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Review

Bioaerosols in Wastewater Treatment Plants: Trends, Recent Advances, and the Influence of SARS-CoV-2 Outbreak

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Abstract: Bioaerosol emission at various WWTP treatment units has drawn attention due to their potential negative impacts on human health. This study conducted a bibliometric analysis of the global research on bioaerosol emissions from WWTPs from 1995 to 2022. The Scopus database was used to identify relevant articles and research trends, major contributors in the field, and recent developments. The study examined 122 articles in the field of bioaerosols in WWTPs. The analysis findings showed that publications and citations peaked in 2022, with values of 25 and 818, respectively. At the beginning of the study period, the USA, Poland, and Italy led the publications' ranking, but with time, China emerged as the most influential country in the field. Recent advances in the field have revealed that spectral intensity bioaerosol sensors have contributed to the faster and more reliable identification and classification of bioaerosols. It was also observed that probabilistic techniques relying on mathematical models and assumptions to ascertain the risks associated with bioaerosols may result in false interpretations. Despite their high cost, epidemiological studies were best for assessing plant workers' health risks. The outbreak has raised questions about accurately evaluating and modeling SARS-CoV-2 persistence, infectivity, and aerosolization over WWTP sites and environmental factors. Finally, the study highlighted the potential of three control treatment approaches: carbon absorption, UV irradiation, and ozone treatments, which proved efficient in reducing bioaerosol emissions.

Keywords: bioaerosols; bibliometric analysis; wastewater treatment plants; SARS-CoV-2

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1. Introduction

In an urban setting, wastewater treatment plants are essential facilities that safeguard the environment and human health. It gathers and treats sewage from nearby communities and releases it into the environment. The sewage collected from communities is treated using mechanical, biological, and chemical methods, and the effluent is either reused or discharged into the environment. In contrast, the resulting emissions are released into the atmosphere [1,2]. Most wastewater treatment plants (WWTPs) employ biological treatment processes that capitalize on the microorganisms in the wastewater. Commonly used biological processes include anaerobic/anoxic/aerobic (A2O), activated-sludge process, trickling filters, aerated lagoons, oxidation ditch (OD), and sequencing batch reactor (SBR) due to their reliable operation, cheap cost, and high treatment efficiency [3–5]. During mechanical agitation and water aeration, the pathogenic microorganisms in the wastewater may be aerosolized, transferred into the air from the water, and take the form of bioaerosols. Bioaerosols from WWTPs can be dispersed across great distances via favorable meteorological conditions, harming living organisms [6]. Several complex factors influence the dispersion of the bioaerosols from the WWTP, such as the capacity of the WWTP, sewage quality and pathogenic load, employed treatment processes, pathogen inactivation rate, and weather conditions (wind direction humidity, temperature, topography, dilution effects, etc.) [4,7].

Primary biological aerosols (PBAs), generally called bioaerosols, are aerosol particles comprised of living or dead organisms (e.g., bacteria), dispersal units (e.g., fungal spores), along with a variety of different biomolecules with an aerodynamic diameter ranging between 0 and 100 µm [8]. When bioaerosols are released into the air, the development and persistence of most microorganisms in bioaerosols depend on factors such as wind speed, relative humidity, oxygen content, and ultraviolet radiation [3]. Early in the 20th century, a relationship between bioaerosol dispersion and disease transmission was established, citing the detrimental effects of bioaerosols on human health due to pathogenic microorganisms entering the human body, mainly by inhalation [9–12]. Additionally, the infectivity of these microorganisms depends on the particle size distribution, species diversity, chemical composition, biological characteristics, and the amount of bioaerosols inhaled [13]. These pathogens are then deposited in the human respiratory tract by impaction, sedimentation, and diffusion mechanisms. Hence, they may cause diseases such as influenza, Legionnaire disease, coccidioidomycosis, measles, inflammation of the nose, fatigue, respiratory problems, diarrhea, dizziness, headache, bacterial pneumonia, and gastrointestinal illness, collectively named "Sewage Worker's Syndrome" to exposed plant workers, surrounding inhabitants, and immunocompromised humans [14–16]. Therefore, investigating bioaerosols linked to wastewater treatment plants (WWTPs) has arisen as an important field of study.

The WWTP-related bioaerosols have not yet been extensively covered in literature review articles [3,16–19]. Additionally, recent articles conducted bibliometric analyses of bioaerosols in general [20] and emerging contaminants from WWTPs [21]. However, this study is the first to present a bibliometric analysis of bioaerosols associated with WWTPs, focusing on the influence of the SARS-CoV-2 outbreak. This study's singular focus yields timely insights into the impact of the pandemic on bioaerosols associated with WWTPs. The bibliometric analysis performed in this study highlights recent contributions to knowledge and advancements in assessing and controlling bioaerosol emissions from WWTPs. This study is intended for researchers, environmental scientists, and policymakers involved in environmental health, virology, and wastewater treatment. It can help individuals understand the field's intellectual structure to track research output growth and dynamics. This type of analysis reveals patterns and clusters in research output, providing a deeper understanding of the topic. Finally, the study also helped identify improvement opportunities and research gaps where environmental policymakers and funding agencies can direct resources toward addressing the gaps. This will help contribute to more sustainable approaches to managing bioaerosols from WWTPs. The research questions addressed in this study are summarized as follows:

RQ1: What trends can be detected when analyzing studies investigating the emissions of bioaerosols in WWTPs?
RQ2: Who are the major contributors to research on bioaerosol emissions from WWTPs?
RQ3: What are the recent advancements and research gaps/future directions?
RQ4: What influence, if any, did the outbreak of SARS-CoV-2 have on research of bioaerosols from WWTPs?

2. Background

Particulate matter of microbial, plant, or animal origin that contains airborne microorganisms (bacteria, fungi, and viruses), metabolites, endotoxins, mycotoxins, high molecular weight allergens, peptidoglycans, pollen, and plant fibers is widely referred to as bioaerosols [22]. WWTPs are potential sources of bioaerosols, as the wastewater and sludge act as carriers of an extensive range of microorganisms [12,23]. The choice of sampling method for bioaerosol measurement is significant as it directly impacts the quantification of microorganisms in the air. The literature survey highlighted that most bioaerosol air samplers in the studies were based on five primary principles: filtration, impaction, impingement, electrostatic precipitation, and gravity settling. While a standardized bioaerosol sampling technique has not been universally established, Andersen's six-stage cascade impactor emerged as a frequently used sampler for bacterial and fungal species, based on the literature. However, there have been limited studies focusing on the sampling of viruses in bioaerosols. Characterization of bioaerosols is critical for accurate detection and quantification. Presently, two primary techniques are employed: the conventional culture-based method utilizing nutrient media for microbial cultivation and the non-culture-based method relying on DNA-based techniques. Several non-culture-based techniques were used in the literature, including polymerase chain reaction (PCR) [24,25], quantitative or real-time polymerase chain reaction [2,14,26–28], and fluorescence in situ hybridization [29].

Research in this field has revealed that different stages of wastewater treatment contribute to varying levels of bioaerosols. Ref. [30] conducted a study on an activated-sludge wastewater treatment plant (WWTP), analyzing bioaerosol emissions at each stage (preliminary treatment, primary clarifiers, aeration tanks, and sludge treatment units). The study noted that pre-treatment and primary clarifiers exhibited the highest bioaerosol emissions. In a separate study from Greece [31], air samples were taken at various stages of activated-sludge wastewater treatment, including pre-treatment, primary settling tanks, aeration tanks, secondary settling tanks, chlorination, and sludge processors. The results indicated that the aerated grit removal stage during pre-treatment exhibited the highest concentrations of airborne microorganisms.

Studies by [11,12,32–39] highlighted the significant contribution of the aeration tank to bioaerosol emissions. As they supply oxygen for biodegradation processes to support the growth and reproduction of microorganisms, the disturbance caused by these mechanical devices encourages particles in the water tank to cross the water–air barrier and reach the atmosphere, forming bioaerosols [36]. Different mechanical agitation systems used in aeration processes can generate different aerosols, with air diffuser aerators generating more aerosols than horizontal rotors and surface turbines [40]. Also, the increase in brush aerator rotational speed was shown to increase bioaerosol emissions, raise the percentage of bacteria adhering to particles greater than 4.7 μm in diameter, and increase the Shannon index of air samples [41]. Similarly, another study showed that the increase in aeration rates in a biochemical reaction tank increased the generation of bioaerosols [42].

Meanwhile, ref. [43] noted higher airborne bacterial concentrations at the grit chambers and bar screen. Another study by [32] involved air sample collection at different stations: the entrance of the treatment plant, aeration basin, grit removal unit, sludge drying bed, and lab. The study observed the highest number of bacteria in primary and secondary sedimentation tanks during summer and winter. Thus, it became evident that pre-treatment, primary settling unit, aeration tank, biological reaction tank, aerobic sludge digester unit, and sludge-thickening units were primary generators of heightened bioaerosol levels.

The seasonal variation in the emission of bioaerosols was a topic of interest in several articles [38,43–47]. These studies highlighted that bioaerosol emissions were notably higher during summer compared to winter. Contrarily, other studies [48] concluded that the winter season had a greater rate of bacterial aerosolization, whereas, for fungi, it peaked in the summer. Moreover, bioaerosols were predominantly indoors rather than outdoors at a WWTP [49,50]. Additionally, research focused on the chemical components of bioaerosols [13,51–54] emphasized that these chemical elements act as a medium for airborne microbe adhesion and provide an ideal habitat for their development and survival in the atmosphere.

Moreover, investigations into the particle size distribution of bioaerosols by [37,55,56] underlined the critical role of particle size in human health risks. Bioaerosols with an aerodynamic size greater than 10 μm tend to be deposited in the upper bronchus. In comparison, those with an aerodynamic size of $\leq$5 μm deeply penetrate the lower bronchus, potentially causing infectious diseases. In parallel, numerous studies have assessed the health risks associated with bioaerosols [45,50,57–64]. These studies consistently found that males faced higher risks than females, and staff members faced higher risks than transient participants. There were fewer health hazards for those wearing personal protective equipment (PPE).

Furthermore, various strategies have been employed in the literature to mitigate bioaerosol emissions at different stages of WWTPs. These measures include proper confinement of the area, provision of effective and adequate ventilation systems, aerator-based control mechanisms, shielding of treatment units, especially aeration basins, filtration, UV irradiation, photocatalytic oxidation, treatment of exhaust air using regenerative thermal oxidation.

UV irradiation is one of the most widely utilized methods for controlling indoor bioaerosols due to its environmentally friendly properties. Ref. [65] explored using ultraviolet radiation to reduce bioaerosols, varying UV intensity from 0 to 110 $\mu W/cm^2$. The study observed the maximum reduction of bacteria and intestinal bacteria at a UV intensity of 110 $\mu W/cm^2$, demonstrating reductions of 68.7% and 78.0%, respectively. In a pilot-scale study by [66], over 85% of airborne bacteria and fungi emitted from the oxidation ditch were adsorbed on activated carbon within 80 h of continuous operation. This suggests that adsorption on granular activated carbon (GAC) could be an efficient method for purifying airborne microorganisms. Moreover, a study conducted in a highly advanced hospital WWTP [2] highlighted that post-treatment processes, including granular activated carbon (9.3 m^3), ozone (3.4 mg O_3/mg DOC), and UV light (45 mJ/cm^2), significantly reduced the risk of exposure to harmful pathogenic bacteria and enteric viruses in the surroundings. These measures led to low concentrations of pathogenic microorganisms in the exhaust air.

3. Methodology

The results presented in this paper are based on a bibliometric analysis of articles published from 1995–2022 on bioaerosol emissions from WWTPs. The extracted data are used to identify publication growth and trends, research contributors, and recent developments in the topic.

3.1. Data Collection

For the bibliometric analysis, the Scopus database was used to retrieve published articles, as it is widely accepted and includes a broad range of high-ranking journals used as research resources for quantitative studies [67]. The first article on the topic was published in 1979. The time frame selected for the study was between January 1995 and December 2022. This time interval was chosen as most articles were published during this period, with only four articles published between 1979 and 1994. Data were retrieved on 20 July 2023. The search queries used for data collection were "bioaerosols" OR "bio-aerosols" OR "aerosols" AND "WWTP" OR "wastewater treatment Plant" when searching for the relevant literature within the "Title, abstract, or author-specified keywords". A total of 200 documents were initially retrieved. To further limit the search query, all articles from the year 2023 were excluded in the bibliometric analysis, as this may give misleading information related to the number of publications and citations in this current year when presenting research growth. Only peer-reviewed journal articles written in English were used for the analysis to limit the search to high-quality scientific research. Preliminary screening of the articles by skim reading was used to exclude non-relevant articles. Some articles were excluded because they were outside of the study period. Others were excluded because they were discussing emissions from WWTPs that were not related to bioaerosols or bioaerosol emissions that were related to wastewater reuse. Also, other excluded articles discussed breathing patterns or different settings where the emissions are being studied. The remaining 122 articles were selected for the detailed bibliometric analysis. Articles retrieved using the same research query for 2023 were considered for reviewing recent advancements and research gaps. The framework for the analysis of the study is illustrated in Figure 1.

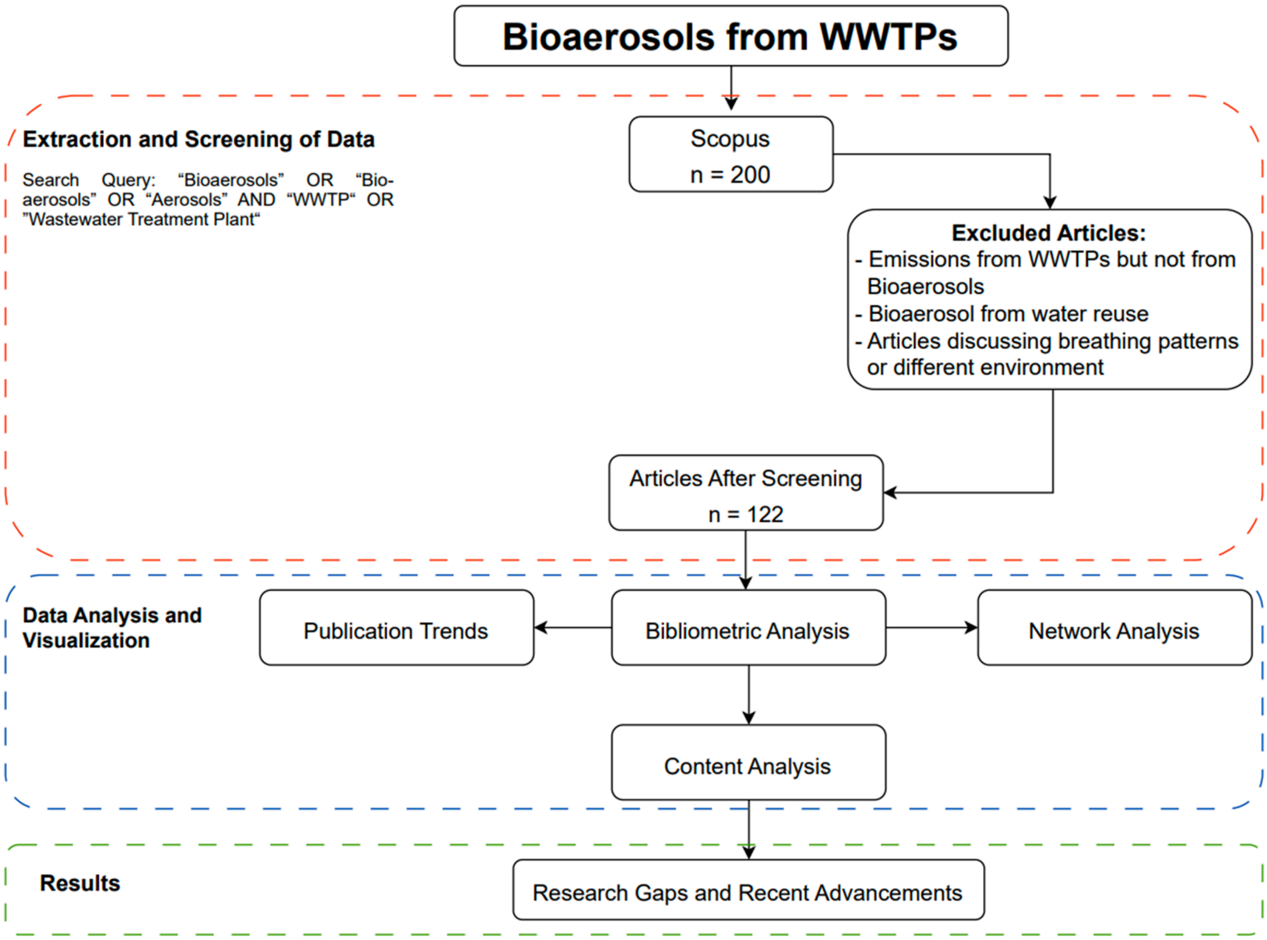

Figure 1. The framework of analysis of the study.

3.2. Data Analysis

The analysis aimed to characterize the dataset of screened articles, encompassing the annual publication count, highly cited journals, countries' contributions, types of publications, and citations per publication [68]. VOSviewer software (version 1.6.19) was employed to represent knowledge maps visually [69]. The software was also utilized to conduct cluster analysis and generate social network maps comprising nodes and links for the most productive nations, prolific authors, and prevalent terms [46]. Author or country contributions to the field were assessed by the total number of publications, while the cumulative citations reflected the prestige and significance of the published journal. Citations per publication were utilized to provide a more refined evaluation of knowledge consistency [70]. Content analysis was conducted on recent articles that focused on advancing bioaerosol assessment and characterization in WWTPs. Additionally, the analysis focused on the content of highly cited articles.

To explore the interconnections among research components, this study utilized co-word analysis, citation analysis, co-citation analysis, and co-authorship analysis. These analytical methodologies were applied to uncover synergistic impacts and structural relationships within the research domains.

4. Results

4.1. Publication Growth

The temporal evolution of articles and citations in the analyzed database is visually presented in Figure 2. Publication trends are depicted through a bar graph, while citations are illustrated using a line graph. Notably, a publication hiatus was observed between 1997 and 1999, and citations ranged from 0 to 4 from 1995 to 2002. Starting from 2003, a consistent upward trend in citations emerged, starting around 7 in 2004 and peaking at 818 in 2022. Concurrently, the annual publication of articles exhibited fluctuations, but an overall increase in published articles was evident.

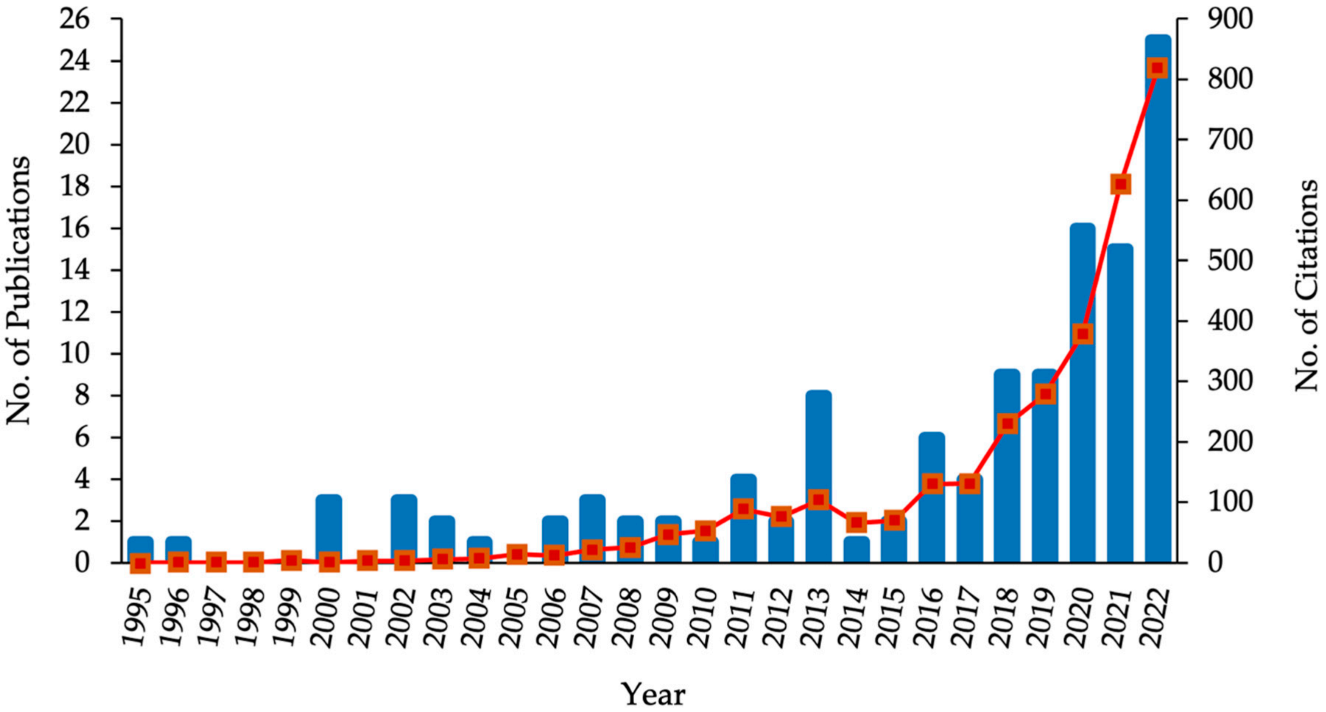

Figure 2. Yearly publications and citations on bioaerosols emissions from WWTPs.

A substantial increase in publications occurred in 2013, likely attributed to heightened awareness and investigations into potential health risks associated with bioaerosols. Several studies published during that year delved into assessing these risks, contributing to increased awareness of the issue. Additionally, the heightened focus on water quality concerns may have driven the peak in publications related to bioaerosols in wastewater treatment plants in 2013. To substantiate this, utilizing the search query "water quality" in Scopus and filtering for review and research articles revealed an annual increase in publications from 2010 to 2013, with respective values of 3693, 4240, 4700, and 5275.

The most prolific years, marked by the most publications, were observed between 2018 and 2022, reaching a zenith of 25 publications in 2022, exhibiting an impressive annual growth rate of 62.5%. This significant upswing in annual article publications may be attributed to the outbreak of the SARS-CoV-2 virus and the growing research interest in studying its presence and transmission within WWTPs.

4.2. Most Relevant Journals

A total of 122 publications were examined, distributed across 52 journals. Table 1 provides a list of the most significant journals contributing to the field based on metrics of total publications (TP), total citations (TC), and citations per publication (CPP). The number of citations a document receives indicates its quality, popularity, and impact within a specific field of study [71,72]. The analysis showed that the Science of the Total Environment journal ranked first in total publications (TP). The Water Research journal also led in total citations (TC) and citations per publication (CPP).

Table 1. Top journals ranked by total publications, total citations, and citations per publication.

Rank	Journal Name	TP *	Journal Name	TC *	Journal Name	CPP * = TC */TP *
1	Science of the Total Environment	11	Water Research	709	Water Research	79
2	Water Research	9	Science of the Total Environment	291	Journal of Hazardous Materials	29
3	Aerobiologia	7	Aerobiologia	182	International Journal of Environmental Research and Public Health	28
4	Ecotoxicology and Environmental Safety	8	Environmental Science and Pollution Research	178	Science of the Total Environment	26
5	Environmental Science and Pollution Research	7	Journal of Hazardous Materials	172	Environmental Monitoring and Assessment	26
6	Polish Journal of Environmental Studies	7	Polish Journal of Environmental Studies	144	Aerobiologia	26
7	Journal of Hazardous Materials	6	Ecotoxicology and Environmental Safety	144	Environmental Science and Pollution Research	25
8	Chemosphere	4	International Journal of Environmental Research and Public Health	113	Chemosphere	23
9	Environmental Monitoring and Assessment	4	Environmental Monitoring and Assessment	105	Polish Journal of Environmental Studies	21
10	International Journal of Environmental Research and Public Health	4	Chemosphere	90	Ecotoxicology and Environmental Safety	18

Note: * TP: total publications, TC: total citations, CPP: citation per publication.

The Science of Total Environment journal featured approximately 11 articles, with a particularly notable study on bacterial populations and chemicals in bioaerosols from sludge-dewatering facilities being the most cited. This study conducted a comparative analysis of bacterial aerosol and chemical emissions from nine distinct municipal WWTPs [13]. Within the array of journals, Water Research exhibited the most significant influence on bioaerosol research related to WWTPs, boasting the highest citation count at 709 out of 3590 and the highest CPP. It also ranked second in article publications, with nine articles. The articles published in this journal covered diverse topics, including bioaerosol emissions from WWTPs, the influence of aeration modes on bioaerosol emissions, seasonal variations in bioaerosols, exposure risks associated with bioaerosols, and bioaerosol emissions from highly advanced WWTPs [2,25,31,37,40,58,73–75].

4.3. Most Contributing Countries, Authors, and Top-Cited Articles

Table 2 presents the nations with the highest research productivity in the field. China emerged as the top contributor in terms of both the total number of publications and citations. However, in terms of citations per publication, China was ranked sixth. This is due to China's publication activity commencing in 2011 and a two-year publication gap from 2014–2015 until the next set of publications in 2016. Nevertheless, there has been consistent progress in publications, reaching the highest count in 2022. On the other hand, despite having a citation per publication (CPP) of 52, Greece had relatively lower total publications (TP) than other countries.

Table 2. Most contributing countries ranked by total publications, total citations, and citations per publication.

Rank	Country	TP *	Country	TC *	Country	CPP *
1	China	42	China	994	Greece	52
2	Poland	15	Italy	541	Denmark	51
3	Iran	13	Poland	484	Italy	45
4	Italy	12	Iran	374	Poland	32
5	The United States	11	The United States	307	Iran	29
6	Canada	6	Greece	156	The United States	28
7	The United Kingdom	5	Denmark	152	China	24
8	India	4	Canada	96	Canada	16
9	Greece	3	The United Kingdom	61	The United Kingdom	15
10	Denmark	3	India	44	India	11

Note: * TP: total Publications, TC: total citations, CPP: citation per publication.

Table 3 displays the top ten most productive authors and their respective countries of origin. In total, 444 authors have contributed to the research on bioaerosols in WWTPs. Authors were ranked based on the total number of publications in the study database.

Table 3. Most contributing authors ranked by publications and citations.

Rank	Name of the Author	Country	TP *	TC *	Academic Institutions
1	Li, Lin	China	16	534	University of Chinese Academy of Sciences
2	Liu, Junxin	China	16	487	University of Chinese Academy of Sciences
3	Han, Yunping	China	13	438	University of Chinese Academy of Sciences
4	Yan, Cheng	China	11	72	China University of Geosciences
5	Wang, Yanjie	China	9	204	Zhengzhou University
6	Yang, Tang	China	8	206	Qingdao University of Technology
7	Yang, Kaixiong	China	7	226	Research Center for Eco-Environmental Sciences Chinese Academy of Sciences
8	Veillette, Marc	Canada	5	90	Quebec heart and lunch institute
9	Duchaine, Caroline	Canada	5	90	Quebec heart and lunch institute
10	Filipkowska, Zofia	Poland	3	175	University of Warmia and Mazury

Note: * TP: total publications, TC: total citations.

Among these authors, two from China secured the top positions with 16 publications each and noteworthy citation counts of 534 and 487, respectively. Li, Lin, and Liu Junxin's initial article was published in 2011, holding the highest total citations of 67 compared to their subsequent articles [55]. It should be noted that the first three authors in the rank work in the same research group and have ongoing research as co-authors, which explains their high number of publications. The list includes authors from China, Canada, and Poland.

4.4. Content Analysis of Top-Cited Articles

This section delves into influential research papers concerning bioaerosols linked to WWTPs. Table 4 presents an overview of scholarly articles that garnered considerable attention, evidenced by their substantial citation counts. The table includes key details such as publication year, journal of publication, total citation count, specific microbial species investigated, and pivotal findings from each study. All papers listed were co-authored except for the article [76].

A seminal article published in 1985 [77] with a total citation count of 80 investigated bioaerosol emissions at varying distances from the aeration tank. The findings of this study likely laid the foundation for subsequent investigations, establishing the primary bioaerosol source in WWTPs, particularly aeration tanks involving mechanical agitation [18,37,39,40,42,57,73,75,77,78]. The research on the influence of different aeration systems on bioaerosol emission at WWTPs marked the first paper to enter the top

10 list, with a notable total citation count of 149. This study engaged six distinct WWTPs to monitor the processes generating substantial bioaerosols. Results indicated that pre-treatment, biological treatment, sludge thickening, and aeration systems such as horizontal rotors and surface turbines produced more significant amounts of bioaerosols than air diffuser aerators [40].

The second most cited article provided insights into various bacterial and fungal species, their drug resistance, and their potential as opportunistic pathogens. This research stood out for pioneering the identification of antibiotic-resistant bacteria and genes, addressing critical environmental concerns and a global threat to public health. Notably, it identified the sludge-thickening basins as the primary source of high bioaerosol emissions and identified approximately 300 bacterial species [15]. Seasonal variations in bioaerosol emissions were examined in various studies, with outcomes varying based on WWTP geographical locations. For instance, emissions were higher in the summer in the Middle East [31,38,79,80]. The final article in the list demonstrated the potential of an advanced wastewater treatment unit in reducing bioaerosol emissions. The study involved sampling inhalable bacteria, endotoxins, and noroviruses (NoV), known pathogenic organisms. The findings were encouraging, revealing the negligible presence of pathogenic bacteria and a minute amount of NoV in the exhaust air, suggesting minimal health risks [2].

Table 4. Content analysis of the top 10 articles based on total citations.

Rank	Article	Microbiological Investigation	TC	Findings
1	Sanchez-Monedero et al. 2008 [40]	Mesophilic bacteria	149	▪ Top bioaerosol producers: sludge thickening, biological treatment, pre-treatment. ▪ Air diffuser aerators: lower bioaerosol output compared to systems with horizontal rotors and surface turbines.
2	Jing Li. et al. 2015 [15]	Bacteria and Fungi	129	▪ Highest bioaerosol levels detected at sludge-thickening basin among 12 sites. ▪ Screen room, sludge-thickening basin, and biological reaction basin pose significant risks for microbial exposure, including airborne antibiotic-resistance genes.
3	Karra and Katsivela 2007 [31]	Total coliforms, Faecal coliforms, *Enterococci* and Fungi	116	▪ Highest airborne microorganism levels found in WWTP's aerated grit removal during sample collection at each stage. ▪ Advanced wastewater treatment led to reductions of 97.4% (mesophilic heterotrophic bacteria), 100% (total coliform, fecal coliforms, and enterococci), and 95.8% (fungi).
4	H. Bauer et al. 2002 [73]	Mesophilic bacteria, TSA-SB bacteria, Mesophilic fungi, and Thermotolerant fungi	112	▪ Higher emissions were observed in the activated-sludge plant's aeration tank compared to the fixed-film reactor. ▪ Aerosolization ratios for cultivable bacteria ranged from 8.4×10^{-11} to 4.9×10^{-9}, with one to three times greater magnitude and significantly different for fungi in the two treatment plant types.
5	Sadegh Niazi et al. 2015 [38]	Bacteria and Fungi	108	▪ Aeration tank: highest bacterial emission; primary treatment: highest fungus emission. ▪ *Bacillus* species were prevalent in summer, whereas *Micrococcus* spp. were most emitted in winter. Throughout all seasons, *Penicillium* and *Cladosporium* species were predominant.
6	G. Brandi et al. 2000 [57]	Bacteria and Fungi	105	▪ Examined two treatment plants with distinct aeration systems; found the highest bioaerosol concentrations above tanks and downwind. ▪ Using a fine diffused aeration system, plants emitted fewer bioaerosols than plants with mechanical aeration systems.

Table 4. *Cont.*

Rank	Article	Microbiological Investigation	TC	Findings
7	Ewa Korzeniewska [78]	Not mentioned	95	▪ The health risk posed by an industrial plant is determined by its type, size, facilities, activities, and meteorology.
8	Leonor Pascual et al. 2003 [30]	Heterotrophic Plate Count, Moulds and Yeasts, Total and Fecal Coliforms	89	▪ Samples from each WWTP stage revealed the highest bioaerosol levels in pre-treatment and primary clarifiers.
9	Pietro Grisoli et al. 2009 [76]	Bacteria and Fungi	83	▪ The study indicated higher bioaerosol emissions in summer compared to winter due to seasonal influence. ▪ Contamination indexes like the global microbial contamination index and amplification index indicate decreasing bioaerosol emissions from upwind to downwind direction, suggesting potential health risks to workers, if not to nearby residents.
10	K. Uhrbrand et al. 2017 [2]	Inhalable bacteria, Endotoxins & Noroviruses	82	▪ Sampling at a hospital WWTP with advanced treatment found no pathogenic bacteria in the exhaust air and low concentrations of NoVs in indoor and exhaust air for inhalable bacteria, endotoxin, and noroviruses (NoVs).

4.5. Keywords Analysis

This analysis examines content by investigating the distribution of frequently used keywords, aiming to shed light on the areas of strength within the disciplinary domains through an accurate depiction of keyword co-occurrence [81]. This co-occurrence network was constructed using VOSviewer software (version 1.6.19), with a specified minimum occurrence threshold value of one to encompass all terms used [82]. The size of the nodes and words in the network represent the nodes' weights. Larger nodes and terms correspond to higher weights, indicating the importance of the keyword. The distance between nodes signifies the level of relationship, with closer distances denoting stronger relationships. Additionally, a line connecting two keywords demonstrates their co-occurrence, with thicker lines indicating a higher frequency of co-occurrences [83].

In this analysis, 340 distinct author keywords were identified. Setting the minimum number of occurrences of a keyword to 2 resulted in 62 keywords (18.24%) meeting the requirement. When selecting the threshold to be 3, 4 and 5, the keywords that met the condition were 35 (10.3%), 22 (6.5%), and 16 (%4.7), respectively.

This reveals research inconsistency and the broad spectrum of study topics [83–86]. The keywords "bioaerosols", "aerosol", "wastewater", and "wastewater treatment plant" were excluded from this analysis as they were the primary search strings used to retrieve data for the bibliometric analysis. The results of this analysis are presented in Table 5, where "quantitative microbial risk assessment" emerged as the author's most frequently occurring keyword with the strongest link. This was followed by "pathogenic microorganisms", "health risk", "airborne bacteria", and "fungi", with respective total link strengths of 47, 45, and 21. A visualization map was generated using VOSviewer software (version 1.6.19) to represent the co-keyword analysis visually, as depicted in Figure 3.

The prominent keywords central to the primary cluster are "quantitative microbial risk assessment", "pathogenic microorganisms", "health risk", and "airborne bacteria." These keywords exhibit thicker connecting lines, indicating their significant relation to a broad spectrum of studies in this domain. These encompass various aspects such as diverse air sampling techniques [87], component analysis of bioaerosols including bacteria, fungi, and viruses [12,87], their particle size distributions [88], dispersion of bioaerosols in the air [57], constituents facilitating bioaerosol survival [89], and the effects of bioaerosol exposure on human health [90].

Table 5. Total link strength of the top 10 reoccurring keywords.

Rank	Keyword	Occurrences	Total Link Strength
1	Quantitative microbial risk assessment	18	57
2	Pathogenic Microorganisms	16	47
3	Health Risk	15	45
4	Airborne bacteria	15	45
5	Fungi	8	21
6	SARS-CoV-2	8	23
7	Disease burden	5	19
8	Monte Carlo simulation	5	18
9	Microbial diversity	4	10
10	Norovirus	4	13

Note: Words of the same meaning and the singular and plural forms of the keywords were combined.

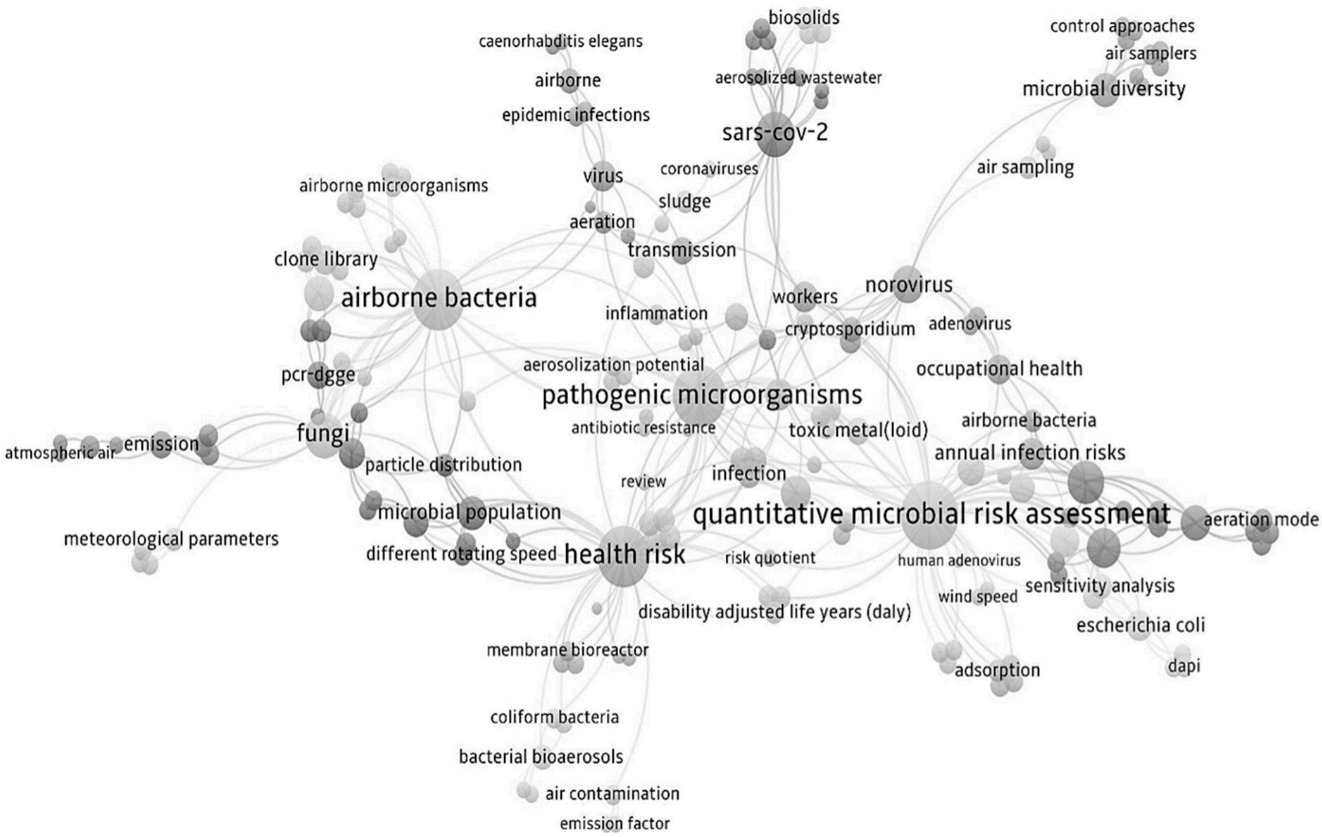

Figure 3. Co-keyword network visualization on bioaerosols emissions from WWTPs. Keywords related to bioaerosols were removed for figure clarity, and synonyms were considered one.

Figure 3 visually portrays the connections between "quantitative microbial risk assessment" (QMRA), "annual infection risks", "health risks", "Monte Carlo simulation", and "pathogenic microorganisms." QMRA represents an approach utilized to estimate and quantify health risks related to bioaerosol exposure, including annual infection risk and disease burden. This is achieved by comparing them with the two prominent health risk benchmarks that are widely used. These benchmarks delineate the acceptable annual infection risk threshold suggested by the United States Environmental Protection Agency (U.S. EPA) ($\leq 10^{-4}$ infection cases per person per year) and the World Health Organization's (WHO's) estimated tolerable disease burden threshold ($\leq 10^{-6}$ DALYs pppy−1) [64]. The QMRA process comprises four key steps: hazard identification, exposure assessment, dose–response assessment, and risk characterization.

The keyword "pathogenic microorganisms" is closely associated with other keywords such as airborne bacteria, fungi, viruses, antibiotic-resistant genes, health risk, and QMRA.

This emphasizes the extensive study of various bioaerosol components to determine the risks associated with exposure, particularly for plant workers.

The keyword "health risk" exhibits multiple connections, likely tied to infection risk studies concerning bioaerosol emissions from oxidation ditches, membrane bioreactors, different aeration modes, aerator speeds, and SARS-CoV-2. Studies have highlighted significant health risks linked to aerosol release during distinct treatment processes in WWTPs. Notably, health risk diminishes with increased distance from the emission source, and risks for males and staff wearing personal protective equipment (PPE) were higher compared to females and staff without PPE. This valuable information can guide appropriate WWTP siting in densely populated areas of developed nations, inform safety regulations for workers, and facilitate the development of control strategies such as ventilation, capping, ultraviolet radiation, and biofiltration. These strategies can optimize bioaerosol release, ultimately mitigating health risks stemming from WWTPs.

A threshold of four instances was employed to delve deeper into the proliferation and development of author keywords, identifying 10 keywords. In Figure 4, these keywords were scrutinized for their growth patterns within distinct five-year intervals. The graph illustrates that the keyword "health risk" was present in all time intervals except between 2001 and 2005. Its frequency peaked between 2016 and 2022, indicating heightened concern among researchers regarding the health risks posed by bioaerosols to employees and residents.

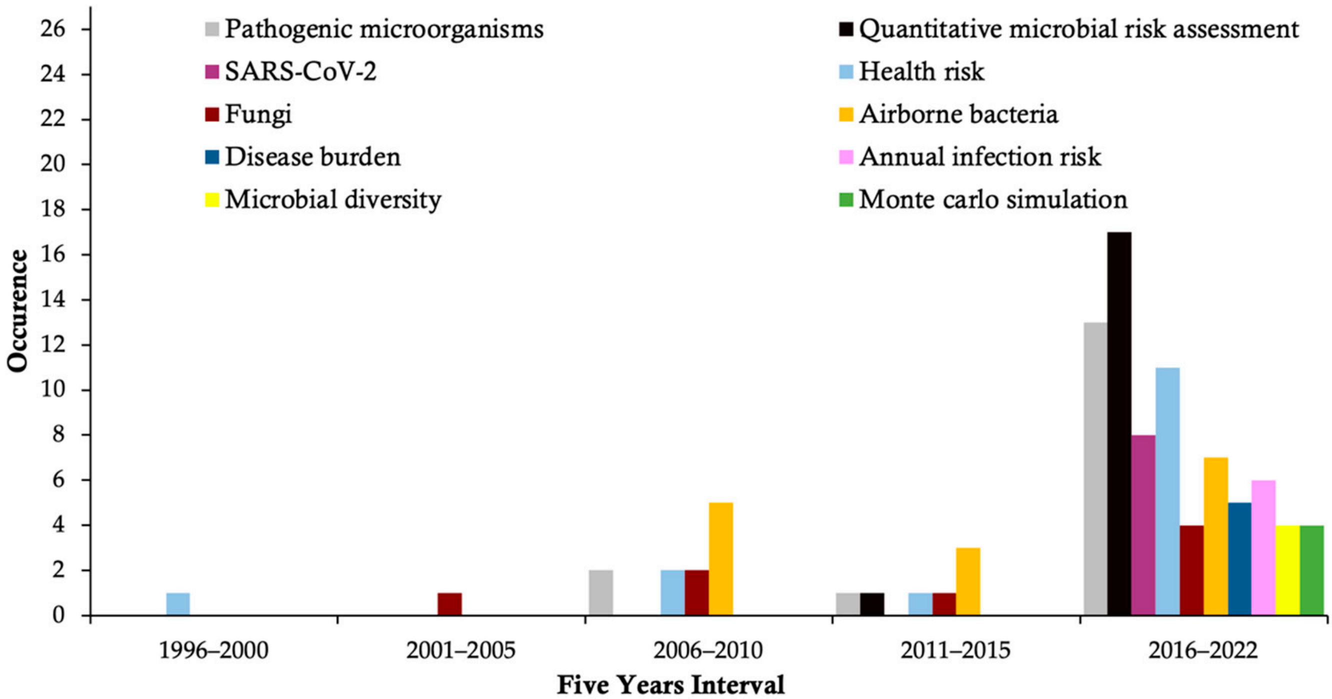

Figure 4. Analysis of recurring keywords in the field of bioaerosols emissions from WWTPs.

The keywords "fungi" and "airborne bacteria" emerged from 2001–2005 and 2006–2010, respectively. Their frequency remained relatively stable throughout the research, signifying their status as common areas of study within the field. The keyword "quantitative microbial risk assessment" debuted between 2011 and 2015, notably dominating all other keywords from 2016 to 2022. This suggests that the scientific community extensively employed this method to assess the harm posed to humans by bioaerosols from WWTPs. Additionally, the term "pathogenic microorganisms" appeared initially between 2006 and 2010, experiencing its peak occurrences during 2016–2022. This trend indicates a shift in researchers' focus from a broad study of bioaerosols to a specific investigation of pathogenic microorganisms, given their significant threat to human health by causing infectious diseases.

Furthermore, keywords like "SARS-CoV-2", "disease burden", "microbial diversity", "Monte Carlo simulation", and "norovirus", along with five other terms, exhibited an upward trajectory in the last five-year interval. This signifies that these topics are current research hotspots within the domain of bioaerosol emissions from WWTPs. This is not surprising, especially in the context of the global SARS-CoV-2 pandemic, as researchers have directed their focus toward comprehending the role of bioaerosols in the transmission of the COVID-19 virus among individuals, including occupants, inhabitants, and temporary visitors [48,60–64,91–101].

4.6. Countries Co-Authorship Analysis

Co-authorship analysis is a pivotal component of bibliometrics, offering insights into research collaboration levels and reflecting the research state within a field [102]. Analyzing co-authorship at a country level can provide valuable indications of international communication levels and the leading nations in their respective research field. We conducted this analysis with a minimum criterion of three publications per country. Out of the total 28 countries, only 12 met this criterion. However, the two countries did not exhibit connections with the other ten, leading to their exclusion from the co-authorship map of countries. It was observed that China and the USA hold the top rankings with a total link strength of 7, underscoring their collaborative efforts with other countries. The United Kingdom (4) and Poland (2) closely follow. Two countries, Greece and Taiwan, displayed no links in this context.

4.7. The Co-Citation Analysis

The co-citation study delves into the concurrent citation of two entities (article, journal, or author) through the citation of a third document, where they appear together in the reference lists of other publications [103]. A threshold value was set, requiring a minimum of 11 citations for a referenced work to be considered. Out of 5846 references, only 7 met this threshold. Table 6 outlines the five most co-cited articles.

Table 6. Top five articles based on co-citation analysis.

Rank	Article	Co-Citations	Total Link Strength
1	Yunping Han et al., 2018 [13]	16	15
2	K.Uhrbrand et al., 2017 [2]	14	13
3	Kaixiong Yang et al., 2019 [104]	13	13
4	Sanchez Monedero et al., 2008 [40]	12	9
5	Tang Yang et al., 2019 [54]	11	11

The most strongly correlated reference was an article discussing bioaerosol emissions from nine sludge-dewatering houses and significant indoor bioaerosol sources [13]. The findings indicated that bioaerosols were generated from the mixed liquor and released into the atmosphere due to the mechanical movement of the belts. Regional variations were observed in airborne bacteria and chemicals, yet certain pathogenic bacteria like *Aeromonas caviae*, *Flavobacterium* sp., and *Staphylococcus lentus* were prevalent across all locations.

The second and fourth articles were among the top 10 prolific journals examining the impact of different aeration systems on bioaerosol emissions and airborne bacteria, along with norovirus (NoV) in advanced hospital WWTPs [2]. The outcomes indicated that horizontal rotors and surface turbine aerators produced higher bioaerosol emissions than air diffuser aerators. Utilizing advanced wastewater treatment technologies, pathogenic bacteria were absent, and only minimal remains of NoV genomes were found within the exhaust air, posing a negligible health risk to the surroundings.

The third article explored airborne bacteria at various stages of WWTPs. The study revealed that the primary sources of airborne bacteria were indoor treatment facilities, treatment units with aeration, and mechanical agitation systems. The percentage of bacteria from wastewater, sludge, atmosphere, and other environments was determined using a source tracker, indicating higher percentages linked to wastewater and sludge in indoor treatment facilities. Conversely, in outdoor installations, more airborne bacteria originate from ambient air. Potential pathogens such as *Micrococcus*, *Bacteroides*, *Chryseobacterium*, *Pseudomonas*, and *Acinetobacter* were found in bacteria, with inhalation being the primary mode of exposure for workers on the site.

The final article highlighted the risks posed by metalloids and heavy metals in bioaerosols through different contact processes like ingestion, dermal contact, and inhalation. The study revealed that most pathogenic bacteria and metalloids were in inhalable fractions, while heavy metals such as arsenic, cadmium, and copper exhibited carcinogenic effects upon exposure.

5. Discussion, Recent Developments, and Research Gaps

In the analyzed dataset, China emerged as the leading contributor among the countries. The country, roughly 20% of the global population, possesses approximately 7% of the Earth's freshwater resources [105]. China has made significant strides to address the increasing demand by constructing 3508 WWTPs [106]. However, these WWTPs face different challenges, including the bioaerosols emissions from the plants. Currently, the country is paying considerable attention to understanding and managing bioaerosol transmission to ensure a safe working environment for WWTP staff and to enhance the air quality in the surrounding areas.

Most of the studied articles focused on bacterial aerosols, with comparatively fewer investigations of fungi and viruses. Bacterial aerosols are prioritized due to their direct health effects and established research base. Bacteria are practical scientific subjects because they are easy to measure and grow. Due to their significance to public health and safety, regulatory agencies prioritize bacterial emission surveillance and regulation. The primary areas of focus have included characterizing bioaerosols, analyzing seasonal variations in their emissions, studying their particle size distribution, and assessing their exposure risks among different demographic groups.

Table 7 provides an overview of recent progress and areas lacking research coverage within four classifications associated with bioaerosol discharges in wastewater treatment plants (WWTPs). Notably, bioaerosols from WWTPs contain various antibiotic-resistance genes, some of which hold clinical significance. Moreover, there is a link between exposure to these bioaerosols and infections and respiratory symptoms among WWTP workers and nearby inhabitants. Hence, it is imperative to formulate robust mitigation approaches to manage bioaerosol emissions from WWTPs.

The recommended actions encompass reducing bioaerosol emissions at their origin and effectively managing their generation, dissemination, and the implementation of efficient collection and treatment systems. Recently, advancements have been made in employing real-time monitoring techniques to assess size-segregated particulate matter (PM) and microbial activity in bioaerosols. These techniques utilize optical particle counters and fluorescence microscopy for monitoring and detailed characterization [107]. Additionally, air samplers have been used to gather varying-sized particles, subsequently undergoing next-generation sequencing for analyzing microbial composition [108]. These advancements have significantly enhanced our ability to precisely and comprehensively characterize bioaerosols in WWTPs. This advancement represents a pivotal initial step in identifying potential environmental or public health hazards associated with bioaerosols.

Table 7. Recent advancements and research gaps in the field of bioaerosols emissions from WWTPs.

Aspect	Research Advancement	Research Gaps
Monitoring and Characterization of Bioaerosols	▪ Size-segregated PM and microbial activity monitored in real-time with optical particle counters and fluorescence microscopy. ▪ Air samplers were utilized to collect particles of varying sizes. ▪ Next-generation sequencing was employed to analyze microbial composition.	▪ Limited understanding of bioaerosol production in different WWTPs. ▪ Lack of knowledge about non-culturable bacterial aerosol distribution and potential risks.
Factors Affecting Bioaerosol Emissions	▪ The impact of different aeration modes on bioaerosol dispersion in WWTPs was studied. ▪ Examination of the interaction between pathogens, bubbles, and virus aerosolization.	▪ Mechanisms of virus aerosolization by bubble properties remain unclear. ▪ Variables affecting airborne virus spread are not well known.
Health and Environmental Impacts of Bioaerosols	▪ Investigation into antibiotic resistance associated with inhalable bioaerosols from WWTPs. Examine how wastewater treatment affects the release and functional changes of microbial aerosol particles that contain Pseudomonas sp.	▪ Limited knowledge about the impact of bioaerosols from WWTPs on human health and the environment. ▪ Specifically, uncertainty about the risks posed by antibiotic-resistant bacteria and the safety of nearby residents.
Mitigation Strategies for Bioaerosol Emissions	▪ Studies investigate the effectiveness of mitigation techniques in reducing bioaerosol emissions from WWTPs. ▪ Techniques include biofilters and bio-trickling filters.	▪ Limited understanding of trade-offs between reducing bioaerosol emissions and other WWTP performance factors. ▪ Uncertainty about optimal design and implementation of mitigation strategies.

However, there remain gaps in research within this domain. Specifically, there is limited understanding regarding the range and possible dangers associated with bacterial aerosols that are not cultivable, constituting a substantial portion, potentially up to 90%, of the total bacterial load in bioaerosols. Another study underscores the notable impact of different types of WWTPs and environmental factors on the characteristics of bioaerosol production, making it challenging to apply generalizations across diverse environmental settings [47,109].

Concerning the factors influencing bioaerosol emissions, recent progress has revealed that diffused aeration, as opposed to surface aeration, can reduce emissions by up to 90% due to its smaller bubble size and slower velocity [107]. Additionally, research has demonstrated that surfactants enhance the aerosolization of bacteria and viruses by reducing surface tension in wastewater. However, there is still a considerable amount to learn, especially regarding comprehending the intricate mechanisms governing virus aerosolization related to bubble properties, encompassing variables such as surface tension, bubble size, and velocity. Furthermore, factors like temperature, humidity, and additional aerosol particles have been shown to influence the ease of virus dispersion in the atmosphere [110]. Nonetheless, more research is required to ascertain the relative significance of these factors and enhance our understanding of the mechanisms governing virus aerosolization and the variables influencing their release into the atmosphere. Additionally, as indicated in a study, the choice of aeration system holds significant importance, with diffused aeration notably reducing bioaerosol emissions by over 80% compared to surface aeration.

Furthermore, research has elucidated the impact of wind direction and speed on the dispersion of bioaerosols, demonstrating that higher wind speeds correlate with increased distribution and reduced concentrations of bioaerosols. At a distance of 50 m from a WWTP, bioaerosol concentrations can be as much as 10 times lower than concentrations at the point of origin. Undoubtedly, these findings will inform the design of effective mitigation plans for viral aerosols in WWTPs. However, to facilitate the development of practical mitigation strategies, this field necessitates further investigation to comprehensively understand the intricate interactions governing bioaerosol emissions and dispersions.

The third aspect highlighted in Table 7 underscores the health and environmental risks associated with exposure to bioaerosols from WWTPs. A study has concluded that individuals working in sludge storage areas are more susceptible to respiratory symptoms and infections when exposed to bioaerosols from WWTPs. Intriguingly, nasal breathers face 2.5 to 3.5 times more risks than oral ones. Another study established a connection between exposure to bioaerosols and an elevated risk of cancer, neurological disorders, and cardiovascular disease [47]. It is essential to acknowledge precise statistics and data concerning bioaerosols' health and environmental impacts, depending on the specifics of each research project and contextual nuances, to formulate effective mitigation plans.

Lastly, the concluding aspect explores various approaches to mitigate bioaerosol emissions from wastewater treatment plants. Research has demonstrated the efficacy of ultraviolet germicidal irradiation (UVGI) in reducing the presence of airborne viruses and bacteria in WWTPs [109]. The article revealed that, in specific cases, applying UVGI can reduce the concentration of airborne bacteria and viruses by up to 99%. Biocides, biofilters, and electrostatic precipitation are additional viable mitigation techniques. However, many questions in this field remain unanswered, particularly regarding the feasibility and effectiveness of diverse mitigation techniques across different scenarios. For example, the effectiveness of diffused aeration may hinge on factors such as the depth and design of the aeration tank, the type of wastewater undergoing treatment, and the local climate conditions. Likewise, the success of UVGI could be contingent on variables like the duration and intensity of UV exposure, the distance between the UV source and the bioaerosol source, and the presence of supplementary aerosol particles. Consequently, further research is essential to enhance our understanding of the practicality and efficacy of various mitigation strategies in varied contexts.

6. Influence of SARS-CoV-2 Outbreak

The recent global pandemic has prompted a surge in the investigation of viruses within wastewater treatment plants (WWTPs). The detection of SARS-CoV-2 in wastewater and its potential to infect plant operators has shifted the focus of researchers toward examining the possibility of SARS-CoV-2 as an occupational health risk. This includes analyzing its concentrations and assessing the threat posed by aerosols as potential transmission agents. Despite the heightened research concerning SARS-CoV-2 in WWTPs, confirming the transmission of SARS-CoV-2 via sewage or wastewater systems remains unproven to date.

The outbreak of SARS-CoV-2 has had a significant impact on the study of bioaerosols originating from wastewater treatment plants (WWTPs). Overall, bacterial abundance has declined due to the widespread use of disinfectants during the pandemic, although chlorine-resistant bacteria tend to survive. Antibiotic resistance genes (ARGs) were 13 times more prevalent during the COVID-19 pandemic compared to the years before, and aerosolized bacteria exhibited increased antibiotic resistance upon exposure to disinfectants [111]. The primary route of exposure to ARGs was through respiratory inhalation, and the potential health risks from this exposure were twice as high as they were before the COVID-19 pandemic. Recent advancements in the field have highlighted the heightened antibiotic resistance of aerosolized bacteria when exposed to disinfectants following the COVID-19 pandemic. Two areas that warrant further investigation are the enduring effects of the pandemic's heightened concentration of ARGs in aerosols and the development of practical mitigation techniques to mitigate the health hazards posed by the spread of antibiotic-resistance genes via aerosols.

Further investigations have brought to light the presence of fragments of SARS-CoV-2 RNA in the wastewater of a WWTP in the Middle East, with a maximum concentration of 1.4×10^5 copies/L. The quantity of these RNA fragments was directly correlated with the number of COVID-19 cases in the region [111]. This discovery underscores the possibility of using wastewater-based epidemiology (WBE) as a viable and cost-effective method for monitoring the virus and identifying infected individuals. Moreover, a positive association was observed between the concentration of SARS-CoV-2 RNA fragments in the wastewater and the ambient air at the WWTP. This suggests the virus can generate bioaerosols during mechanical and operational processes at the WWTP.

An examination was conducted on the study's database, taking into account the submission dates for the articles. This supported the assertion regarding the increase in publications investigating viruses detected at WWTPs. The WHO officially declared the onset of the pandemic in January 2020. The variation in publications linked to this event can be observed in Figure 5.

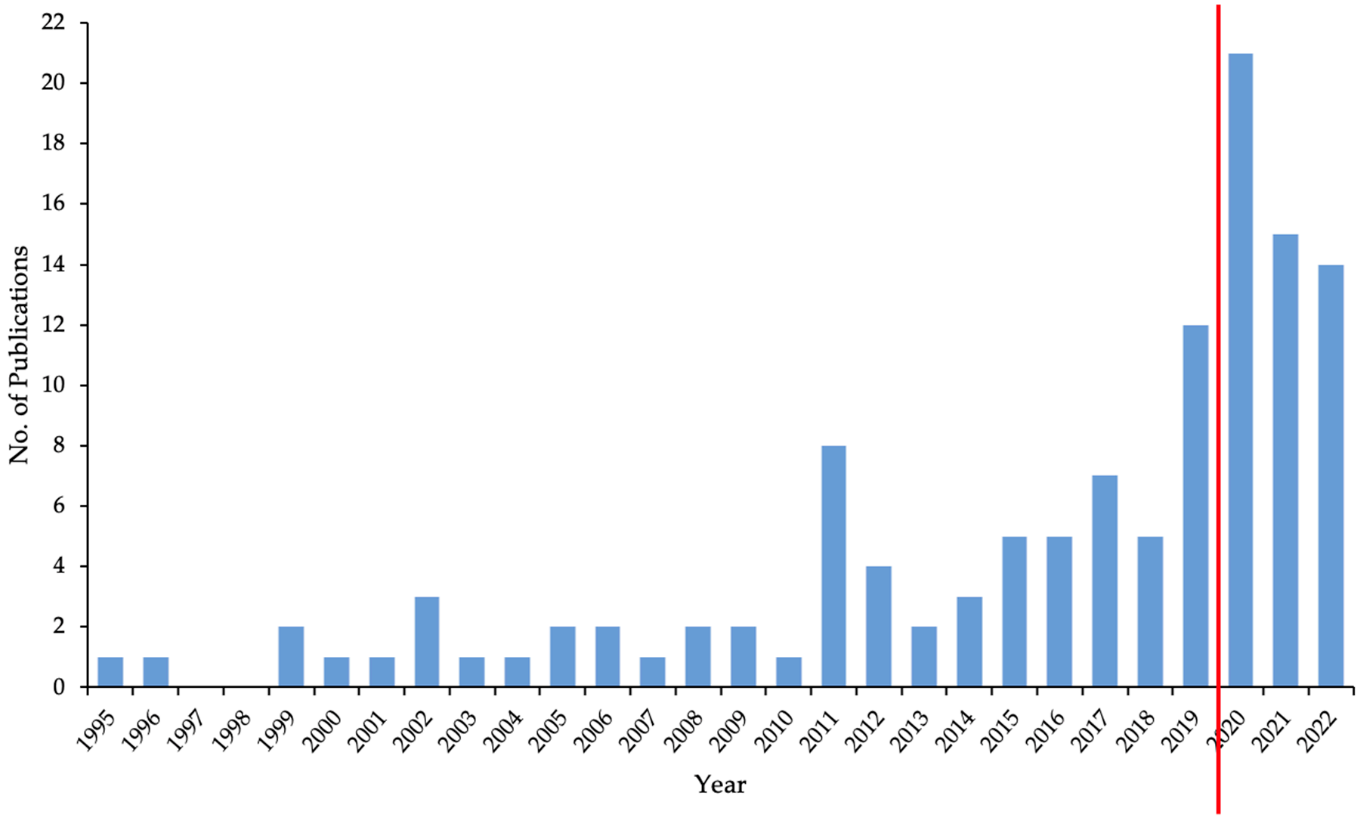

Figure 5. Publications before and after SARS-CoV-2.

Of the 12 articles received in 2019, only 2 were received after the first confirmed case of SARS-CoV-2. The preliminary nature of data reported in the literature suggests further research is needed, mainly emphasizing modeling studies accounting for SARS-CoV-2 persistence, infectiveness, and aerosolization across various WWTP settings and environmental variables for more accurate risk estimation.

7. Conclusions

The bibliometric analysis of bioaerosols from WWTPs revealed significant insights into research trends, major contributors, and recent advancements in this field. The study was based on data retrieved from the Scopus database. The topic has demonstrated notable growth from 1996 to 2022, with a small number of publications in the initial years of the selected time frame to a substantial increase of 25 publications in 2022. Initially, leading research was conducted by developed countries such as the USA, Poland, and Italy; however, as time progressed, China emerged as a primary publisher of articles related to this study. Collaboration between countries was found to be weak and may benefit from improvement. Most of the research was led by developed countries, underscoring the need for increased collaboration between developing and developed countries. The most influential journals in total publications were Science of Total Environment, Water Science, Aerobiologia, and Ecotoxicology and Environmental Safety. Key recurring keywords in the analysis included "quantitative microbial risk assessment", "pathogenic microorganisms", "health risk", and "airborne bacteria."

Recent advancements in bioaerosol studies related to WWTPs have revealed several areas that warrant further research. The spectral intensity bioaerosol sensor (SIBS) method can potentially overcome outdated sampling techniques with inherent limitations, which require less time and labor for sampling collection. Moreover, the type and concentration of suspended solids in pre-treatment and activated-sludge aeration tanks influenced bioaerosol emissions from these units. The quantitative microbial risk assessment model was recognized as a common approach to assessing infection risk probability, offering a valuable tool for implementing control strategies to mitigate dangers of exposure for neighboring households and employees of the facility. The recent pandemic has fueled interest among researchers in investigating the presence of SARS-CoV-2 in WWTPs. Additionally, implementing various control techniques has effectively reduced bioaerosol emissions in WWTPs.

Identifying research gaps in bioaerosols associated with WWTPs can provide valuable insights into knowledge deficiencies and guide future research. From the analysis, several future research opportunities have been identified:

1. Explore additional data analysis tools for the spectral intensity bioaerosol sensor (SIBS) to develop and validate a library, enhancing the identification and classification of bioaerosols.
2. Investigate aerosolization rates and bioaerosol emissions in real WWTPs using varying types and concentrations of suspended solids (SS) and different species of microorganisms.
3. Conduct epidemiological research to comprehensively understand WWTP workers' diverse health risks, moving beyond the QMRA model that relies on mathematical models and assumptions for risk assessment.
4. Undertake modeling studies to analyze SARS-CoV-2 persistence, infectiveness, and aerosolization across diverse WWTP settings and environmental variables, aiming for a more precise estimation of SARS-CoV-2 risk.
5. Investigate different control treatment processes at various wastewater treatment (WWT) stages to minimize exposure effects on occupants and nearby residents.

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