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MICROORGANISMS AS INDOOR AND OUTDOOR AIR BIOLOGICAL POLLUTION

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Abstract: Air pollution is a major threat to human health. Biological air pollution is predominantly caused by the pollen of plants, fungi, bacteria and viruses. The main sources of microorganisms in the air include soil, water and the decomposition of organic matter, while anthropogenic sources are represented by landfills, wastewater treatment plants, composting facilities and traffic. Microorganism populations in the air can be seasonal or relatively constant, but the most frequent increase in their occurrence is recorded in the summer and autumn. Studies show that humidity, the presence of carbon monoxide and ozone concentrations are the main factors affecting the diversity of bacteria and the percentage of pathogenic bacteria present in outdoor air. Microorganisms in the air inside residential buildings are primarily concentrated on dust particles. Approximately 60% of dust microbiota are spores of mould fungi. The key emitters of microorganisms into the atmosphere are municipal wastewater treatment plants. The bacteria and pathogens released are potentially resistant to antibiotics, rendering the bioaerosols of wastewater treatment plants a possible hazard to human health. There is a need for further research aimed at explaining the magnitude of impacts of air microorganisms on human health.

MIKROORGANIZMY JAKO BIOLOGICZNE ZANIECZYSZCZENIA WEWNĘTRZNEGO I ZEWNĘTRZNEGO POWIETRZA

Streszczenie: Zanieczyszczenia powietrza stanowią jedno z głównych zagrożeń dla zdrowia człowieka. Zanieczyszczenia biologiczne powietrza to w głównej mierze pyłki roślin, grzyby, bakterie oraz wirusy występujące w powietrzu jako tzw. bioaerozole biologiczne. Do głównych źródeł zanieczyszczeń mikrobiologicznych powietrza należą źródła naturalne, takie jak gleba, woda czy rozkład materii organicznej, a do źródeł antropogenicznych składowiska odpadów, oczyszczalnie ścieków, kompostownie, a także ruch uliczny. Pojawiające się w powietrzu populacje mikroorganizmów mogą mieć charakter zarówno sezonowy, jak również całoroczny, jednakże najczęściej, wyraźne nasilenie narażenia ich występowania następuje w okresie letnim i jesiennym. Badania wskazują, że wilgotność, obecność tlenku węgla i stężenie ozonu są głównymi czynnikami, wpływającymi na różnorodność bakterii i odsetek występujących tam bakterii chorobotwórczych w powietrzu zewnętrznym. Mikroorganizmy w powietrzu wewnątrz budynków mieszkalnych znajdują się przede wszystkim w kurzu. Jak wykazano, około 60% mikrobioty kurzu stanowią zarodniki grzybów pleśniowych. Do istotnych emiterów drobnoustrojów do powietrza atmosferycznego należą oczyszczalnie ścieków komunalnych. Obecność uwolnionych bakterii i patogenów opornych na antybiotyki w oczyszczalni ścieków sprawia, że bioaerozole oczyszczalni stanowią potencjalne ryzyko zdrowotne. Koniecznością staje się prowadzenie kontroli oraz stałe monitorowanie jakości powietrza, zarówno w pomieszczeniach zamkniętych, jak i przestrzeniach otwartych, pod względem występujących w nim mikroorganizmów, szczególnie w odniesieniu do ich wpływu na zdrowie człowieka. Ponadto istnieje potrzeba dalszych badań, dla wyjaśnienia roli mikroorganizmów przenoszonych przez powietrze i ich wpływu na zdrowie człowieka.

Key words: air, bacteria, health, human, microorganisms
Słowa kluczowe: bakterie, człowiek, mikroorganizmy, powietrze, zdrowie

1. Introduction

Atmospheric air, including indoor air, is a basic factor affecting the proper functioning of the human body. Air pollution constitutes one of the main threats to the environments in which people live. World Health Organization (WHO) and European Environment Agency (EEA) report that environmental risks such as air or water pollution have a significant impact on human health [26, 108]. Air pollution includes all substances in the Earth's atmosphere that are not natural components, as well as natural substances in significantly

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increased quantities. The Earth's atmosphere is composed of gases and vapours of chemical compounds, acid rain, airborne ashes, dust, trace elements and biological contaminants [26].

Biological air pollutants, also known as bioaerosols, include pollen, fungi, bacteria and viruses [5]. Most are microorganisms that colonise the soil, water bodies, plant surfaces, rocks and buildings [85]. The components of the bioaerosol that make up the dispersed phase are particles ranging in size from 1 to 200 μm. For example, single bacterial cells with dimensions of 0.5–2.0 μm can constitute *Bacillus* sp. or *Pseudomonas* sp. In turn, many spores of mould are sized between 3.0 and 17 μm [71]. In addition, bacterial toxins, mycotoxins, enzymes and fragments of plant and animal tissues are present in the air [18]. Bioaerosols ranging from 1.0–5.0 μm usually float in the air, while larger ones tend to settle on surfaces [97]. The greatest implications to human health has so called respirable bioaerosol, which is defined as fraction smaller than 7 μm. This fraction can penetrate the human respiratory tract with the inhaled air. The smallest particles can even reach pulmonary brioncholes [18]. Larger particles of bioaerosols tend to deposit in the upper airways [22].

Microorganisms are found in virtually every environment, including at extreme temperatures, pressures, salinity and acidity. The atmosphere has been described as one of the last biological limits on Earth. The composition and biodiversity of the microbial community in the atmosphere is still poorly researched [85]. Interestingly, bacteria and fungi have been detected in various atmospheric layers, such as the boundary layer (up to 1.5 km high), the upper troposphere (up to 12 km high) and even the stratosphere at an altitude of over 20 km above sea level [35, 105]. In addition, the fungi *Penicillium notatum* have been collected at an altitude of 77 km, and *Micrococcus albus* and *Mycobacterium luteum* bacteria at an altitude of 70 km [44]. The movement of airborne microorganisms at different distances and through wind and precipitation means that they can spread in all ecosystems. Some may be pathogens or transmit allergens and consequently endanger the health of the population [36].

As we know, one of the main factors influencing human health is air quality. Therefore, the presence of microorganisms, in particular those causing infectious diseases, in both ambient air and indoor environments can be particularly dangerous. Scientists have demonstrated that most often, microorganisms living in the air are responsible for irregularities in the immune system, such as allergies and infections [23, 31, 41, 50]. WHO alerts that the exposure to mould and other dampness-related microbial agents increases the risks of hypersensitivity pneumonitis, allergic alveolitis or chronic rhinosinusitis which is supported by *in vivo* and *in vitro* studies [107]. In addition, as Cabral [14] claim, the number of microorganisms present in the air as well as indoors is one of the main indicators of air pollution. It should also be noted that a threat is posed not only by the presence of pathogenic microorganisms or toxins of microbiological origin in the air, but also by an excess of saprophytic microorganisms, especially when their composition is quite undifferentiated and dominated by microorganisms belonging to a single species [14, 23, 31, 41, 107].

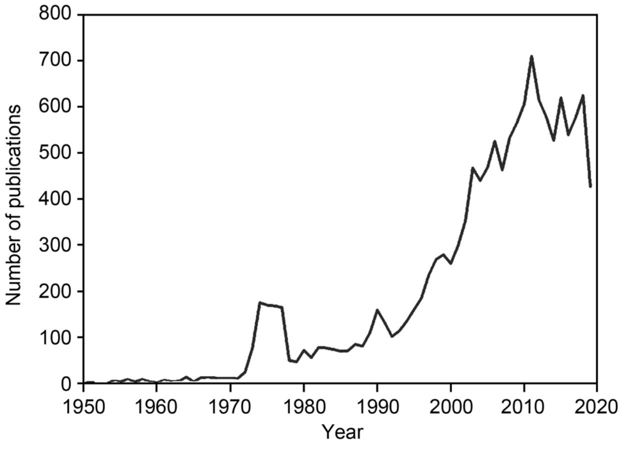

Fig. 1. Yearly number of scientific publications with keywords 'air' and 'microorganism' in the Scopus database – state for 13 Sept. 2019

Information on the presence of microorganisms in the air remains incomplete and numerous questions still need to be answered. The recent report of European Environment Agency (EEA) [26] describes sources and types of air pollution along with the potential health and ecosystem impacts, however it does not refer to biological agents in the air. Research on microorganisms in air have emerged only recently as documented by number of scientific publication. Figure 1 presents number of scientific papers in the Scopus database having 'microorganism' and 'air' as keywords. They are scattered within various research domains with environmental science, medicine, immunology and biochemistry subjects most often represented (Fig. 2).

The goal of this article was to provide recent information on presence of microorganisms in outdoor and indoor air, across residential, industrial and public spaces, with implications to human health.

2. Sources, transport and factors affecting the presence of microorganisms in the outdoor air

Viruses, cells or fragments of bacterial cells, as well as fragments of mycelium and fungal spores and protozoa are found in atmospheric air [5, 34]. The composition of atmospheric air in microbiological terms has become the subject of considerable research. The

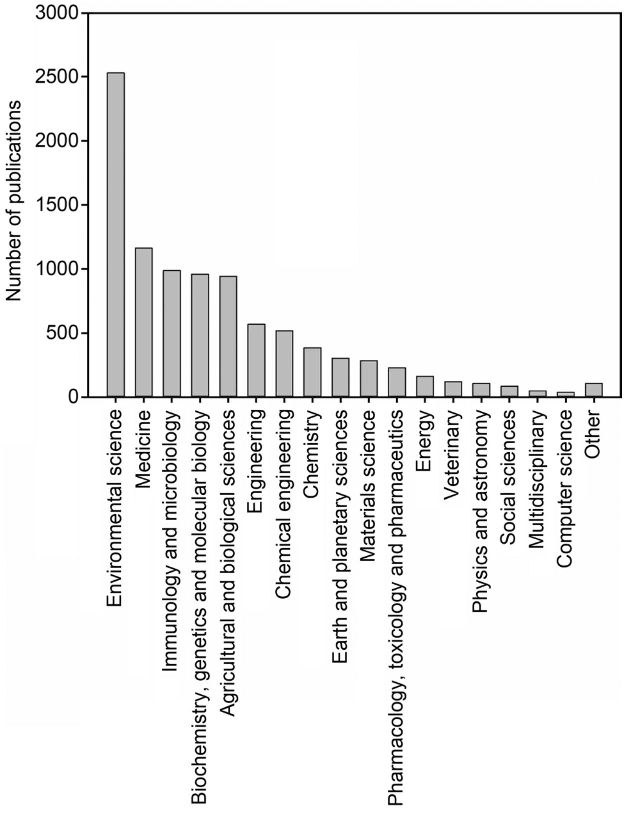

Fig. 2. Number of scientific publications with keywords 'air' and 'microorganism' as recorded in the Scopus database (date 13 Sept. 2019) across research subjects

main sources of microbiological air pollution include natural sources such as the soil, water, and plant surface contamination, including microorganisms transmitted by gusts of wind, dust in rain drops or insects. In turn, anthropogenic sources include various types of landfill, sewage treatment plants, composting plants and traffic. It should be mentioned that the microbiological composition of the atmosphere is significantly influenced not only by the source, but also by the volume of emissions, the distance from places of increased emissions, the type and survival of microorganisms, their spread and climatic conditions [11, 14, 31, 38, 90].

In addition, it is important to note that the main source of air pollution is human activity, including the transport. Humans also contribute to the production of biological aerosols through sneezing, coughing or physical exertion by introducing significant amounts of microorganisms into the atmosphere [33]. Further potential sources of microorganisms include domestic activities such as cooking or vacuuming as well as domestic objects like carpets. They can release spores of fungi of the genera *Alternaria* or *Cladosporium*. The soil of potted plants grown in apartments can also be a source of increased concentrations of fungal spores, for example from the genus *Aspergillus*. Sources of bioareozoles include rooms with high levels of humidity, which can also promote the development and subsequent spread of mould. Cellars, bathrooms and holiday cottages are all extremely attractive places for fungi to rapidly develop. Moreover, dust in places of everyday use can encourage fungi's development [40, 95]. Zyska [115] reports that fungal spores in the domestic environment constitute between 5 and 20% of household dust. Aquariums may also be a potential source of bioaerosols [82], as water fungi live on fish excrements, food remains or dead fish. Furthermore, some species of bacteria have learnt to function on the skin, gills and digestive tract of fish [1]. Mbareche *et al.* [70] reported the broad range of fungi pathogens including many known genera like *Aspergillus*, *Acremonium*, *Alternaria* and *Fusarium* in bioaerosols within dairy farms in Eastern Canada.

Recently, biological air pollution inside buildings has attracted growing attention of researchers. According to the literature, biological matter scattered in the air, such as microorganisms, their products, fragments of plants and animals directly connected with fine solid particles or droplets of liquid can constitute from 5 to approximately 34% of all contaminants present in the internal air [71, 30]. Overall assessment of the concentration of bioaerosols is very difficult, depending on numerous indicators that have a direct or indirect impact on general airborne microorganisms [15, 61].

Scientific research conducted over the years has rendered to link exposure to bioaerosols with the occurrence of adverse health effects, including infectious diseases, acute toxic effects, allergies and cancers [7–9, 23, 32, 97, 106].

According to literature data, in addition to harmful effects on health, bioaerosols can play an important role in the development, evolution and dynamics of ecosystems and atmospheric processes. Airborne bacteria, fungal spores and other bio-particles play a key role in the reproduction and spread of organisms, such as the long-range transportation of pollen over long distances and across geographical barriers. Furthermore, the dispersion of plant, animal and human pathogens can have serious consequences for agriculture and public health. Bioaerosols are therefore very important for the spread of organisms, allowing the exchange of genetic material in different habitats and latitudes [28]. Desert sandstorms have been shown to constitute an important source and the most efficient transport mechanism for bioaerosols, enabling the spread of microorganisms over distances greater than 5,000 km [54]. Therefore, an increasing number of studies are seeking to characterise the temporal and spatial variability of bioaerosols and their interactions with human health and air pollution.

Liu *et al.* [67] conducted an air quality study on the total number of bacteria and pathogenic bacteria. Air samples containing dust with different particle size and index values (relative humidity, carbon monoxide, ozone concentration) of different quality were collected in Hangzhou (China) and then 16S rRNA sequencing was applied to classify the bacteria present. The study revealed that at the genus level, *Thiobacillus*, *Methylobacterium*, *Rubellimicrobium* and *Paracoccus*, all belonging to *Proteobacteria*, were the most abundant: with average relative abundances of 2.6%, 2.5%, 1.8% and 1.7% of the total community, respectively. The most dominant pathogenic bacteria genera in the airborne particulate matter (PM) samples were: *Staphylococcus*, *Bacillus*, *Clostridium*, *Enterobacter* and *Klebsiella* [67]. Their research demonstrated that humidity, carbon monoxide presence and ozone concentration were the main factors influencing the diversity of airborne bacteria and the percentage of pathogenic bacteria. Similar observations were made by Tsai and Liu [102], whose analyses showed that the air humidity index is an extremely important factor for the development of microorganisms. Analysis of the state of air pollution with PM dust of various diameters while considering the chemical state (relative humidity, carbon monoxide, ozone concentration) allowed the researchers to state that in the PM2.5 and PM10 samples (PM2.5 with particle diameters up to 2.5 µm and PM10 with particle diameters up to 10 µm), the relative abundance of pathogenic bacteria was greatest in heavy and medium air pollution, respectively [67].

As mentioned above, high concentrations of airborne particulate pollutants are one of the main environmental factors that have a harmful impact on the health of the population. Hwang *et al.* [43] conducted research on airborne concentrations in ambient air, dust and bacteria at day care facilities in Seoul (South Korea) in the context of indoor environmental factors. Room tests were carried out for temperature and relative humidity, PM10 dust in rooms with two or more windows, and PM10 dust concentration in heating, ventilation and air-conditioning systems. The aim of this study was to measure concentrations of PM10 and bacteria in the air in order to determine how they correlated with a specific indoor environment. The research demonstrated that certain meteorological factors are strongly related to the structure and diversity of pathogenic bacteria. The bacterial count was significantly higher in day care rooms, as they only had one window and solely relied on window ventilation. In addition, a correlation between relative air humidity and the number of bacteria was observed. In turn, Fröhlich-Nowoisky *et al.* [28] have stated that relative humidity affects the number of fungi occurring in an environment to a greater extent than temperature. Moreover, the bacterial level in ventilated areas is lower than in areas without ventilation systems [102].

As shown by Zhen *et al.* [113], meteorological factors have a greater impact on airborne bacteria than air pollution. During their study in Beijing (China), the authors analysed the relationship between the total number of bacteria in the atmospheric air and the structure of their population and weather factors. The examined factors included air temperature and humidity, wind speed, atmospheric pressure and the level of air pollution, expressed by indicators such as concentration of PM, ozone, oxides of sulphur and nitrogen as well as carbon monoxide. The factors exerting the greatest influence were atmospheric pressure (in winter), air temperature and relative humidity (in spring and summer) and water vapour pressure in the air (in autumn). Moreover, meteorological factors explained changes in the structure of the bacterial community to a greater extent than air pollution.

Xie *et al.* [110] have characterised the microorganisms present in the air of different levels of quality. Air samples were collected in Xi'an (China) from April 2016 to February 2017. The abundance of all microorganisms in the air, showed significant seasonal variations. In addition, the average bacterial content in the air on foggy days was much higher than on days without fog. Interestingly, the amount of bacteria in the air in the first period of research increased, before slightly decreasing with a deterioration in air quality. The largest number of microorganisms was observed at a moderate level of air pollution. In addition, it was noted that snowfall can improve air quality by reducing number of bacteria.

The movement of dust particles, microorganisms and other contaminants over long distances is caused by the movement of the air. Research on the quantity and species composition of airborne microorganisms is a key factor in explaining the ways in which they spread and interact [10, 54, 88]. Previous literature has confirmed that some bacteria (e.g. from the genus *Bacillus*) and most of fungi have endospores or latent forms. The resting form enables organisms to survive in adverse conditions (drought, low temperatures and nutrient deficiencies) [79]. In addition, it has been shown that many bacteria isolated from the air exhibit intense colouration associated with high pigment content involved in protecting the microorganisms from ultraviolet radiation [29, 53, 100]. In addition, microorganisms, pollen or mineral dust particles are removed from the atmosphere by natural physical phenomena such as mutual attraction [65, 66, 103].

Rajasekar and Balasubramanian [86] focused their research on the occurrence and structure of microbial populations in different places, depending on the prevailing conditions: temperature, relative humidity, population density, location (indoor/outdoor) and the presence of air conditioning. They showed that bacteria constituted 50.5% and fungi 49.5% of bioaerosol composition in the internal environment, while in the external environment they constituted 20.6%

on average, while the proportion of fungi was 79.4%. Moreover, they noted that population density is a factor that significantly affects the concentration of bacteria in the air. It should be noted that in the case of indoor air samples, measurements were taken at two points in each location, i.e. the bedroom, kitchen, toilet or work area. At these points, air samples were taken from a height of about 1 m above floor level. However, in the case of outdoor air, samples were taken from outside the place of residence at a height of 1.5 m. The authors summarised that the type of ventilation and activities performed in different places in the household may affect the level of biological pollutants in the air. Furthermore, the results of this study suggest that the cycle of replacement of used air with fresh air from outside and the types of human activities carried out in the outside environment represent important factors. The ventilation system should be continuous and efficient, reflected in its impacts on levels of endotoxins in the air and airborne microorganisms.

The speed of multiplication, resistance to adverse environmental conditions and differentiation of preferences as to environmental conditions allow for the spread of these bacteria in the environment. Hara and Zhang [39] conducted a study on the presence of airborne Asian dustborne bacteria in southwest Japan. Aerosol particles were collected in the form of dust on the campus of the University of Kumamoto in both high and low concentrations. The results of the study indicated that the total concentrations of bacterial cells in the dust were higher in the dusty air. In addition, it was found that dead bacterial cells were superior to dead bacterial cells in dust, but the concentrations of living bacterial cells were comparable or higher than in non-dusty air. Bacterial cells transported with airborne dust can be exposed to many adverse factors, including changes in the weather such as temperature and humidity, atmospheric pressure, wind speed and direction as well as solar radiation intensity. It should be noted that the presence of living bacterial cells may be dispersed over long distances [89].

3. Microorganisms in the air inside residential buildings

In residential buildings, biological factors in direct contact with humans may affect the human health. Due to the time spent in residential or office buildings (approximately 90 percent of time is spent by human in closed rooms) [109], indoor air quality has a greater impact on people's well-being and health than does outdoor air quality. The construction techniques of the 21st century, such as the installation of air-tight windows or ventilation systems, are causes of increased biological pollution. In addition, these pollutants can come both from inside residential buildings and from outside, flowing directly through chimneys, open windows, cracks or other leaks [56].

Nabrdalik i Latała [78] undertook research aimed at the quantitative and qualitative characterisation of fungi present in building structures. They demonstrated that it is necessary to develop test methods and criteria to assess the degree of air pollution in residential buildings due to the presence of potentially harmful fungi in the analysed objects. Excessive humidity, poor ventilation and insufficient insulation are reasons for the excessive development of filamentous fungi [107].

Microorganisms in the air inside residential buildings are mainly found in dust, which has become an increasingly frequent subject of research aimed at assessing the quality of indoor air. Dust is primarily characterised by the presence of organic compounds, trace elements and metabolites of mites, responsible for many hypersensitivity reactions in the form of allergies [75]. It has been shown that about 60% of dust microbiota are spores of mould fungi of the genera: *Alternaria, Aspergillus, Cladosporium, Fusarium, Mucor*, *Penicillium* and *Rhizopus*. On the other hand, bacteria constitute about 1 to 25% of microbiota, and about half of the bacterial population are *Micrococcus* spp. Moreover, staphylococci, rods and aerobic spore-forming bacteria from the genus *Bacillus* are quite common. Yeast, in turn, is represented by species of the genera *Rhodotorula* and *Candida* [16, 46, 104].

Staszowska [96] conducted research on dust samples from dwellings and educational institutions from a microbiological perspective. Samples were collected from various objects in Lublin (Poland). Dust samples were collected from places such as the floor (carpet) and from shelves with books from a height of 1 m. The most common bacteria were Gram-positive *Bacillus* spp. In dust samples from educational institutions, *B. subtillis* was characterised by the highest concentration. The second largest recognised group was Gram-positive bacteria, with *Micrococcus* sp. predominating, especially the *M. luteus*. Among fungi the dominant genera were *Aspergillus* (especially *A. nigeri*) and *Penicillium*. Fungi of the genus *Cladosporium* were also found.

Other authors have also demonstrated the presence of *Penicillium* sp. and *Aspergillus* sp. Fungi, as well as Gram-positive and Gram-negative bacteria, with the aim of assessing the effect of selected factors on the presence of microorganisms in the dust and the relationship between their presence indoors and human respiratory health. Significant dependence of concentration of microorganisms on humidity in rooms and presence of animals was shown. Both factors affect the functioning of the respiratory system, including the appearance of asthma [99]. Li *et al.* [63] have highlighted the greater presence of fungi in bathrooms than in bedrooms whereas Buttner and Stezenbach [13] have similarly noted that the number of fungi in living rooms are higher than in bedrooms, which may be associated with the greater activity of people in the former.

Al-Hunaiti *et al.* [3] studied bacteria and fungi in floor dust in residential buildings and education rooms. Dust samples were taken from closed rooms in Amman (Jordan). Genetic identification of selected fungal and bacterial groups and chemical analysis of dust were undertaken. Concentrations of bacteria and fungi were directly correlated with those of polycyclic aromatic hydrocarbons (PAHs). The lowest concentrations of bacteria and fungi were found in the apartment that was the least used. The concentration of Gram-positive bacteria in the educational building was found to be lower than that observed in the living quarters. Gram-positive bacteria showed a positive correlation with phenanthrene concentration, while Gram-negative bacteria showed a positive correlation with fluoranthene and pyrene concentration. The content of fungi of the genera *Penicillium* and *Aspergillus* was moderately or strongly negatively correlated with concentrations of such hydrocarbons as anthracene, fluoranthene, benzo (b) fluoranthene and benzo (j) fluoranthene. The total number of fungi showed negative correlations with the concentration of anthracene, benzo (a) anthracene and dibenzo (a-h) anthracene. This means that there may be a high risk of exposure to the appearance of metabolites of aromatic hydrocarbons, which are carcinogens, in these rooms. The results obtained undoubtedly necessitate further study on the biodegradation of PAHs in house dust and on the presence of potentially health hazardous metabolites of PAHs, including a whole range of measurements with chemical and biological analysis of floor dust contamination and its occurrence in confined spaces.

4. Microorganisms in indoor air in office and public spaces

In enclosed spaces with a fairly high density of people, the number of microorganisms is much higher than in open spaces. The air inside buildings is richer in various microorganisms, including pathogenic microorganisms. Humans constitute a source of indoor bacteria. Bacteria are emitted from the human respiratory airways through talking, coughing or sneezing. They also get into the air with skin scales. Therefore the level of air contamination with bacteria is dependent on the number of people inside a room and efficiency of ventilation system [73]. Kubera *et al.* [60] carried out microbiological air quality tests in selected kindergartens. Air samples were collected in classrooms, both in the morning and in the afternoon. Samples collected outside the kindergartens were a control sample and the quality of indoor air was assessed according to Polish standards (PN-Z-04111-01:1989; PN-Z-04111-02:1989; PN-Z-04111-03:1989) based on groups of indicator microorganisms. Regardless of the research method applied, the total number of heterotrophic bacteria and staphylococci in the air exceeded the permissible values, although no excessive contamination with mould fungi was found. These studies suggest that air sanitation should be controlled with particular regard to biological agents that may directly or indirectly affect the health of indoor residents.

In the course of studies carried out in offices and laboratories, 20 different species of bacteria have been detected, belonging to genera such as *Bacillus*, *Staphylococcus*, *Microccocus*, *Microbacterium*, *Acinetobacter*, *Arthrobacter*, *Shigella*, *Escherichia*, *Moraxella*, *Paenibacillus*. Several genera of fungi have been also detected, including: *Alternaria*, *Penicillium*, *Aspergillus* and *Cladosporium* [114].

The results of biological research of air pollution in office buildings in Warsaw (Poland) equipped with or without air conditioning systems were presented by the team of the Central Institute for Labour Protection – National Research Institute [33]. Two genus of fungi predominated: *Penicillium* and *Aspergillus*. Such presence did not exceed the acceptable standards proposed by a panel of experts on the factors of recommended concentrations of microorganisms and endotoxins in indoor air. More concerning, however, was that the presence of fungi belonging to the risk group responsible for the occurrence of adverse health conditions for workers was found. In this group there were, among others, *Streptococcus viridans*, *Candida* spp*., C. glabrata, A. fumigatus*, *A. flavus* and *A. niger*. These fungi are classified as allergenic and toxic factors and cause infectious and invasive diseases. Research conducted in the 1980s by Morey *et al.* [76] demonstrated that the ventilation system affects the composition and concentration of bioaerosols in offices. Warm and dry conditions favour the passive dispersion of fungal spores such as *Cladosporium* sp. Spores in dry air conditions are mostly blown naturally by the air, and their concentrations are higher in places without a ventilation system.

Xu and Hao [111] reviewed the available literature on air quality in public metro spaces. Their data show that air quality in subways is particularly alarming. Airborne particles, aromatic hydrocarbons and airborne bacteria are classified as major air pollutants within metro systems. Despite the fact that metro passengers usually spend only a dozen or so minutes there, they are exposed to pollution emitted from various sources as well as directly from metro passengers. The harmfulness of the underground internal microenvironment is associated with the exposure time resulting from its frequent and even daily use [55].

Subway air quality was also the subject of research by Robertson *et al.* [87], who determined the composition and diversity of microorganisms in bioaerosol samples in the New York metro. Their analyses showed the presence of various genera and species of microorganisms, characteristic of the soil and water environment and inhabiting the human body, including those occurring mainly on the skin. Among these latter microorganisms, 99% identity of the rRNA sequence with such species as *Staphylococcus* spp., *S. epidermidis* (the most abundant group of bacteria colonising the human skin surface, contributing to its protection against pathogen penetration) and *S. hominis*, *S. cohnii*, *S. caprae* and *S. haemolyticus* were found. The authors did not produce results regarding the presence of microorganisms that pose a threat to public health.

5. Microorganisms in the air of industrial facilities

Perez-Martin *et al.* [84] measured the abundance of lactic acid bacteria and yeasts in the air and wine produced in vineyards in order to assess the potential for microbial migration. Samples were taken from a vineyard in Castilla-La Mancha (Spain). The sampling period was as follows: air and wine samples were collected in September and November 2011 and September and October 2012. The *Leuconostoc mesenteroides* species was the most common lactic acid bacteria in the air, while the *Oenococcus oeni* species dominated in wine. *Saccharomyces cerevisiae* was the most frequently isolated yeast species in both air and wine. Furthermore, the presence of the same microorganisms in samples from the vineyard's air as well as in the wines themselves has confirmed that the transfer of microorganisms between the two environments is possible.

Tsai and Liu [102] conducted a study on workers' exposure to bioaerosols at a pasta plant in central Taiwan. The biological risks associated with pasta production had not previously been studied. The aim of the study was to characterise the biological composition and concentrations of bioaerosols at different locations in the pasta factory over a period of one year, as well as to investigate the impact of various environmental factors on the concentration of bioaerosols. Air samples were collected twice daily. Nine species of fungi were identified in the air and strains from the genera *Cladosporium*, *Penicillium* and *Aspergillus*, commonly regarded as fungi that cause allergic reactions, were the dominant fungi isolates in indoor and outdoor air samples. *Micrococcus* spp. and *S. xylosus* predominated among isolated strains of bacteria. In addition, significant seasonal differences were observed for most fungi, except for the genus *Fusarium*. The number of fungi of the genera *Aspergillus* and *Rhizopus* differed significantly during two sampling times (morning, afternoon), as did the levels of *Micrococcus* spp. and *S. arlettae*. It was found that the concentration of bacteria was higher in the morning; similar results have been obtained in other studies [27, 60, 65]. These results were important for the exposure assessment of workers. Due to the findings, increased ventilation was recommended to improve sanitation. Although study of Hwang [43] demonstrated the need for further detailed studies, especially regarding the relationship between the composition of bioaerosols and the possibility of allergic reactions.

Czerwińska and Piotrowski [20] conducted a risk assessment of microbiological contamination in a bakery in Koszalin (Poland). Microbiological analyses of the air were carried out in the warehouse, production hall and the room where the bread was stored in order to cool it down. Their results demonstrated that air pollution in the bakery area was negligible, with the most frequently appearing microorganisms being bacteria of *Bacillus* genus. Microbiological analyses of the presence of fungi indicated a fairly large variation in the occurrence of these microorganisms depending on the season (December – 1st term, March –X 2nd term). Analyses performed in the first term indicated contamination with these microorganisms, while in the second term the number of isolated fungi did not arouse any objections.

In 2016, a paper on bacterial and fungal aerosol concentration in the air in the production halls of a meat plant was published [11]. In the examined air the following indicators were determined: total number of bacteria and fungi, as well as *E. coli* and bacteria from genus *Listeria*. The studies showed that the presence of bacterial and fungal aerosols may potentially be a source of indirect microbiological contamination. The dominant fungi were *Aspergillus* sp., *Alternaria* sp. and *Cladosporium* sp. The presence of *E. coli* and *Listeria* spp. was also found. Moreover, genetic identification confirmed the presence of *L. monocytogenes*.

6. Bioaerosols within the sewage treatment plants

As previous research has shown, municipal sewage treatment plants are significant emitters of microorganisms to atmospheric air. The presence of microorganisms in wastewater entering the air together with the droplets of wastewater that are formed during mixing and aeration creates a certain pathogenic risk. Breza-Boruta *et al.* [12] reported the sewage treatment plant originated pathogens in air samples collected 150 m from the plant border. However, it should be remembered that not every treatment plant poses a risk to the environment [12, 47, 51, 52]. Paśmionka *et al.* [81] conducted research on microbiological atmospheric air pollution in the area of the Group Wastewater Treatment Plant in Chrzanów (Poland). The plant was identified as a potential cause of an increase in the number of microorganisms present in the air and of a localised deterioration in the sanitary conditions within the plant. Relationships between the number of microorganisms in the air and weather conditions were observed. The number of fungi in the air did not exceed the permitted values according to Polish standards (PN-Z-04111/02,1989; PN-Z-04111/03,1989). It should be noted, however, that the results attained indicated the existence of bioaerosols within the sewage treatment plant, which may adversely affect the state of the air and consequently pose a potential health hazard.

Of particular concern are pharmaceutical wastewater treatment plants, which have been identified as important reservoirs and sources of emissions to the natural environment of bacteria resistant to antibiotics [112]. Moreover, antibiotic-resistant microorganisms migrate within and around wastewater treatment plants in the form of biological aerosols, mainly produced by aeration equipment, which is the main reason for the release of bioaerosols in wastewater treatment plants [42, 57, 91].

According to Zhang *et al.* [112], pharmaceutical wastewater treatment plants are potential locations for concentrations of pathogenic bacteria resistant to antibiotics, which can be transferred to the natural environment. Analyses have shown that almost half (45%) of the bacteria floating in the air within the pharmaceutical wastewater treatment plant studied were resistant to three or more antibiotics. Some pathogenic strains were resistant to up to 16 different antibiotics. This fact proves that wastewater treatment plants are an important source of antibiotic-resistant bacteria. The dominant genera of such bacteria isolated from all bioaerosol samples are: *Acinetobacter*, *Alcaligenes*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Pantoea*, *Pseudomonas* and *Sphingomonas*. Previous research has indicated that measurements of bioaerosol emissions in pharmaceutical wastewater treatment plants are required, while sites inside and around wastewater treatment plants should be constantly monitored.

The presence of released antibiotic-resistant bacteria and pathogens in sewage treatment plants renders the bioaerosols of such plants a potential health risk. Despite new reports, there remains little systematic research regarding the exact composition, possible health consequences and resistance to antibiotics of pathogenic bacteria found in bioaerosols from sewage treatment plants. Existing studies primarily focus on the antibiotic resistance of pathogenic Gram-negative bacteria [4, 21, 37, 58, 59].

Gram-negative bacteria usually constitute a small proportion of the bacteria present in bioaerosols. It should also be remembered that pathogenic bacteria living in bioaerosols function in conditions that are not always favourable to them, affecting their survival. The air represents a challenging environment for such microorganisms. Variable meteorological conditions, such as sunlight and humidity, can inhibit their growth and even kill them [2, 59].

Korzeniewska *et al.* [58] conducted research on bacteriological atmospheric pollution in the area and surroundings of a mechanical-biological municipal sewage treatment plant, including sewage from the dairy industry. The study recognised a number of heterotrophic, psychrophilic, psychrotrophic and mesophilic bacteria as well as selected physiological groups of *Enterobacteriaceae* family, *Staphylococcus* and *Enterococcus* genera, *P. fluorescens* and *P. aeruginosa*, haemolytic bacteria and *Actinomycetes*. Significant differences were found in the number of groups of tested microorganisms depending on the season and the method of air sampling applied (sedimentation and collision method) at six sites located in the area of the treatment plant as well as five sites located in its vicinity. The exceptions were psychrophilic bacteria and *Enterobacteriaceae* family bacteria, in which no significant variability was found. The highest average numbers of microbial groups selected for the study were observed in air samples collected via the sedimentation method, especially in the autumn (with the exception of *Actinomycetes*, whose maximum numbers were seen in spring). The lowest average numbers were observed in winter and/or summer. In addition, it was shown that the concentration of bacteria in the air collected at various sites located in the area of sewage treatment plants differed. Higher bacterial numbers were observed in the following areas: sandstone, phosphate chamber, nitrification and denitrification chambers and secondary settling tank. In general, air in both the area and the surrounding environment of mechanical-biological sewage treatment plants was classified as slightly polluted, whereas in the spring and autumn there were more psychrophilic, psychrotrophic and mesophilic bacteria.

Korzeniewska *et al.* [57] determined the microbiological composition of air in a sewage treatment plant with a closed bioreactor. The air samples showed the presence of heterotrophic, psychrophilic, psychrotrophic, mesophilic and hemolytic bacteria, *Enterobacteriaceae* family bacteria and *Staphylococcus*, *Enterococcus* and *Pseudomonas* genera, as well as colonies of *Actinomycetes*. The presence of fungi, including moulds, yeasts and yeast-like fungi was also demonstrated. The largest concentrations of microorganisms were detected in air collected in and near the bioreactor itself, as well as near the truss. The authors demonstrated that the presence and diversity of *Enterobacteriaceae* family (including *Shigella* spp., *Yersinia enterocolitica*, *E. coli, K. pneumoniae* and *K. ozaenae*) in or near the bioreactor may potentially affect the health of people working with and staying by the bioreactor for an extended period of time.

Seasonal changes in the number of bacteria occurring in the air within sewage treatment plants are mainly observed in the summer, when higher emissions are recorded due in particular to lower precipitation. Szyłak-Szydłowski *et al.* [98] carried out bioaerosol studies at a plant in different seasons of the year, collecting air samples using different technological processes at the plant. The most intensive emissions of microorganisms to the air were recorded in the summer, while among the technological processes, the highest concentrations of bacteria were generated by the primary settling tank and the sludge tank. Among the microorganisms isolated from the air, the presence of heterotrophic bacteria, mesophilic bacteria and coliforms was found.

7. Air microorganisms as an important factor influencing human health

At present, awareness is growing with regard to external and internal air pollution, as evidenced among others by recent studies on the influence of air quality on the prenatal and postnatal development of children [17, 19, 98, 101]. One example is a study by Shehab and Pope [93], which demonstrates the relationship between air quality and human cognitive performance. Air quality was assessed through annual average levels of nitrogen dioxide and indoor air by means of data on humidity and exposure to secondhand smoke. In terms of biological air quality, studies show that exposure to microorganisms and their derivatives (endotoxins, mycotoxins) in early childhood has a significant impact on the development of the immune system and the emergence of allergies, asthma and atopy [64, 77, 92]. Based on epidemiological data, it has been found that children living in rural areas on a farm are much less likely to develop allergies and asthma than are children raised in the same rural area but without exposure to farming environment [25].

Air is often considered an important carrier of bacterial pathogens such as *S. pneumoniae*, *S. pyogenes, Mycoplasma pneumoniae*, *Haemophilus influenzae*, *K. pneumoniae*, *P. aeruginosa* and *Mycobacteriaceae* family. In order to ascertain the source of the presence of these health-endangering pathogens in the air, it is important to identify the environment in which they live and reproduce. For more information on their ability to exist in the air and to spread, continuous monitoring and proper control are required. Undoubtedly, the occurrence of pathogenic bacteria in the air is characterised by seasonality. For example, meningococcal foci have been found throughout sub-Saharan Africa, where climatic conditions with strong droughts and sandstorms ending with the rainy season contribute to the occurrence of these life-threatening bacteria, which can cause dangerous invasive diseases [74, 94].

Numerous studies have shown that bioaerosols in the air may be potential causes of bodily dysfunctions. Airborne microorganisms, especially bacteria and fungi, can cause inter alia asthma, hay fever, bronchitis, chronic lung failure, lung cancer, cardiovascular diseases, gastrointestinal tract infections, tuberculosis, allergic reactions, sinus and conjunctivitis and acute viral infections [45, 62, 69]. As many as 80 types of fungi can cause respiratory allergies, and as many as 100 types of fungi can cause severe infections not only in humans and animals, but also in plants. These include fungi-representing genera such as *Cladosporium*, *Alternaria*, *Aspergillus* and *Fusarium* [48].

Aside from pathogenic microorganisms in the air, attention should be paid to their biologically active products, such as endotoxins (toxins occurring in the outer membrane of Gram-negative bacteria) or mycotoxins (secondary metabolic products of mould fungi, which can also have a significant impact on human health). Airborne biological agents are becoming important issues for human safety and health [24, 68].

Pastuszka *et al.* [83] performed a study to determine the concentrations of bacterial and fungal bioaerosols in different types of buildings and to determine the risk of specific diseases that they can cause. They showed that in buildings with mould problems there was a risk of asthma symptoms. This risk most likely increased through the inhalation of fungal particles and other airborne compounds. In houses without mould, the relative concentration of the observed species, including *Penicillium* spp., ranged from 3 to about 50% of the fungal population, while in houses with mould, the highest concentration of *Penicillium* spp. was found in 90% of all fungi.

Biological factors are one of the main causes of the so-called "sick building syndrome" (SBS). The term is used to describe a situation in which residents of a given building experience health problems and a range of symptoms such as e.g. skin rashes, headache, itchy eyes, throat irritation, dry skin, rashes, dry cough, hoarseness of voice, asthma attacks and other. It is assumed that biological pollutants such as viruses, bacteria, dust, pollen, toxic black mold, insect body parts contribute to the SBS along with chemical pollutants, ozone produced by printers, electromagnetic radiation, inadequate ventilation [6]. A low level of fresh air in a room, a concentration of living organisms, poor ventilation, elements of building equipment and human physiology all affect the quality of life in buildings.

In general, factors responsible for so called thermal comfort in buildings are also decisive for biological quality of indoor air. They include ambient temperature and its fluctuations, relative humidity, air movement, average radiant temperature and personal parameters, describing metabolic activity or clothing [80]. The optimal humidity is defined in a range between 40–60%. In too dry conditions harmful substances enter human organism faster whereas in high humidity reproduction of microorganisms is accelerated. Ambient temperature most recommended for the thermal comfort is in the range 20–24°C, which is also conducive to good air quality [80]. Mniszek i Rogiński [72] reviewed the literature on structural defects in buildings as the causes of fungal infections in rooms. They concluded that moulds attack not only old, unheated houses but also new or recently renovated buildings. This is due to numerous factors appearing even in the initial stages of construction (incorrect construction of walls, roofs with inadequately protected wood, lack of adequate ventilation, leakage of water system, etc.). Moisture, which results in the appearance of mould, is linked to the presence of mycotoxins produced by certain species of mould from the genera *Aspergillus, Penicillium, Fusarium, Rhizoctonia, Claviceps* and *Stachybotrys*. They are classified as dangerous or even carcinogenic and mutagenic substances that can cause changes in the proper functioning of the body. They inhibit DNA synthesis or instigate alterations in RNA metabolism. Their toxic secondary metabolites belong to the group of the most potent poisons. Mycotoxins can cause acute and chronic poisoning, allergies, mycoses, respiratory, digestive and liver diseases, as well as weakening of the immune system. These toxic substances disturb a number of vital functions, including disorders of the central nervous system, immune mechanisms, liver and kidneys. Metabolites produced by moulds can cause dermatitis and poisoning, with symptoms including headaches and diarrhoea [33, 72]. As such, exposure to moisture and mould has an undeniable impact on respiratory health [46].

Currently there are no binding legal instruments regulating the permissible content of microorganisms in Poland in both atmospheric and indoor air. Previous standards, such as PN-89/Z-04008/01; PN-89/Z-04008/08; PN-89/Z-04111/02; PN-89/Z-04111/03 have been waived and they have not been replaced by new instruments until now. However the waived standards are still occasionally used to interpret the results [18]. For example, the standard PN-89/Z-04111/02 specified the strong air contamination with bacteria as state when their total content exceeded 3000 CFU m^{-3} or the content of *P. fluorescens* was above 50 CFU m^{-3}. The contents of fungi were specified in the PN-89/Z-04111/03 – the strong contamination meant the total content of fungi above 10000 CFU m^{-3} [18].

There are no safe level values for exposure to microbiological contamination on the air specified by WHO. The WHO guidelines [107] specify the conditions that contribute to the health risk, emphasizing that microbial growth may result in greater numbers of spores, cell fragments, allergens, mycotoxins, endotoxins, β-glucans and volatile organic compounds in indoor air. The guidelines were formulated by WHO, such as the following: persistent dampness and microbial growth on interior surfaces and in building structures should be avoided or minimized; indicators of improper conditions include the presence of condensation on surfaces, visible mould, mouldy odour and frequent water leakage or penetration. The document also states that no quantitative health-based guideline values or thresholds can be recommended for acceptable levels of microorganisms in indoor air [107].

8. Conclusions

The composition of atmospheric air is an extremely important factor affecting human health. This review of literature from Poland and elsewhere has shown that biological aspects of the air significantly reflect the state of the environment in which we live and function on a daily basis. Humidity is an important indicator of air quality, because in most cases it contributes to the development of microorganisms, both naturally occurring and those that operate under conditions of excessive humidity. This can in turn cause allergic reactions. The most frequently mentioned allergens in the air include fungi such as *Cladosporium* sp., *Penicillium* sp. and *Aspergillus* sp. Microbial populations in the air may be either seasonal or all-year in character, although their occurrence is generally most intense in summer and autumn. Further factors shaping the number and diversity of microbial communities include atmospheric phenomena (i.e. clouds, fog, rain and snow) and proximity to municipal sources of bioaerosol such as e.g. wastewater treatment plants. It is therefore essential to monitor air quality in both indoor and outdoor environments for microorganisms, in particular with regard to their effects on human health. Increased awareness of the threats posed by biological air pollution will enable greater care of the conditions in which people live every day. In particular, the proper ventilation of buildings, ventilation of rooms and prevention of excessive air humidity are all essential.

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