Rodac plates). Additionally, API identification of bacteria and fungi was carried out. The highest concentration of total bacteria count (3617 CFU/m^3) and fungi in bioaerosols (5386 CFU/m^3) was detected in the air around the sewage pumping station, close to the aeration tanks. *P. fluorescens* was found in the air around the grit chamber (78 CFU/m^3). The majority of the examined indoor air samples were characterized with different levels of microbiological contamination – from non-polluted to moderately polluted. The number of total bacteria counts ranged from 180 to 4679 CFU/m^3. The highest estimated indoor fungi concentration was 4022 CFU/m^3. The controlled surfaces were mostly contaminated with the *Actinomycetes* and *Coliform* bacteria. No *Salmonella* sp. were detected. The bacteria from the *Enetrobacteriaceae* family were commonly isolated from the indoor and outdoor air samples. The obtained data can be used to devise further guidelines facilitating control and management of WWTP to avoid or minimize the staff exposure.

Keywords: microbial air quality, wastewater treatment plant, bioaerosols.

INTRODUCTION

The facilities of municipal wastewater treatment plants (WWTPs) are a recognized source of emission to the air of bacterial and fungal bioaerosols and pathogenic microorganisms (viruses, protozoa) [Li et al. 2016]. Bioaerosol emissions take place during the wastewater treatment process and the treatment of sewage sludge. The emission of microbiological pollutants by wastewater treatment plants depends on: the content of microorganisms in raw wastewater, the technology of wastewater treatment used, including the method of aeration and the method of sewage sludge management in particular. The meteorological conditions (temperature, wind direction and speed, the thermodynamic equilibrium of the atmosphere) are equally important, as they influence the spread of bioaerosol in the area surrounding the WWTP [Guo et al., 2014].

The source of microorganisms involves both the sewage itself and the products of its treatment, i.e. sewage sludge and gases. Especially at various stages of processing, such as compaction, drying, dewatering on presses [Fathi et al., 2017; Li et al., 2011]. Due to the climatic conditions, the highest bioaerosol emission in the treatment plant occurs in the summer months and the lowest in winter [Fraccia et al., 2006; Niazi et al., 2015]. During the wastewater treatment process, the highest concentrations of bacteria are recorded near the grit chambers in the mechanical part of the treatment plant and in the vicinity of the activated sludge chambers in the biological part, which are intensively aerated [Yang et al., 2019; Wang et al., 2019].

In the case of the sludge part of the wastewater treatment plant, the highest emission of airborne microorganisms occurs at the dewatering stations [Han et al., 2018]. Despite the proven harmful effect of microorganisms emitted in the area of wastewater treatment plants, in many countries, including Poland, there are still no regulations regarding the permissible concentrations of bioaerosols in the outdoor air. In Poland, the wastewater treatment plants operating in a mechanical and biological system are dominant. The most common types of air-tight sealing are the grit chambers, sand traps, sludge thickeners and preliminary settling tanks. On the other hand, the biological part, involving the activated sludge chambers, where the processes of aeration and mixing of wastewater take place, are the objects open to the atmosphere. These emissions are well known and documented in the scientific literature [Lou et al., 2021; Singh et al., 2021;Yang et al., 2019]. However, there are no scientific reports on the influence of bioaerosols from the outdoor air on the microbiological quality of indoor air in the premises of WWTP, including the rooms where the staff is present. In the case of WWTP workers, there is a possibility of occupational exposure during the inhalation of bacterial and fungal bioaerosols or contact through the skin. Endotoxins and exotoxins of gram-negative bacteria and peptidoglycans of gram-positive bacteria are also dangerous biological factors present in the bioaerosols emitted from wastewater. They cause inflammatory reactions and gastric problems. On the other hand, fungal mycotoxins have mutagenic, carcinogenic and hepatotoxic properties [Liu et al., 2020; Lu et al., 2020]. This paper presents (i) evaluation of outdoor and indoor air contamination by different types of microorganisms emitted during technological operations at the municipal wastewater treatment plant and (ii) results of surface cleanliness control in technological and social rooms located in the area of this facility.

MATERIALS AND METHODS

Microbiological air quality tests were carried out in 12 monthly measurement series in the municipal mechanical – biological wastewater treatment plant (WWTP) located in the Lubelskie Voivodeship, Poland. The average daily sewage flow is approx. 90,000 m^3/d. The treatment plant covers an area of 63 ha. The facility was put into operation in 1992. Since then, the treatment plant has been constantly modernized. The studied plant treats only household wastewaters from city of 350 000 inhabitants and a few surrounding villages.

Description of sampling points

The technological facilities where the bioaerosol samples were collected were designated by the management of the WWTP. The air samples were collected at the following points: grit chamber (S1), raw sludge pumping station (S2), work room under mesophilic anoxic digester (S3), duty room at the blower station (S4), excess sludge thickening station (S5), centrifuge station (S6), thermal sludge drying station (S7), work room at the thermal sludge drying station (S8), duty room at the main entrance in administrative building (S9). For each of the measuring points, the concentration of bioaerosols in the outdoor air was determined, the so-called background (windward side). Additionally, the cleanliness of the surfaces was checked in the work rooms. In the case of the grit building, the control panel was examined, and desk tops were checked in the social rooms.

Bacterial and fungal aerosol air sampling

The outdoor and indoor air samples were collected to assess the presence of selected physiological groups of indicator airborne microorganisms. The air samples for microbiological analyses were collected using the sedimentation and impaction method (MAS 100 Eco air sampler, Merck). In addition, in the crew work rooms, measurements were made with the six-stage Andersen impactor in order to determine the particle distribution of the inhaled fraction. However, the results of these studies are not discussed in this paper. All samples were collected 1.5 m above the ground or floor surface, i.e. in the breathing zone. The microbiological tests for the presence of bacteria and fungi (media, culture conditions and quantification) were performed in accordance with the guidelines contained in the Polish standards PN-89/Z-04111/02 and PN-89/Z-04111/03 (Table 1). At least three sets of plates with an appropriate culture media were used in each of the measuring points located inside and outside the buildings. The sampling time was assumed to be 20 minutes.

In qualitative (taxonomic) analysis, the grown colonies of bacteria and Actinomycetes that dominated in the studied environment were identified by means of additional biochemical API tests (Biomerieux) in order to determine their systematic affiliation. Moreover, the surface cleanliness was controlled with the use of RODAC contact plates (55 mm). Relative humidity (%), indoor and outdoor air temperature (°C), as well as wind speed and direction were recorded during survey. The measurements of bioaerosol emissions in the outdoor air were not performed when the outdoor temperatures fell below 5 °C or during rainy and very windy weather.

Table 1. Media used in the detection of studied microorganisms (Biomaxima, Poland)

Indicator microorganisms	Medium
Heterotrophic bacteria	TSA agar
Hemolytic bacteria	Sheep blood agar
Mannitol-positive and mannitol-negative staphylococcus	Chapman agar
Actinomycetes	Pochon medium
Pseudomonas fluorescens	King B agar
Fungi (including yeast and filamentous species of molds)	Sabouraud agar
Enterobacteriaceae[a]	Mac Conkey agar

[a] – Not included in Polish standards.

RESULTS AND DISSCUTION

The assessment of the microbiological purity of the outdoor air has not been legally regulated in Poland to this day. Despite the withdrawal of the PN-89/Z-04111/02 and PN-89/Z-04111/03 standards in 2015, they have not been replaced by new documents to date. However, in the case of indoor air quality, such provisions have never been in force. Only the recommendations of the maximum concentration limits are available, which, however, are not supported by legal acts.

The results from this study indicated that the WWTP could be a source of airborne microorganisms to the air (Tables 2 and 3) and be a cause of occupational exposure to workers. However, comparing the results from this study with the reports of other Polish authors, the determined concentrations of bioaerosols were at low levels [Korzeniowska et al., 2009; Michałkiewicz et al., 2018; Szylak-Szydłowski et al., 2016].

The total number of bacteria and fungi determined in the outdoor air allows classifying the air quality as moderately polluted to non-polluted. Seasonal changes in the concentration of the determined bioaresols are similar to the results of the works of other researchers [Michałkiewicz et al., 2018]. Under temperate climate conditions, the highest concentrations will occur in the summer months and the lowest in winter. Regardless of the season, the highest concentrations of bioaerosols were recorded for the air in the room of the grit chamber and the sewage sludge dewatering station as well as the sludge drying plant and

Table 2. Total number of bacteria (range min-max in CFU/m^3)

Sampling point	Spring	Summer	Autumn	Winter
S1 O I	644–803 1494–1850	336–453 2846–3326	681–844 1544–1982	322–410 826–1120
S2 O I	560–786 383–550	3260–3617 844–1106	835–1887 653–826	621–786 216–275
S3 O I	997–1258 390–590	1990–2583 1148–1309	2104–3051 880–1140	299–432 180–248
S4 O I	662–865 348–535	1690–1926 770–904	1040–1282 491–613	367–550 210–220
S5 O I	456–786 329–444	1563–2084 2989–4679	1230–1515 853–1152	272–334 205–314
S6 O I	549–609 562–798	2018–2241 780–916	1042–1317 612–798	234–287 302–452
S7 O I	1532–1730 1874–2068	2560–2791 3358–4073	1430–1532 1939–2280	853–1022 1207–1400
S8 O I	1089–1230 1203–1395	2100–2318 1539–1845	1085–1252 856–932	340–743 219–294
S9 O I	885–1073 558–641	1021–2044 1230–1985	748–963 544–907	844–924 432–629

Note: O – outdoor air, I – indoor air.

Table 3. Total number of fungi (range min-max in CFU/m^3)

Sampling point	Spring	Summer	Autumn	Winter
S1 O	421–3283	130–3450	459–3774	664–740
I	722–1270	430–786	330–521	135–410
S2 O	553–747	3290–5386	624–798	399–511
I	302–484	1356–1612	890–1596	245–484
S3 O	667–885	2453–3066	4120–4934	536–649
I	502–680	724–916	1890–2186	220–273
S4 O	245–393	2540–2712	2856–3263	310–389
I	250–338	865–1152	437–980	270–340
S5 O	560–649	894–1225	1893–2044	298–433
I	210–326	3658–4200	560–731	402–523
S6 O	1190–1317	3053–4072	2784–3400	321–432
I	810–955	1540–1769	1204–1540	240–373
S7 O	459–649	6504–8099	720–944	325–540
I	2163–2398	3352–4022	2001–2422	289–444
S8 O	230–534	1399–1754	1490–1534	301–399
I	129–186	548–673	590–749	145–193
S9 O	1100–1455	2395–3557	3421–4011	1162–1258
I	1230–1612	998–1228	1240–1409	990–1120

Note: O – outdoor air, I – indoor air.

their surroundings. These emissions are caused by the technological processes carried out at the stage of sewage treatment and sludge management in the WWTP [Xu et al., 2018].

Indoor air samples were collected between 12 p.m. and 2 p.m. on a normal working day. The windows in the rooms were closed from the evening of the previous day. All crew rooms and quarters were equipped with gravity ventilation. There were 4 service stations in each of the rooms. Thus, it can be assumed that the only source of bioaerosols could be outdoor air infiltration or workers' clothing.

Hemolytic staphylococci were not present in the indoor or outdoor air. On the other hand, mannitol negative staphylococci were common in the concentrations ranging from 52 to 472 CFUm^3. This allows evaluating the air quality as heavily polluted. Two measuring points where their presence was not recorded included the sludge pumping station and a work room at mesophilic anoxic digester. The highest concentrations of these organisms were detected in the indoor air samples at the thermal sludge drying station (average 250 CFU/m^3). *P. fluorescens* was measured sporadically, i.e. in one out of 12 measurement series, and the concentrations did not exceed 78 CFU/m^3. On the other hand, the bacteria from the *Enterobacteriaceae* family were common, both in indoor and outdoor air. They originate from treated sewage and dewatered sludge. In the outdoor air, they ranged from 230 to 1120 CFU/m^3. In the case of indoor air, they were also a common component of bioaerosols. However, their concentrations did not exceed the value of 52 CFU/m^3.

The Polish regulations governing occupational health and safety conditions for the people

Table 4. The results of the assessment of surface cleanliness control in technological and social rooms (CFU/plate)

Sampling point	Bacteria	Fungi	*Coli* form	*Enetrobacteriacea*	Staphylococci
Grit chamber – control panel	3–12	5–7	0	0–2	0–1
Social room next to blower station	0–33	11–47	0–3	0–11	0–4
Social room next to excess sludge dewatering station	50–106	5–44	0–2	1–4	2–62
Social room next to thermal sludge drying station	67–91	49–59	0–6	0–12	11–38
Work room with mesophilic anoxic digester	0–12	0–5	0–2	0–2	0–3
Duty room at the main entrance	10–46	8–20	0–2	0–2	21–33

Note: MIN - MAX.

employed in wastewater treatment plants do not include surface cleanliness testing. Thus, it is not a routine procedure used on the premises of such facilities. The results of the conducted measurements are presented in Table 4. Similar concentrations of the determined indicator organisms were obtained in all the measurement series.

Single coliform bacteria were detected in the samples from each series. The presence of *Salmonella* and *Pseudomonas* was not recorded. The presence of intestinal bacteria and staphylococci can be defined as numerous. During determination of the microbiological safety, the duty room at the main entrance to the administration building and the social room at the thermal sewage sludge drying station turned out to be the least clean of the controlled areas. This state of affairs can be explained by the lack of sufficient hygiene among employees or insufficient cleaning of the surface. It is also possible that an additional source of bioaerosols in the tested rooms may be the clothing of employees, on the surface of which they may have deposited during the work near technological facilities. Ultimately, periodic disinfection of these rooms should be considered.

The identification of selected bacterial and fungal strains grown on the media used in the experiment was carried out in the collected samples of indoor and outdoor air bioaerosols. Identification of bacteria was based on microscopic observation of the preparations stained with the Gram method, determination of biochemical properties and API tests (Biomerieux). In the case of fungi, the identification was carried out on the basis of the macroscopic and microscopic appearance of the grown fungal colonies based on the taxonomic key.

In all measurement series carried out at the WWTP, cocci and gram-positive rods were the most common bacteria in indoor and outdoor air. *Microccocus luteus* and other species of the genus *Micrococcus* were the most common cocci in all the measurement series. *Kocuria rosea, Kocuria kristinae* and *Kocuria varians* were common species of this genus. Kernels belonging to the genus *Staphylococcus* have also been identified. The most abundant staphylococci species were *Staphylococcus simulans* and *Staphylococcus xylosus*. Species such as *Staphylococcus haemolyticus*, *Staphylococcus hominis, Staphylococcus cohnii ssp.* Urealyticus or *Staphylococcus capitis* also appeared frequently. The *Staphylococcus aureus* species were also found in the indoor and outdoor air. Other identified species included: *Aerococcus viridans*, *Aerococcus urinae, Enterococcus avium*, *Gemella haemolysans* and *Leuconostoc spp*. In the conducted studies, Gram-positive spores of the genus *Bacillus* were dominant in the total number of bacteria. The most common species were Bacillus subtilis, *Bacillus cerus* and *Bacillus lincheniformis*. In addition to the species mentioned, strains such as *Bacillus pumilis* and *Bacillus megaterium* were also found. Bacteria of the genus *Microbacterium*, *Brevibacterium* and *Rhodococcus* were also identified in the air. The air also contained non-enteric gram-negative rods. The most common species were *Pseudomonas fluorescens* and *Aeromonas hydrophila*. The other bacteria in this group are: *Aeromonas sobria, Pseudomonas stuzeri, Sphingomonas paumobilis, Moraxella spp.,* and *Agrobacterium radiobacter*. The presence of Gram-negative rods belonging to the *Enterobacteriaceae* family in the air was checked on MacConkey and ENDO medium. After the Api E tests, the following strains were identified: *Pantoea spp., Burkholderia cepacia, Ewingella americana, Citrobacter freundii, Serratia rubidaea* and *Escherichia coli*. The species of the genus *Pantoea* were the most numerous. In the case of fungi, in all the studies carried out, the most common strains of the genus *Penicillium, Alternaria, Cladosporium* and *Aspergillus* were found. There were also fungi of the genus *Mucor, Fusarium, Bipolaris, Chaetomium Rhizopus*, and yeasts.

CONCLUSIONS

In this study, the outdoor and indoor bioaerosol concentrations at the premises of WWTP were determined. The research results can be summarized as follows. In the outdoor air, the highest concentrations of bacteria were recorded in the summer months and the lowest in winter. The highest concentrations of bacterial and fungal bioaerosol were found in the room of the grit chamber building and sludge dewatering station (next to aerations chambers). The number of microorganisms recorded in indoor and outdoor air allows classifying the air as moderately to non-polluted. In the indoor air, the species composition of microorganisms was unstable and differed from season to season.

REFERENCES

1. Fathi S., Hajizadeh Y., Nikaneen M., Gorbani M. 2017. Assessment of microbial aerosol emissions in an urban wastewater treatment plant operated with activated sludge process. Aerobiologia, 33, 507–515.
2. Fraccia L., Pietronave S., Rinaldi M., Martinotti M.G. 2006. Site-related airborne biological hazard and seasonal variations in two wastewater treatment plants. Water Research, 40, 1985–1994.
3. Guo X., Wu P., Ding W., Zhang W. Li L. 2014. Reduction and characterization of bioaerosols in a wastewater treatment station via ventilation. Journal of Environmental Science, 26, 1575–1583.
4. Han Y., Wang Y., Li L., Xu G., Liu J., Yang K. 2018. Bacterial population and chemicals in bioaerosols from indoor environment: Sludge dewatering houses in nine municipal wastewater treatment plants. Science of the Total Environment, 618, 469–478.
5. Korzeniowska E., Filipkowska Z., Gotkowska-Płachta A., Janczukowicz W., Dixon B., Czułowska M. 2009. Determination of emitted air borne microorganisms from a BIO-PAK wastewater treatment plant. Water Research, 43, 2841–2851.
6. Li J., Zhou L., Zhang X., Xu C., Dong L. 2016. Bioaerosol emissions and detection of airborne antibiotic resistance genes from a wastewater treatment plants. Atmospheric Environment, 12, 404–412.
7. Li L., Gao M., Liu J. 2011. Distribution characterization of microbial aerosols emitted from a wastewater treatment plant using the Orbital oxidation ditch process. Process Biochemistry, 46, 910–915.
8. Liu M., Nobu M., Ren J., Jin X., Hong G., Yao H. 2020. Bacterial composition in inhalable particulate matters from indoor and outdoor wastewater treatment processes. Journal of Hazardous Materials, 385, 121515.
9. Lou M., Liu S., Gu C., Hu H., Tang Z., Zhang Y., Xu C., Li F. 2021. The bioaerosols emitted from toilet and wastewater treatment plant: a literature review. Environmental Science and Pollution Research, 28, 2509–2521.
10. Lu R., Fredriksen M., Uhrbrand K., Li Y., Østergaard C., Madsen A.M. 2020. Wastewater treatment plant workers' exposure and methods for risk evaluation of their exposure. Ecotoxicology and Environmental Safety, 205, 111365.
11. Michałkiewicz M., Kruszelnicka I., Widomska M. 2018. The variability of the concentration of bioaerosols above the chambers of biological wastewater treatment. Ecological Chemistry and Engineering S, 25(2), 267–278.
12. Niazi S., Hassanvand M.S., Mahvi A.H., Nanizadeh R., Alimohammadi M., Nabavi S. 2015. Assessment of bioaerosol contamination (bacteria and fungi) in the largest urban wastewater treatment plant in the Middle East. Environmental Science and Pollution Research, 22(20), 16014–16021.
13. Polish Standard PN-89/Z-04111/02. Protection of air cleanness. Microbiological study. Enumeration of bacteria in atmospheric air during samples collection by impact and sedimentation methods.
14. Polish Standard PN-89/Z-04111/03. Protection of air cleanness. Microbiological study. Enumeration of fungi in atmospheric air during samples collection by impact and sedimentation methods.
15. Singh N.K., Sanghvi G., Yadav M., Padhiyar H., Thanki A. 2021. A state-of-the-art review on WWTP associated bioaerosols: Microbial diversity, potential emission stages, dispersion factors, and control strategies. Journal of Hazardous Materials, 410, 12686.
16. Szylak-Szydłowski M., Kulig A., Miaśkiewicz-Pęska E. 2016. Seasonal changes in the concentrations of air borne bacteria emitted from a large wastewater treatment plant. International Biodeterioration & Biodegradation, 115, 11–16.
17. Xu G., Han Y., Li L., Liu J. 2018. Characterization and source analysis of indoor/ outdoor culturable airborne bacteria in a municipal wastewater treatment plant, 74, 71–78.
18. Yang K., Li L., Wang Y., Xue S., Han Y., Liu J. 2019. Airborne bacteria in a wastewater treatment plants: Emission characterization, source analysis and health risk assessment. Water Research, 149, 596–606.
19. Yang T., Han Y., Liu J., Li L. 2019. Aerosols from wastewater treatment plants using oxidation ditch process: characteristics, source apportionment, and exposure risks. Environmental Pollution, 250, 627–638.
20. Wang Y., Li L., Xiong R., Guo X., Liu J. 2019. Effect of aeration on microbes and intestinal bacteria in bioaerosols from the BRT of an indoor wastewater treatment facility. Science of the Total Environment, 648, 1453–1461.