

Airborne Bacteria from Wastewater Treatment and Their Antibiotic Resistance: A Meta-Analysis

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ABSTRACT

The treatment of domestic and industrial wastewater is a source for a large number of airborne microorganisms, which can be released at the environment during mechanical aeration. A meta-analysis of up to 14 industrial and municipal treatment plants was performed to determine the bacterial count in bioaerosols, as well as antibiotic resistance, at different stages of the wastewater treatment (pretreatment, primary, secondary and tertiary treatment), in comparison with bacterial counts of the ambient air surrounding the treatment plants. The highest contamination of the air with microorganisms was observed in the raw sewage inlet and at the biological reactor. In most analyzes, the air in the wastewater treatment plant was characterized by a higher content of microorganisms than at the control point. Bioaerosols from water treatment might be an important source of antibiotic resistance genes, which can be transported considerable distances and can represent a potential risk to humans. Therefore, employees should recognize the health risks associated with the absence of personal protective equipment, such as masks or respirators.

Keywords: bioaerosols, resistome, SARS-CoV-2, meta-analysis

INTRODUCTION

Bioaerosols are atmospheric microparticles suspended or attached to dust or water droplets (Burdsall et al., 2021; Calero-Cáceres et al., 2019; Lou et al., 2021). They are composed of fungi, bacteria, viruses, pollen, plant debris, endotoxins, allergens and mycotoxins and originate in a wide variety of productive activities: markets, slaughter of animals, waste management, wastewater treatment; indoor activities in hospitals and laboratories (Bruni et al., 2019; Burdsall et al., 2021). Bioaerosols can be transported long distances due to their tiny size (1 to 100 nm), as well as environmental effects: temperature, humidity, wind speed, and molecular diffusion (Moran-Zuloaga et al., 2021; Yadav et al., 2020). Exposure to bioaerosols has been associated with various public health effects, including infectious diseases, allergies, and cancer (Rocha-Melogno et al., 2020;

Song et al., 2021). Wastewater carries different pathogenic and non-pathogenic microorganisms that can be dispersed in the environment. Workers who frequent wastewater treatment plants may therefore be exposed to aerosols containing a high concentration of potentially dangerous biological agents, or they may come into direct contact with contaminated material. Pathogenic bacteria such as *Klebsiella pneumoniae*, *Legionella* spp., *Acinetobacter* spp. have been detected in bioaerosols from water treatment plants (Górny, 2020).

Currently, bacterial superinfection in intensive care units (ICU) has intensified due to outbreaks of acute respiratory syndromes such as SARS-CoV-2 (Bardi et al., 2021; Elsamadony et al., 2021). And due to the lack of data on technological measures for disinfection and sterilization, and the lack of information for the general public, the capacity for antibiotic resistance remains. Thus, the optimal control of pathogens

with antibiotic resistance is of public health concern. Since the beginning of the coronavirus pandemic (COVID-19), great attention has been paid to the spread of SARS-CoV-2 in bioaerosols. At the same time, the presence of SARS-CoV-2 in wastewater it is believed that contributes to the spread of the disease (Han & Yoo, 2020; Robotto et al., 2021).

It is hypothesized that the intensive use of antibiotics in the clinical, industrial and agricultural sectors has fostered the evolution of bacteria resistant to these drugs since their first deployment since the 1930s (Robertson et al., 2019). However, industrial antimicrobial agents (detergents, soaps, and other cleaning products) add to the causes of this resistance (Smets et al., 2016). Given the problem raised, herein this review article, through analysis of scientific publications of the last 20 years, will focus on evaluating causes, effects and case studies on antibiotic resistance of microorganisms contained in bioaerosols, from wastewater treatment plants.

META-ANALYSIS OF AIRBORNE BACTERIA

The peer-reviewed literature was systematically searched to identify quantitative studies on the presence and concentration of bacteria in the environment of domestic and industrial wastewater treatment plants. Published articles were selected through ISI Web of Science Core Collection and Elsevier Scopus from the last 20 years.

Three criteria to select information were used. Criterion 1: articles were searched with results of bacterial counts in colony forming units (CFU) for each cubic meter of ambient air in industrial and domestic wastewater treatment plants. Studies related to spores of fungi and other species, as well as laboratory experiments, and microcosm were excluded. Criterion 2: for the meta-analysis, the bacterial count in different stages of the wastewater treatment (pretreatment, primary, secondary and tertiary treatment) was compared based on the bacterial count of the ambient air surrounding the treatment plants. Only studies with very strong evidence were included, as a means of ensuring the high quality of the results. Criterion 3: studies related to the identification of genes with antibiotic resistance of bacteria present in the particulate material of treatment plants were selected.

ORIGIN OF ANTIBIOTIC RESISTANCE

Most antibiotics are bioactive compounds, synthesized either by bacteria (mainly actinobacteria such as *Streptomyces spp.*) or fungi, most of them coming from the soil (Nesme & Simonet, 2015). Antibiotics are among the most widely used drugs worldwide, with more than 70 billion doses administered in 2010, representing a 36% increase in consumption compared to 2000 (Crofts et al., 2017). These medications are invaluable due to their ability to cure bacterial infections. However, since their massive application in the 1930s, microorganisms have generated antibiotic resistance, which represents a serious global threat of growing concern to human and animal health (Wright, 2007). Thus, the term “resistome” is referred to as the collection of antibiotic resistance genes (ARGs) (Sultan et al., 2018).

Causes of antibiotic resistome are overpopulation, excessive use of antibiotics in clinics and in animal production, poor waste management, and a poor sewage system (Aslam et al., 2018). In addition, it is due to the appearance, spread and persistence of multi-resistant bacteria or “superbugs”. However, the microbial defense mechanisms against antibiotics date back millions of years, according to metagenomic analysis of ancient DNA collected from Beringia permafrost, where the presence of genes for resistance to the antibiotics β -lactams, tetracyclines and glycopeptides was demonstrated (Perry et al., 2014; Crofts et al., 2017).

Soil is one of the largest and most diverse microbial habitats on earth, and is increasingly recognized as a vast deposit of ARG (Forsberg et al., 2012). Therefore, it is highly relevant to investigate whether ARGs are the result of rapid adaptation through gene mixing or mutation, or are rather acquired through horizontal gene transfer (Perry et al., 2014).

Wastewater treatment plants

The wastewater treatment is composed of (i) pretreatment, where the wastewater comes from the sewer systems and then coarse solids are removed; (ii) primary treatment to remove suspended solids by physical (sedimentation) and chemical methods (coagulation-flocculation); (iii) secondary treatment to remove suspended and dissolved solids by anaerobic and aerobic processes; (iv) finally, tertiary treatment to remove dissolved

particles through chemical oxidation with chlorine or ozone, and physical treatments with filtration (Lou et al., 2021). Workers and nearby residents are vulnerable to wastewater treatment plants (WWTPs) because they are exposed to bioaerosols that can contain a wide variety of bacterial, viral and fungal species; therefore, exposure to bioaerosols in WWTPs generates occupational health risks for workers. The application of sludge from wastewater treatment and animal manure to agricultural lands to improve their properties and fertility, is also considered important sources of heavy metals, antibiotics and ARGs (Calero-Cáceres et al., 2019).

In developing countries, the treatment of wastewater is performed by oxidation ponds, where not quality and environmental control whatsoever is taken. In Figure 1, there is an example of an oxidation pond from an Ecuadorian food industry, which odors are a big concern for the community. All chemicals, organic matter and grease from that Ecuadorian industry are therein incorrectly disposed without proper wastewater management.

Bacterial count

It must be considered that only up to 15% of the microbial cells (viable microorganisms) transported in the air can be enumerated; the remaining species, which are viable but not cultivable,

cannot be isolated and identified (Elsamadony et al., 2021; Nair, 2021; Song et al., 2021). Due to cultivable bacteria represent only up to 20% of the total bacterial diversity, more recent studies in the literature have focused on DNA-based detection systems, which allow the examination of all microorganisms (live, dead and non-culturable) (Tian et al., 2020; Xu et al., 2020). The sampling of cultivable bioaerosols is based on impactors, where the microorganisms are collected directly on a culture medium, or by air filtration methods, where the microorganisms are collected on a filter (Gong et al., 2020; Lou et al., 2021).

For the following meta-analysis, the Polish standard for atmospheric air (PN-89Z-04111/02) will be taken into account (Kowalski et al., 2017). This standard indicates that there is no contamination when the number of bacteria is less than 1000 CFU/m³, there is average contamination between 1000 to 3000 CFU/m³ and there is strong contamination when there is more than 3000 CFU/m³.

Wastewater inlet: the sewerage system is a biological reactor that transforms feces, detergents, and other pollutants from various human activities into ammonia, nitrites, nitrates, phosphates, and dissolved organic matter. In various studies, the highest levels of bacteria present in bioaerosols have been determined in the reception of wastewater. In one cubic meter of air, up to 4,795 CFU of viable bacteria and 2,233 CFU of intestinal bacteria have been counted (Li et al.,



Figure 1. Oxidation pond from an Ecuadorian food industry

2021; Yazdanbakhsh et al., 2020). In Spain, in municipal plants, up to 5,600 CFU/m³ have been determined in the pre-treatment section (Sánchez-Monedero et al., 2008). The review of 12 scientific articles allowed to establish an average of 3042 CFU/m³ in bioaerosols sampled in WWTPs of Poland, Portugal, China, India, Mexico, Spain and Iran (Figure 2). It is observed that the average concentration of culturable airborne heterotrophic bacteria at the aerated tanks was 87 times higher than in other subsequent treatment processes and 35 times higher than the values measured in the control site ambient air (Fathi et al., 2017; Dehghani et al., 2018; Korzeniewska & Harnisz, 2018; Bruni et al., 2019).

Primary and secondary treatment: Because the mechanical mixing of wastewater increases through aeration, the levels of bioaerosols in the air increase (Gangamma et al., 2011; Teixeira et al., 2016). Maximum levels of 17,900 CFU/m³ were found in bioaerosols during the aeration stage (Ruiz-Gil et al., 2020). However, as water pollution decreases, so does the number of bacteria in the air: Splashing and bubble popping that occur as a result of forced aeration in activated sludge processes release a large number of bacteria into the air (Hsiao et al., 2020). In Figure 2, the bacteria count between pretreatment, primary and secondary treatment is between 1,383.38 and 880.40 CFU/m³, which indicates that there is a decrease in bacteria in the air as the water stabilizes through the different physical, chemical and biological treatments. Taking into account

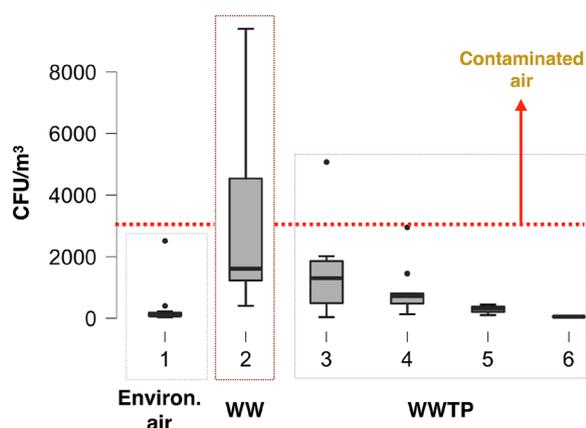


Figure 2. Airborne bacteria count in CFU/m³ between pretreatment, primary and secondary treatment

Where: WW = Samples of bioaerosol taken at the wastewater inlet, WWTP = Samples of bioaerosol taken at the wastewater treatment plant.

the maximum abundances of bacteria according to the Polish standards PN-89/Z-04111/02 and PN-89/Z-04111/03, a strong periodic air pollution with bacteria is established in the area of each WWTP.

Tertiary treatment: Advanced wastewater treatment by filtration, dosing of chlorine, ozone and UV leads to the reduction of potential health risks associated with the emissions of potentially pathogenic microbes (heterotrophic bacteria, and total coliforms), inhalable particles and hydrogen sulfide, confirming the importance and the need for adequate wastewater treatment (Han & Yoo, 2020). A decrease in bioaerosol concentrations of up to 60 times was observed, during advanced wastewater treatment from the chambers the entry of wastewater to the primary sedimentation tanks.

Table 1 presents a descriptive analysis of the results of the bacterial count in bioaerosols from up to 14 treatment plants from different countries. The highest contamination of the air with microorganisms was observed in the raw sewage inlet and in the biological reactor. The highest concentration of bioaerosols in the treatment plant is observed near the aeration chambers and sludge deposits (Paśmionka, 2020). At the untreated wastewater inlet, the highest number of bacteria was 9,400 CFU/m³, while at the control point (ambient air outside the radius of the WWTP), the lowest value was 34 CFU/m³.

Species of microorganisms isolated from the air

As mentioned above, bacteria are one of the most studied bioaerosol components, showing average concentrations of 10² to 10⁶ cells per cubic meter of air and with very high taxonomic diversity (Katsivela et al., 2017). In the air samples obtained in WWTP facilities, 25 species of Enterobacteriaceae were isolated, including the pathogenic bacteria *Salmonella spp.*, *Klebsiella pneumoniae* and *Escherichia coli* (Michałkiewicz, 2018). Therefore, exposure to wastewater can cause illnesses such as gastrointestinal disorders, respiratory problems, skin disorders, fever, eye irritation, headaches, nausea, and fatigue (Mania et al., 2018). Additionally, endotoxins derived from Gram-negative bacteria cause various problems, including diarrhea, fatigue, nose irritation, respiratory symptoms, and impaired lung function in wastewater treatment plant workers (Burdshall et al., 2021).

Table 1. Descriptive Statistics of airborne bacterial count

	CFU/m ³						
	1	2	3	4	5	6	
Valid	13	14	13	10	3	1	
Missing	0	0	0	0	0	0	
Mean	309.62	3042.00	1383.38	880.40	287.67	50.00	
Std. Error of Mean	185.36	727.50	358.35	257.49	100.33	NaN	
Std. Deviation	668.34	2722.06	1292.04	814.27	173.77	NaN	
Shapiro-Wilk	0.43	0.85	0.79	0.76	0.97	NaN	a
P-value of Shapiro-Wilk	2.89e-6	0.02	4.73e-3	4.89e-3	0.69	NaN	a
Minimum	34.00	400.00	36.00	130.00	100.00	50.00	
Maximum	2510.00	9400.00	5070.00	2950.00	443.00	50.00	

^a All values are identical

Where,

1 = Environmental air as control,

2 = Wastewater inlet from sewage system,

3 = Aeration tank inlet,

4 = Aeration treatment tank,

5 = Secondary sedimentation tank

6 = Chlorination tank

Different studies show that the percentage of Gram-negative bacteria is higher compared to Gram-positive bacteria in bioaerosols, especially in winter since Gram-positive bacteria are more tolerant of dryness and, consequently, can survive longer in the air.

In Figure 3, isolated genera and species of bioaerosols are summarized, with their respective phylum:

- Proteobacteria (Brucellaceae, Alcaligenaceae, Neisseriaceae, Moraxellaceae, Aeromonadaceae, Pseudomonadaceae, Xanthomonadaceae y Enterobacteriaceae),
- Firmicutes (Staphylococcus, Bacillaceae y Staphylococcabacterias),
- Bacteroidetes (Flavobacteriaceae),
- Actinobacteria (Corynebacterium) (Han & Yoo, 2020).
- Among the most frequent types of bacteria, the genera and Bacillus also stand out. Among species, the following have been identified:
- Cocci Gram-positive: *Staphylococcus gallinarum*, *Staphylococcus lentus*, *Staphylococcus xylosus*, *Kocuria rosea*, *Staphylococcus sciuri*, *Staphylococcus auricularis*, *Micrococcus luteus*, *Micrococcus spp.*, *Kocuria varians*, *Staphylococcus cohnii*;
- Bacilli Gram-positive: *Brevibacterium spp.*, *Microbacterium spp.*, *Rothia mucilaginosa*, *Corynebacterium spp.*;
- Bacilli Gram-positive: *Bacillus firmus*, *Bacillus mycoides*, *Bacillus cereus*; mesophilic

Actinomycetes: *Streptomyces spp.*, *Nocardia spp.*;

- Bacilli Gram-negative: *Pseudomonas spp.*, *Pseudomonas stutzeri* (Kowalski et al., 2017; Michałkiewicz, 2018)
- The most abundant Proteobacteria were members of the Enterobacterial orders (Enterobacter, Pantoea, Escherichia, Shigella, Klebsiella and Serratia genera) and Pseudomonades (*Pseudomonas*, *Acinetobacter* and *Moraxella*) (Gangamma et al., 2011; Ruiz-Gil et al., 2020).

Antibiotic resistance

As a consequence of the wide application of antibiotics in human and veterinary medicine, it has led to the large-scale dissemination of antibiotic-resistant bacteria in different elements of the environment (Zieliński et al., 2021). The main sources of resistant bacteria are animal manure and liquid manure, as well as human excretions. In sludge from wastewater treatment, the highest resistance rates were found with the following antibiotics: penicillin (ampicillin and piperacillin), cephalosporins (cephalothin and cefuroxime), quinolone (nalidixic acid) and trimethoprim/sulfamethoxazole, and paratetracycline (Crofts et al., 2017; Elsamadony et al., 2021; Han & Yoo, 2020; Małeczka-Adamowicz et al., 2019; Wright, 2007; Zieliński et al., 2021). In Figure 3, a list of airborne bacteria and antibiotics with limited effect is summarized, where it is presented that

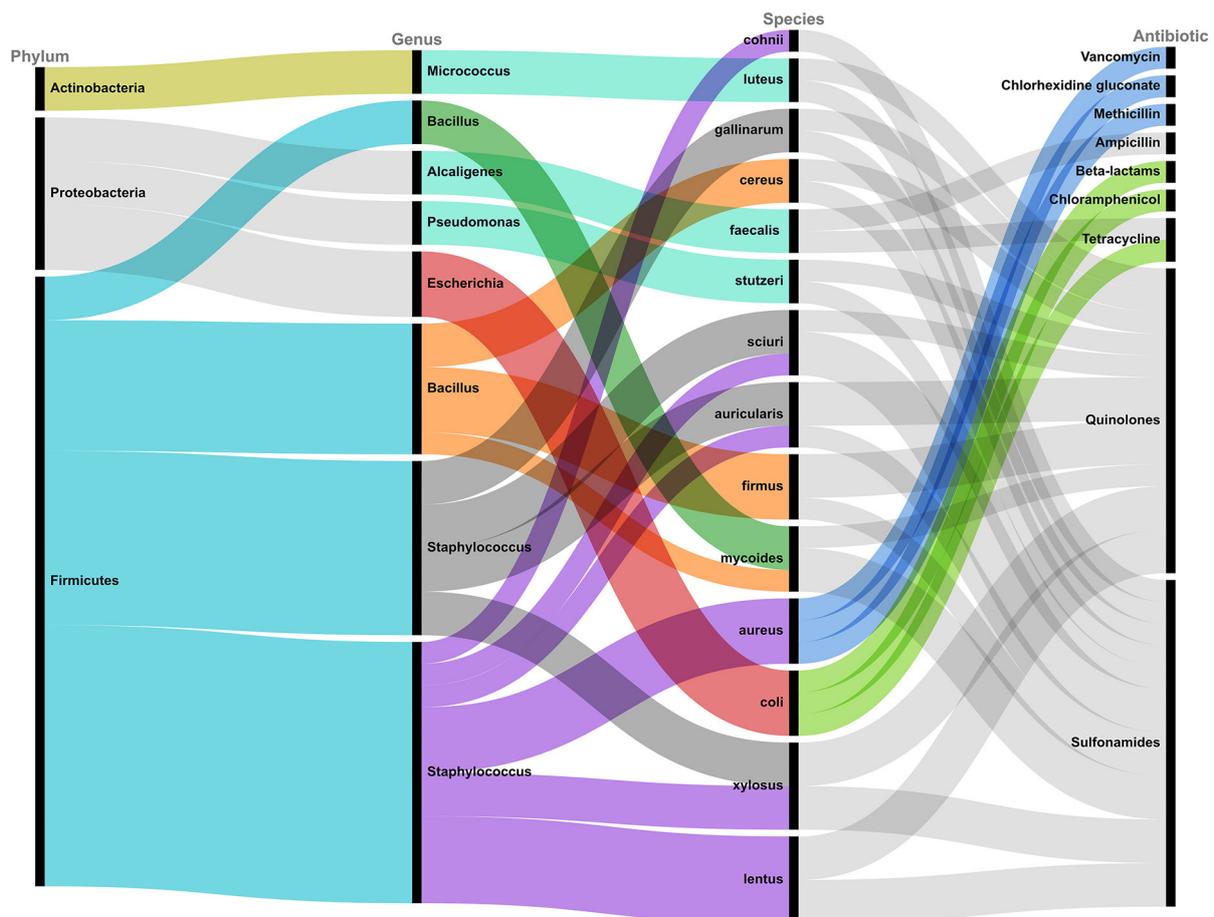


Figure 3. Phylum, genus, species of airborne bacteria which show resistance activity to antibiotics.

bacteria such as *E. coli* and *Alcaligenes faecalis* are resistant to tetracycline.

Among the isolated airborne bacteria, the most antibiotic resistant traits are present in *Bacillus* species like *B. mycoides* (Kowalski et al., 2017; Liang et al., 2020; Nnadozie & Odume, 2019). The group of bacteria most sensitive to antibiotics is *Kocuria sp.*, except for the influence of quinolones, sulfonamides, and nitrofurantoin (Kowalski et al., 2017). *Acinetobacter iwoffii* showed a resistance profile against the antibiotics tetracycline, chloramphenicol, erythromycin, streptomycin, penicillin, vancomycin, among others. *Micrococcus luteus* was resistant to fosfomycin and aztreonam (Bruni et al., 2019).

Air samples analyzed harbored antibiotic resistance genes *tet(A)*, *tet(B)* and *tet(M)* that encode resistance to tetracyclines, as well as the *blaTEM* and *blaAMP-C* genes that encode resistance to beta-lactams (Ding et al., 2019; Hernando-Amado et al., 2019; Osińska et al., 2021; Pazda et al., 2019). The highest number of ARGs (presence of 10 genes per sample) was determined in the samples collected in winter air; data

indicated that 88% of the bacteria showed resistance to 8–15 antibiotics and 3% of the strains were resistant to 19 antibiotics tested (Osińska et al., 2021). The most effective antibiotics were penicillins, cephalosporins, and aminoglycosides, which are commonly used in antibiotic treatment (Kowalski et al., 2017; Xu et al., 2020). However, to this date, the published studies on the resistance to antibiotics of bioaerosols emitted in wastewater treatment plants are limited.

Environmental interactions

Microbe concentrations in bioaerosols are the result of the dynamic equilibrium of the contamination source and the sink that contains it, which varies according to geographic location, season, and climatic conditions (Niazi et al., 2015). Furthermore, studies suggest that the relative abundance of pathogenic and total bacteria is positively correlated with the concentration of particles and pollutants in the air (Rocha-Melagno et al., 2020). However, environmental factors contribute to the diffusion of aerosols (Moran-Zuloaga et al., 2021). Temperature and

atmospheric pressure were positively correlated with bacterial diversity (Ruiz-Gil et al., 2020). Wind speed has also been positively correlated with the concentration and diversity of bacteria in several studies and has been associated with an important factor for improving the generation of bioaerosols (Song et al., 2021). In summary, the strongest effects on the appearance of bacteria are the air humidity, temperature, wind speed and UV index (Paśmionka, 2020).

A statistically significant difference was observed between the concentration of bioaerosols in autumn and spring seasons, while no significant difference was observed between autumn and winter or between winter and spring. The highest mean value for most microorganisms (mesophilic, psychrophilic, and actinobacterial bacteria) occurred in summer or fall. Investigations in municipal landfills, the highest number of bacteria was recorded in the spring and summer season (Nair, 2021).

In general, many meteorological conditions have been shown to influence the ability of bioaerosols to survive in the atmosphere. Among these factors, relative humidity played a key role in increasing concentrations of airborne bacteria in the air of WWTPs (Smets et al., 2016). A significant correlation between the concentration of bacteria and the relative humidity in autumn and spring, because the high relative humidity promotes the formation of bacteria in spring (Dehghani et al., 2018).

On average, the concentrations of culturable/non-culturable bacteria range between 10^4 and 10^6 cells per cubic meter of air (Gong et al., 2020; Zhou & Mancl, 2007). For example, up to 76% of bacteria in atmospheric cloud water samples have been found to be metabolically active and could have important effects on cloud chemistry (Lee, 2011). Therefore, bacteria in bioaerosol particles are believed to indirectly affect global change and potentially affect atmospheric chemistry (Gong et al., 2020).

The survival of specific microbial strains can be highly correlated with both temperature and humidity (Osińska et al., 2021). Generally, many environmental factors influence the ability of microorganisms to survive in the air, the most important of which include ultraviolet radiation, microorganism species, relative humidity, and temperature (Dehghani et al., 2018). Pigmentation, which protects cells from ultraviolet radiation and contributes to survival at low

temperatures, was revealed to be a very common characteristic among bacteria grown in air (DasSarma et al., 2020).

CONCLUSIONS

The highest contamination of the air with microorganisms was observed in the raw sewage inlet and at the biological reactor. In most analyzes, the air in the wastewater treatment plant was characterized by a higher content of microorganisms than at the control point. The results of the research carried out indicate that air pollution by microorganisms, including pathogens, occurs in the treatment plant. The bioaerosol generated can contribute to the deterioration of the atmospheric air quality and negatively affect human health and the environment.

Bioaerosols from municipal WWTPs might be an important source of antibiotic resistance genes, which can be transported considerable distances and can represent a potential risk to humans. Inhalation of bioaerosols may be the primary means of exposure to antibiotic resistance genes and drug resistant pathogens. The relevant health risks are particularly high among WWTP employees who are exposed to organic dust and bioaerosols containing high concentrations of microorganisms. Therefore, employees should recognize the health risks associated with the absence of personal protective equipment, such as masks or respirators.

Studies have shown the impact of atmospheric conditions on the number of microorganisms in the air. However, the use of ultraviolet radiation and other disinfection methods allow a significant reduction in the concentration of bioaerosols generated in the wastewater treatment process.

Acknowledgments

The authors are grateful to Dr. Aida Maile De la Cruz Balón (Environmental Engineering – ES-PAM) for her invaluable support.

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