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WYDZIAŁ OCEANOGRAFII I GEOGRAFII

CYANOBACTERIA AND MICROALGAE IN ATMOSPHERIC AEROSOLS IN THE COASTAL ZONE OF THE GULF OF GDANSK

SINICE I MIKROGLONY W AEROZOLACH ATMOSFERYCZNYCH W STREFIE BRZEGOWEJ ZATOKI GDAŃSKIEJ

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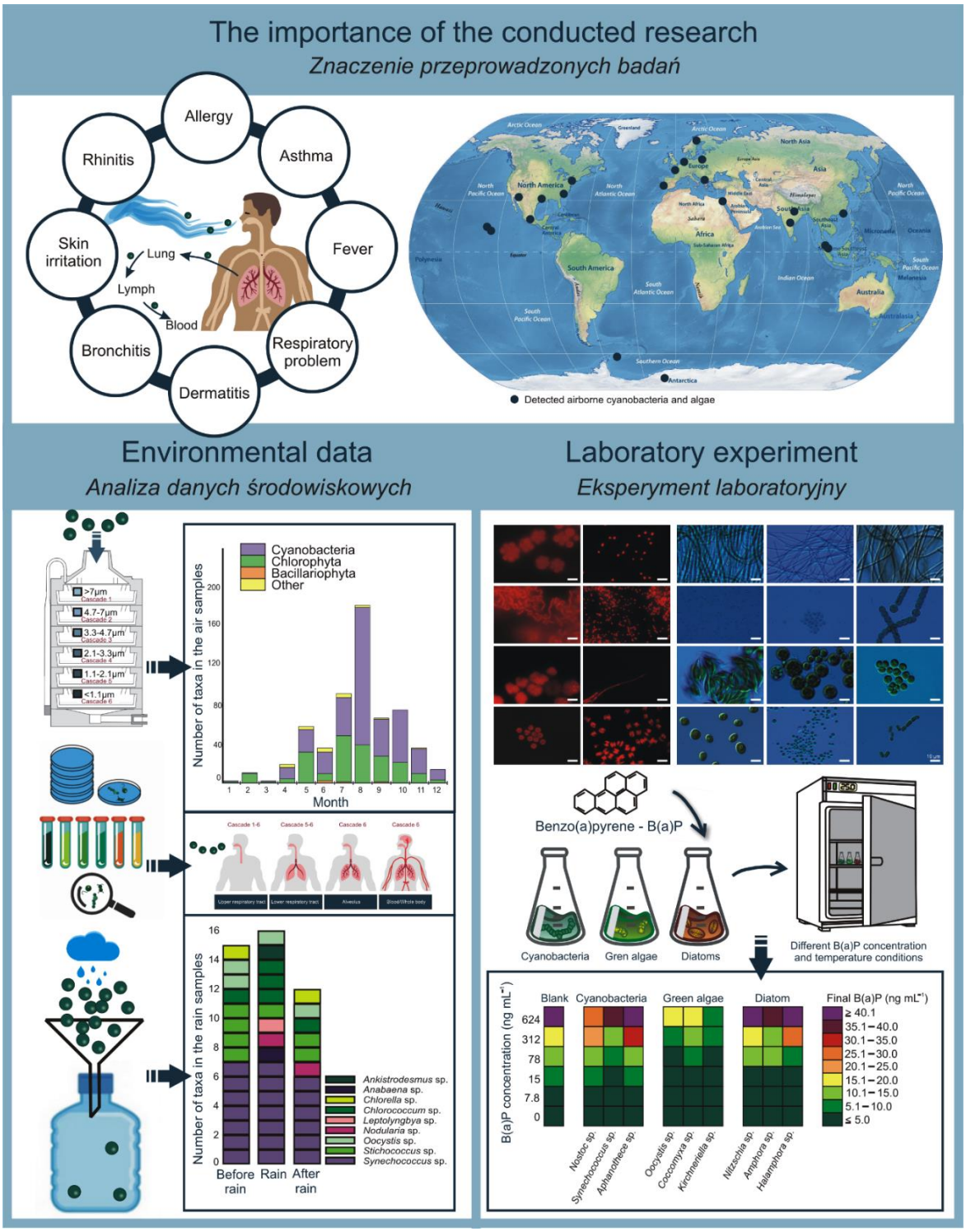

Keywords: bioaerosols; airborne cyanobacteria and microalgae; benzo(a)pyrene; harmful taxa; microcystin

Słowa kluczowe: bioaerozole; sinice i mikorglony przenoszone drogą powietrzną; benzo(a)piren; gatunki toksyczne; mikrocystyna

2 ABSTRACT IN POLISH

2 STRESZCZENIE W JĘZYKU POLSKIM

Rozprawa doktorska podejmuje temat obecności sinic i mikroglonów w aerozolach atmosferycznych w strefie brzegowej Zatoki Gdańskiej. Po przeprowadzeniu szczegółowego przeglądu literatury światowej na temat aktualnego stanu wiedzy o sinicach i mikroglonach obecnych w powietrzu, procesach jakim podlegają oraz aspektach wymagających dalszych badań naukowych, wyznaczono cele i zadania badawcze oraz postawiono hipotezy. Zostały one następnie zweryfikowane podczas badań środowiskowych i eksperymentów laboratoryjnych. Na ich podstawie, wykorzystując specjalistyczną aparaturę naukowo - badawczą, określono ilość oraz skład taksonomiczny sinic i mikroglonów w aerozolach różnych rozmiarów w cyklu dobowym, jak i sezonowym. Dodatkowo, przeprowadzono jakościową i ilościową analizę tych mikroorganizmów w opadach deszczu w okresie największej produktywności fitoplanktonu w Zatoce Gdańskiej. Wykazano ponadto, które z czynników meteorologicznych determinują zmienność występowania sinic i mikroglonów w powietrzu. Istotną kwestią prowadzonych badań było wskazanie, czy sinice i mikroglony obecne w atmosferze strefy brzegowej Zatoki Gdańskiej mogą stanowić potencjalne zagrożenie dla zdrowia człowieka. Celem badań było ustalenie, czy występują między nimi organizmy szkodliwe i czy są one zdolne do produkowania toksycznej mikrocystyny-LR. Ostatni cel pracy związany był z określeniem wpływu sinic i mikroglonów na obecność w powietrzu benzo(a)pirenu, który stanowi indykator stopnia jego zanieczyszczenia wielopierścieniowymi węglowodorami aromatycznymi.

Realizacja celów pozwoliła zweryfikować następujące hipotezy:

H1. Sinice i mikroglony są obecne w atmosferze strefy brzegowej Zatoki Gdańskiej przez cały rok, prawdopodobnie jako konsekwencja obserwowanego wzrostu temperatury powietrza w ostatnich dekadach.

H2. Wśród czynników meteorologicznych warunkujących występowanie sinic i mikroglonów w atmosferze strefy brzegowej Zatoki Gdańskiej największe znaczenie odgrywa opad deszczu.

H3. Unoszące się w powietrzu sinice i mikroglony mogą stanowić potencjalne zagrożenie dla zdrowia ludzi, jako źródło toksyn oraz nośnik benzo(a)pirenu, który jest wskaźnikiem zanieczyszczenia powietrza wielopierścieniowymi węglowodorami aromatycznymi.

W niniejszej pracy ustalono, że:

- Ilość sinic i mikroglonów w powietrzu strefy brzegowej Zatoki Gdańskiej waha się od 0 do 1685 komórek m^{-3}. W deszczu ich ilość wynosi od 100 do 342×10^3 komórek L^{-1}.
- Organizmy te są obecne w powietrzu atmosferycznym przez cały rok. Największa ich ilość jest odnotowywana w atmosferze w lipcu, co może być konsekwencją wzmożonej produkcji pierwotnej w morzu. Warunki sprzyjające występowaniu sinic i mikroglonów w atmosferze są analogiczne do warunków sprzyjających toksycznym zakwitom w Morzu Bałtyckim w okresie letnim. Procesowi temu towarzyszy wzrost temperatury powietrza oraz niska prędkość wiatru.
- Najbardziej efektywnym czynnikiem meteorologicznym, który prowadzi do usuwania z atmosfery aż do 87% sinic i mikroglonów jest opad deszczu.
- W atmosferze w rejonie Zatoki Gdańskiej dominują sinice, zielenice oraz okrzemki. Badania pozwoliły zidentyfikować 29 taksonów sinic i mikroglonów, z czego 60% stanowiły sinice. Wraz z opadem deszczu organizmy te są wymywane z atmosfery bez względu na skład taksonomiczny.
- Wśród obecnych w atmosferze sinic i mikroglonów występują taksony potencjalnie niebezpieczne dla zdrowia ludzi. Mogą być one deponowane w najgłębszych odcinkach układu oddechowego człowieka, tj. w oskrzelikach płuc. Jednak zdecydowana większość tych organizmów (70%) odnotowywana jest w cząstkach dużych (średnica > 7 µm), mniej niebezpiecznych dla zdrowia.
- Obecne w atmosferze sinice są zdolne do produkowania mikrocystyny - LR, a jej stężenie jest zmienne dla poszczególnych szczepów i waha się od wartości poniżej limitu detekcji do 420 fg $kom.^{-1}$.
- Niskie stężenie B(a)P może prowadzić do wzrostu liczby komórek sinic i mikroglonów w atmosferze, a także do zmian zawartości barwników asymilacyjnych i zdolności do przeprowadzania procesu fotosyntezy. Dodatkowo ustalono, że obecne w atmosferze zielenice są prawdopodobnie zdolne do degradowania benzo(a)pirenu.

3 ABSTRACT IN ENGLISH

3 STRESZCZENIE W JĘZYKU ANGIELSKIM

The doctoral dissertation addresses the presence of cyanobacteria and microalgae in atmospheric aerosols in the coastal zone of the Gulf of Gdansk. After conducting a comprehensive review of the global literature on the current state of knowledge regarding airborne cyanobacteria and microalgae, the processes they undergo, and the aspects that require further scientific research, research objectives and tasks were determined, and hypotheses were formulated. They were verified during environmental studies and laboratory experiments. Based on them, utilizing specialized scientific research equipment, the quantity and taxonomic composition of cyanobacteria and microalgae in aerosols of various sizes were determined in both diurnal and seasonal cycles. Additionally, a qualitative and quantitative analysis of these microorganisms was conducted in rainwater during the peak productivity period of phytoplankton in the Gulf of Gdansk. Furthermore, it was demonstrated which meteorological factors determine the variability of cyanobacteria and microalgae occurrence in the air. An important aspect of the conducted research was to determine whether the cyanobacteria and microalgae present in the atmosphere of the Gulf of Gdansk coastal zone could pose a potential threat to human health. The objective of the study was to establish the presence of toxic organisms among them and to assess their ability to produce toxins, exemplified by microcystin-LR. The final objective of the research was to determine the influence of cyanobacteria and microalgae on the presence of benzo(a)pyrene in the air, which serves as an indicator of the level of contamination with polycyclic aromatic hydrocarbons.

The achievement of these objectives has allowed for a verification of the hypotheses:

H1. Cyanobacteria and microalgae are present in the atmosphere of the coastal zone of the Gulf of Gdansk throughout the year, probably due to increase in air temperature in recent decades.

H2. Among the meteorological factors determining the presence of cyanobacteria and microalgae in the atmosphere of the Gulf of Gdansk coastal zone, rainfall is the most significant.

H3. Cyanobacteria and microalgae suspended in the air can pose a potential threat to human health as a source of toxins and through the transfer of benzo(a)pyrene, which is an indicator of air pollution by polycyclic aromatic hydrocarbons.

In this study, it was determined that:

• The quantity of cyanobacteria and microalgae in the air of the coastal zone of the Gulf of Gdańsk varies from 0 to 1685 cells m^{-3}. In rainwater, their quantity ranges from 100 to 342×10^3 cells L^{-1}.

• These organisms are present in the atmosphere throughout the year. The highest quantity of microorganisms in the atmosphere is observed in July, which is a result of increased primary production in the Baltic Sea. The conditions favoring the occurrence of cyanobacteria and microalgae in the atmosphere are analogous to the conditions favoring phytoplankton blooms in the Baltic Sea during the summer period. This process is accompanied by an increase in air temperature and low wind speed.

• The most effective meteorological factor that leads to the removal of up to 87% of cyanobacteria and microalgae from the atmosphere is rainfall.

• In the Gulf of Gdansk region, cyanobacteria, green algae, and diatoms are dominant in the atmosphere. The research let to identify 29 taxa of cyanobacteria and microalgae, with cyanobacteria accounting for 60% of the total. With rainfall, these organisms are washed out of the atmosphere regardless of their taxonomic composition.

• Among the cyanobacteria and microalgae present in the atmosphere, there are taxa that are potentially hazardous to human health. They can be deposited in the deepest parts of the human respiratory system, such as the bronchioles of the lungs. However, most of these organisms (70%) are observed in larger particles (diameter > 7 μm), which are less harmful to health.

• The cyanobacteria present in the atmosphere can produce microcystin-LR, and its concentration varies for different strains, ranging from values below the detection limit to 420 fg $cell^{-1}$.

• Low concentration of B(a)P can lead to an increase in the number of cyanobacteria and microalgae cells in the atmosphere, as well as changes in the pigments content and the photosynthesis performance. Additionally, it has been established that the green algae present in the atmosphere are probably capable of degrading benzo(a)pyrene.

4 LIST OF PUBLICATIONS CONSTITUTING THE DOCTORAL THESIS

4. LISTA PUBLIKACJI STANOWIĄCYCH ROZPRAWĘ DOKTORSKĄ

I. **Wiśniewska K.**, Lewandowska A., Śliwińska-Wilczewska S. 2019. *The importance of cyanobacteria and microalgae present in aerosols to human health and the environment – Review study.* Environment International, 131, 104964. DOI: 10.1016/j.envint.2019.104964.

II. **Wiśniewska K**., Śliwińska-Wilczewska S., Savoie M., Lewandowska A. 2022. *Quantitative and qualitative variability of airborne cyanobacteria and microalgae and their toxins in the coastal zone of the Baltic Sea*. Science of The Total Environment, 826, 154152. DOI: 10.1016/j.scitotenv.2022.154152.

III. **Wiśniewska K**., Śliwińska-Wilczewska S., Lewandowska A. 2022. *Airborne microalgal and cyanobacterial diversity and composition during rain events in the southern Baltic Sea region*. Scientific Reports, 12, 2029. DOI: 10.1038/s41598-022-06107-9.

IV. **Wiśniewska K.,** Lewandowska A.U., Śliwińska-Wilczewska S., Staniszewska M., Budzałek G. 2023. *The ability of airborne microalgae and cyanobacteria to survive and transfer the carcinogenic benzo(a)pyrene in coastal regions.* Cells, 12, 1073. DOI: 10.3390/cells12071073.

Table 4.1 A metric summary of publications constituting the doctoral thesis
Tablica 4.1 Zestawienie metryczne publikacji stanowiących rozprawę doktorską

Article	IF	5-years IF	MS&HE*	No. of citations	Year of publication	Own contribution
I	13.352	13.238	140	67	2019	60%
II	10.754	10.237	200	3	2022	60%
III	4.996	5.516	140	8	2022	60%
IV	7.666	7.677	140	0	2023	50%
Sum	36.768	36.668	620	78	-	-

* Ministry of Science and Higher Education

5 OTHER ORIGINAL PUBLICATIONS FROM JCR LIST

5 LISTA POZOSTAŁYCH PUBLIKACJI Z LISTY FILADELFIJSKIEJ

I. **Wiśniewska K**., Lewandowska A.U., Witkowska A. 2017. *Factors determining dry deposition of total mercury and organic carbon in house dust of residents of the Tri-city and the surrounding area (Baltic Sea coast).* Air Quality Atmosphere and Health 10, 821–832. DOI: 10.1007/s11869-017- 0471-2.

II. Lewandowska A. U., Bełdowska M., Witkowska A., Falkowska L., **Wiśniewska K**. 2018. *Mercury bonds with carbon (OC and EC) in small aerosols (PM1) in the urbanized coastal zone of Gulf of Gdansk (southern Baltic)*. Ecotoxicology and Environmental Safety 157, 350-357. DOI: 10.1016/j.ecoenv.2018.03.097.

III. **Wiśniewska K**., Lewandowska A., Staniszewska M. 2019. *Air quality at two stations (Gdynia and Rumia) located in the region of Gulf of Gdansk during periods of intensive smog in Poland*. Air Quality Atmosphere and Health 12, 879–890. DOI: 10.1007/s11869-019-00708-6.

IV. Śliwińska-Wilczewska S., Konarzewska Z., **Wiśniewska K.,** Konik, M. 2020. *Photosynthetic Pigments Changes of Three Phenotypes of Picocyanobacteria Synechococcus sp. under different Light and Temperature Conditions.* 2020. Cells, 9(9), 2030. DOI: 10.3390/cells9092030.

V. **Wiśniewska K.,** Śliwińska-Wilczewska S., Lewandowska A. 2020. *The first characterization of airborne cyanobacteria and microalgae in the Adriatic Sea region.* Plos One, 15(9):e0238808. DOI: 10.1371/journal.pone.0238808.

VI. **Wiśniewska K**., Śliwińska-Wilczewska S., Lewandowska A., Konik M. 2021. *The effect of abiotic factors on abundance and photosynthetic performance of airborne cyanobacteria and microalgae isolated from the southern Baltic Sea region.* Cells, 10(1), 103. DOI: 10.3390/cells10010103.

VII. Śliwińska-Wilczewska S., **Wiśniewska K**., Konarzewska Z., Cieszyńska A., Felpeto A., Lewandowska A., Latała A. 2021. *The current state of knowledge on taxonomy, modulating factors, ecological roles, and mode of action of phytoplankton allelochemicals.* Science of the Total Environment, 773(1),14581. DOI: 10.1016/j.scitotenv.2021.145681.

VIII. Budzałek G., Śliwińska-Wilczewska S., **Wiśniewska K**., Wochna A., Bubak I., Latała A., Wiktor J.M. 2021. *Macroalgal defense against competitors and herbivores.* International Journal of Molecular Sciences, 22(15), 7865. DOI: 10.3390/ijms22157865.

IX. Buch J.K., Lewandowska A.U., Staniszewska M., **Wiśniewska K.A.,** Bartkowski KV. 2021. *The Influence of Transport on PAHs and Other Carbonaceous Species' (OC, EC) Concentration in Aerosols in the Coastal Zone of the Gulf of Gdansk (Gdynia).* Atmosphere, 12(8), 1005. DOI: 10.3390/atmos12081005.

X. Stojanowska A., Górka M., Lewandowska A.U., **Wiśniewska K**., Modelska M., Widory D. 2021. *Can Abies alba Needles Be Used as Bio-passive Samplers to Assess Air Quality?* Aerosol and Air Quality Research, 21, 210097. DOI: 10.4209/aaqr.210097.

XI. Budzałek G., Śliwińska-Wilczewska S., Klin M., **Wiśniewska K.,** Latała A., Wiktor J.M. 2021. *Changes in growth, photosynthesis performance, pigments, and toxin contents of bloom-forming cyanobacteria after exposure to macroalgal allelochemicals.* Toxins, 13(8), 589. DOI: 10.3390/toxins13080589

Table 5.1 A metric summary of other original publications
Tablica 5.1 Zestawienie metryczne pozostałych publikacji autora

Article	IF	5-years IF	MS&HE*	No. of citations	Year of publication
I	5.804	4.585	70	11	2017
II	7.129	6.263	100	21	2018
III	5.804	4.585	70	19	2019
IV	7666	7.677	140	14	2020
V	3.752	3.272	100	20	2020
VI	7.666	7.677	140	15	2021
VII	10.754	10.237	200	26	2021
VIII	6.208	6.628	140	10	2021
IX	3.110	3.222	70	5	2021
X	4.53	3.668	70	3	2021
XI	5.075	5.305	100	2	2021
Sum	67.498	63.119	1200	146	-

*Ministry of Science and Higher Education

6 JUSTIFICATION FOR TACKLING SCIENTIFIC RESEARCH

6 UZASADNIENIE PODJĘCIA BADAŃ NAUKOWYCH

Bioaerosols are microorganisms or their fragments emitted from the surface of the sea or terrestrial environment to the atmosphere. These organisms include bacteria, viruses, fungi, plant fragments, as well as cyanobacteria and microalgae (Genitsaris et al., 2011; Urbano et al., 2011). So far, scientific research has focused much more on viruses, bacteria, and fungi, which has led to a weaker recognition of the presence of cyanobacteria and microalgae in the air compared to other microorganisms. Therefore, research publications often state that cyanobacteria and microalgae present in the air are the least studied group of organisms in both phycology and aerobiology (Sharma et al., 2007; Després et al., 2012; Sahu and Tangutur, 2014). Before undertaking work on cyanobacteria and microalgae in the air of Gulf of Gdansk coastal zone, there was only one review article focusing on the occurrence of these organisms and their impact on human health (Genitsaris et al., 2011), as well as one preliminary study on the presence of these microorganisms in the southern Baltic Sea region (Lewandowska et al., 2017). A thorough analysis of literature data, which was collected and published by Wiśniewska et al. (2019), formed the theoretical basis for the present study (**Publication I**) but also allowed to formulate hypotheses, set research goals, and plan tasks necessary for their implementation.

The first studies on cyanobacteria and microalgae present in the air date back to the 1840s. During his journey across the Atlantic Ocean, Darwin collected and identified 18 genera of diatoms found in the air (Sharma et al., 2007; Genitsaris et al., 2011). Since then, studies have mainly focused on determining the taxonomic composition and identifying in what proportions these organisms occur in the air (Meier and Lindbergh, 1935; Schlichting, 1969; Rosas et al., 1989; El Gamal, 2008; Genitsaris et al., 2011; Singh et al., 2018). Scientists have used various methods to collect and cultivate these organisms, including car or airplane measurement campaigns (Carson and Brown, 1976; Lee and Eggleston, 1989). However, determining the number of microalgae in the air remains a problem due to the research techniques used and the lack of a clear methodology (**Publication I**). As a result, studies on the quantitative composition of cyanobacteria and microalgae are rare compared to studies determining the taxonomic composition of these organisms (Reisser, 2002; Després et al., 2012; **Publication I**). According to a review by Després et al. (2012), the number concentration of cyanobacteria and microalgae ranges from 100 to 1000 per m^{-3} of the air. It has also been established that humans inhale up to 1000 algal cells per liter of air (Tesson et al., 2016). Regarding the taxonomic composition of cyanobacteria and microalgae present in the air, Guiry et al. (2012) argue that the number of recognized taxa is still underestimated, and thousands of organisms living in the atmosphere have not yet been described. Similarly, in terms of research areas, there are many countries where such studies have never been conducted (**Publication I**). From the studies conducted so far, it appears that cyanobacteria are the only group that has been recorded in the air regardless of the research area. This means that so far, no region has been found where cyanobacteria are not present in the air (**Publication I**). The other two dominant groups in the air are green algae and diatoms but cryptophytes, chrysophytes, and dinoflagellates can sporadically occur in the atmosphere as well (Genitsaris et al., 2011; Després et al., 2012; **Publication I**). It is very probable that the domination between cyanobacteria and green algae depends on season (Sharma et al., 2006; 2006b; Lewandowska et al., 2017). Measurements conducted by Lewandowska et al. (2017) from April to November 2015 in the region of the southern Baltic Sea (Poland) let to identify 41 taxa overall, but their highest number occurred in April. The diversity stayed high until June and later started to drop up to only one taxon. The authors suggested that the variability in noted number of taxa was due to the vegetation period of algae and cyanobacteria in the Baltic Sea. In the case of diatoms during a warm spring, Cyanobacteria phylum dominated significantly over green algae, while from June to November Chlorophyta was the most abundant

group. Since the research was of a preliminary nature and did not provide information about the variability of taxa throughout the year or their quantity in aerosols, there was a need to continue the study.

Scientists have confirmed the presence of cyanobacteria and microalgae in various matrices, such as soil, snow, rain, aerosols, and clouds (van Overeem, 1937; Carson et al., 1976; Sharma et al., 2007; Genitsaris et al., 2011; Lewandowska et al., 2017). Research on these organisms has been conducted e.g., in the United States, Spain, Greece, India but also in Poland (Tormo et al., 2001; Sharma et al., 2007; Genitsaris et al., 2011, Facciponte et al., 2018, Lewandowska et al., 2017; Singh et al., 2018, **Publication I**).

It is important to note that scientists have been able to determine the role that cyanobacteria and microalgae present in the atmosphere play in the environment. Above all, these organisms are involved in processes occurring in the atmosphere and contribute to climate change (Tesson et al., 2016). Bioaerosols can act as cloud condensation nuclei as well as ice nuclei and influence the hydrological cycle and climate (Hoose and Möhler, 2012; Després et al., 2012; Tesson and Šantl-Temkiv, 2018). In addition, they affect the Earth's radiation balance by absorbing and scattering solar radiation (Després et al., 2012).

So far, there is little described in the world literature about the size of particles in which cyanobacteria and microalgae can occur in the atmosphere. Research conducted by Lewandowska et al. (2017) revealed that the size of bioaerosols depends on the studied area. In Poland, over the land microalgae and cyanobacteria occurred frequently in particles not exceeding diameter of 3.3 μm, while over the sea bioaerosol particles had a diameter of above 3.3 μm. The particle size information is important, above all, due to their transport in the atmosphere. Smaller particles can be transported further in the atmosphere (Marshall and Chalmers, 1997, Briffa et al., 2020). The size of the particle is also significant when it comes to its impact on human health. The smaller the particle, the deeper it can be deposited in the human respiratory system, and therefore poses a greater danger to health (Luo et al., 2016). The smallest particles can settle in air sacs and bronchi, and even enter the bloodstream, causing many diseases (Franck et al., 2003, Fröhlich-Nowoisky et al., 2016; Lewandowska et al., 2017; Facciponte et al., 2018). In the case of bioaerosols, including certain cyanobacteria and microalgae, particle fragmentation may occur during transport, resulting in smaller fragments that can ultimately reach deeper parts of the human respiratory system (Després et al., 2012).

There are studies confirming the negative impact of cyanobacteria and microalgae present in the atmosphere on human health (Genitsaris et al., 2011; Murby and Haney, 2015; Facciponte et al., 2018; Wiśniewska et al., 2019, Hofbauer 2021, Juay et al., 2023). Breathing air containing these organisms can result in allergies, inflammatory response, runny nose, skin irritation, burning eyes, or respiratory tract irritation (Bernstein and Safferman, 1966; Sharma and Rai, 2008; Genitsaris et al., 2011, Hofbauer 2021, Juay et al., 2023). Genitsaris et al. (2011) suggested that even 15% of airborne cyanobacteria and microalgae have a negative impact on human health. Among the dangerous airborne organisms are the commonly occurring green algae *Chlorella* sp., as well as many other microalgae and cyanobacteria such as *Amphora* sp., which belongs to diatoms, or the cyanobacteria *Synechococcus* sp. (Genitsaris et al., 2011). Moreover, some microorganisms inhabiting the atmosphere can produce toxins. Although the concentrations of toxins in the atmosphere still require further scientific research, it is important to note that toxins inhaled by humans can cause negative effects on health even at lower doses compared to other routes of entry (Genitsaris et al., 2011; Sahu and Tangutur, 2014). There is still no clear answer as to whether and how these organisms transport toxins, heavy metals, or pesticides into the human respiratory system. This is an area that requires further scientific investigation (Sharma et al., 2007; Lewandowska et al., 2017; Singh et al., 2018; May et al., 2018; **Publication I**). Similarly, in the case of pollutant transformations, there are studies demonstrating the ability of phytoplankton to transform benzo(a)pyrene (**Publication IV**), but as noted by Burge and Rogers (2000), airborne cyanobacteria and microalgae may undergo the same chemical processes as aerosols, and thus chemical pollutants may adsorb onto them. However, confirmation of this hypothesis requires further scientific research.

7 THESIS OBJECTIVES AND HYPOTHESES

7 CEL I HIPOTEZY POSTAWIONE W PRACY

The main aim of this thesis was to provide comprehensive knowledge on the presence of cyanobacteria and microalgae in atmospheric aerosols in the coastal zone of the Baltic Sea. **The research goals focused on:** *the quantity and taxonomic composition of these organisms in aerosols and rain, the factors influencing their variability in the air in daily and seasonal scale, as well as their potential role as threat to human health.*

The following research hypotheses were postulated in the doctoral dissertation:

H1. Cyanobacteria and microalgae are present in the atmosphere of the coastal zone of the Gulf of Gdansk throughout the year, probably due to increase in air temperature in recent decades.

H2. Among the meteorological factors determining the presence of cyanobacteria and microalgae in the atmosphere of the Gulf of Gdansk coastal zone, rainfall is the most significant.

H3. Cyanobacteria and microalgae suspended in the air can pose a potential threat to human health as a source of toxins and through the transfer of benzo(a)pyrene, which is an indicator of air pollution by polycyclic aromatic hydrocarbons.

To achieve the goals add verify the formulated hypotheses, the following research tasks have been defined:

- Sampling of bioaerosols in various size ranges in the Gulf of Gdansk coastal zone using a method that allows qualitative and quantitative analysis of cyanobacteria and microalgae in a daily and seasonal cycle,
- Sampling of atmospheric precipitation for qualitative and quantitative analysis of cyanobacteria and microalgae in rain during the period of highest primary production in the Baltic Sea,
- Simultaneously with collecting samples of aerosols and precipitation, collecting meteorological data (wind direction and speed, air temperature and relative humidity, atmospheric pressure, precipitation amount) and determining the air mass trajectories (https://www.ready.noaa.gov),
- Collecting an ecohydrological model data (http://model.ocean.univ.gda.pl) of blue green algae biomass, total phytoplankton primary production and basic biogenic compounds (NO_3^- and PO_4^3) in the Gulf of Gdansk seawater,
- Determination of the taxonomic composition and quantitative analysis of cyanobacteria and microalgae in aerosols of various size ranges and in rain samples,
- A review of scientific literature aimed at determining the range of B(a)P concentrations in atmospheric aerosols in the coastal zone of the Gulf of Gdansk,
- Conducting laboratory experiments aimed at determining the relationship between selected cyanobacteria and microalgae isolated from the atmosphere and benzo(a)pyrene concentration, which is an indicator of air pollution with polycyclic aromatic hydrocarbons.

8 MATERIALS AND METHODS

8 MATERIAŁY I METODY BADAWCZE

8.1 BIOAEROSOLS AND RAIN SAMPLE COLLECTION

Based on the identified literature (**Publication I**), it was possible to select a location for conducting measurements of airborne cyanobacteria and microalgae in the coastal zone of Gulf of Gdansk. Both, precipitation, and aerosol samples were collected in Gdynia at a research station located on the roof of the Faculty of Oceanography and Geography building, the University of Gdansk (54°31′ N, 18°48′E), at an altitude of 20 meters above sea level (**Publication II**, **Publication III**). This building is situated about 1 km from the coastal zone of the Gulf of Gdansk, while being in the city center. The height of the building enabled the collection of microalgae and cyanobacteria in aerosols and rain samples above the level of tree canopies and buildings. It also allowed for the collection of samples from mixed masses of air originating from both land and sea. In addition, this height reduced the risk of contamination of the samples with cyanobacteria and microalgae reemitted directly from the ground. The station was previously used to collect bioaerosols, particulate matter (PM_x) and wet precipitation samples (e.g., Witkowska et al., 2016 a and b; Lewandowska et al., 2017; Lewandowska et al., 2018; Skalska et al., 2019; Wiśniewska et al., 2019b; Buch et al., 2021).

The research material consisted of samples of cyanobacteria and microalgae in aerosols (**Publication II**, **Publication III**) and in wet deposition (**Publication III**) (Table 8.1).

Table 8.1 Biological materials used in doctoral dissertation
Tablica 8.1 Zestawienie materiału badawczego wykorzystanego w rozprawie doktorskiej

Kind of material	Sampling period	Number of samples	Publication
Aerosols	2018	180	Publication II and III
	2019	234	
	2020	800	
Rain	2019	13	Publication III
	2020	7	

Samples of airborne cyanobacteria and microalgae used for pilot studies were collected with varying frequency between 2018 and 2019 to refine the sampling method used by Lewandowska et al. (2017) and (Wiśniewska et al., 2021). Samples used in **Publication II** were collected at least 4 times per month during both day and night in the year 2020. A total of 1214 aerosol samples were collected (Table 8.1). To determine whether rainfall effectively washes out cyanobacteria and microalgae from the atmosphere, samples were collected during two measurement campaigns in the period of highest primary production in Baltic Sea (**Publication III**). The first campaign lasted from May to September 2019. The second campaign of seven days took place from August 27th to September 2nd, 2020, during which rainfall occurred almost every day.

Atmospheric aerosols were always collected before and after each rainfall. A total of 20 rain samples were collected (Table 8.1).

8.2 Analysis of bioaerosols and rain samples

8.2.1 Techniques used for bioaerosols and rain sample collection

Before collecting bioaerosol samples for quantitative and qualitative analysis, sterile F/2 culture medium (Guillard, 1975) was prepared and calibrated using seawater with a salinity of 8 PSU. The salinity was measured using salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany) (**Publication II, Publication III**). The modified method of bioaerosol sampling was applied from the combination of methods used by Lewandowska et al. (2017) and Wiśniewska et al. (2020) (**Publication II**, **Publication III**). A constant amount of liquid F/2 culture medium (6 mL) was placed on Petri dishes, which were then placed in a microbiological impactor (Tisch Environmental, Inc). The impactor consists of six cascades, allowing for the collection of particles of different diameters depending on the diameter of the impactor cascade nozzles: >7 μm (1), 4,7–7 μm (2), 3,3–4,7 μm (3), 2,1–3,3 μm (4), 1,1–2,1 μm (5) and ≤1,1 μm (6). The impactor was calibrated by the manufacturer (Tisch Environmental, Inc.) in such a way that all collected particles, regardless of their physical characteristics, could be classified aerodynamically, like they would be deposited in different parts of the human respiratory tract. The air flow through the impactor was 28.3 L min^{-1}. Impactor was exposed between 30 minutes and 6 hours, depending e.g., on the rain duration. The precise sampling time was recorded to calculate the volume of air sampled. Separate samples were collected in a 24 h period representing daytime and nighttime samples (**Publication II**, **Publication III**).

The rain gauge consisted of a 1 dm^3 polyethylene bottle and a Teflon funnel with a surface area of 0.314 m^2 (**Publication III**). The bottle was connected to the funnel and sealed with a Teflon ring. Prior to sample collection, each bottle was treated with 1.0 M hydrochloric acid for 24 hours, rinsed three times with distilled and deionized water, and then dried. To cultivate microalgae and cyanobacteria present in rainwater samples, F/2 medium components were added to 20 ml of rainwater in at least one repetition depending on the sample volume. The rain sampler was exposed from 30 min to 48 h depending on the rainfall duration. The collector was removed as soon as possible after it stopped raining (**Publication III**).

After collection, all samples were incubated under constant temperature and light conditions for 30 days (**Publication II**, **Publication III**). The incubation temperature was maintained at 20°C (±1°C), while the lighting cycle was set at 16:8 hours light:dark, with a photosynthetically active radiation (PAR) intensity of 10 μmol photons m^{-2} s^{-1}. The PAR intensity was measured using a quantum meter (LI-189, LI-COR Inc., Nebraska, USA) with a cosine collector. Fluorescent lamps (Cool White 40 W, Sylvania, OH, USA) were used as the radiation source. This method was previously used by Lewandowska et al. (2017) and Wiśniewska et al. (2020).

8.2.2 Meteorological data and ecohydrological model parameters

Meteorological data was obtained using a Vaisala WXT520 weather sensor (Vaisala Inc., Woburn, MA) and data from ARMAAG Foundation (The Foundation: Agency of Regional Air Quality Monitoring in the Gdansk metropolitan area, https://armaag.gda.pl/) (**Publication II**, **Publication III**). Meteorological data was collected simultaneously with sampling of aerosols and rain and included air temperature and relative humidity, wind speed and direction, atmospheric pressure, and precipitation amount. Additionally, 48h

backward trajectories were determined using the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT) model (https://www.ready.noaa.gov) to determine the air mass origin (**Publication III**).

An ecohydrological model (http://model.ocean.univ.gda.pl) was used to obtain results of blue green algae biomass and total primary production, as well as NO_3^- and PO_4^{3-} concentrations in the Gulf of Gdansk sea water (**Publication II**, **Publication III**).

8.2.3 Quantity and quality composition of airborne microorganisms

The taxonomic composition and number of identified taxa were determined using a light microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) equipped with a camera (Nikon DSU2, objective Plan Apo VC 100; magnification x1000) (**Publication II**, **Publication III**). An epifluorescence microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) with UV-2A, B-2A, and G-2A band-pass filters was used to verify the microscopic results and the presence of chlorophyll a in the identified taxa (**Publication II**, **Publication III**). The organisms were identified at the species or genus level using light and epifluorescence microscopes with the help of keys and appropriate literature (**Publication II**, **Publication III**). The number of vegetative cells of cyanobacteria and microalgae in bioaerosols was determined using flow cytometry (BD Accuri™ C6 Plus; BD Biosciences, San Jose, California, USA) according to the method described by Śliwińska-Wilczewska et al. (2018) (**Publication II**, **Publication III**).

8.2.4 Microcystin-LR analysis

Quantitation of Microcystin-LR equivalents were performed using a colorimetric MC-LR, enzyme-linked immunosorbent assay (ELISA) kit (Abnova, Taipei, Taiwan) (**Publication II**). The quantitative analysis of microcystin-LR was performed using a colorimetric kit, according to the method described by Perez and Chu (2020). The detection limit of the kit was 0.1 $\mu g\ L^{-1}$ and was described in more detail by Kumar et al. (2020). The absorbance was measured at a wavelength of 450 nm using a microplate reader Thermo Scientific Multiscan Go (Thermo Scientific, Waltham, MA, USA).

8.3 Monocultures of airborne cyanobacteria and microalgae analysis (Experimental part of the thesis)

8.3.1 Criteria for selecting experimental organisms from airborne cyanobacteria and microalgae

The analyzed airborne cyanobacteria and microalgae strains were isolated and maintained in the Culture Collection of Baltic Algae (CCBA)- Airborne Algae (AA) (https://ccba.ug.edu.pl/pages/en/home.php). In total, 61 strains of microorganisms were isolated (Wiśniewska et al., 2021). Three representatives of cyanobacteria, green algae, and diatoms were selected from them and subjected to laboratory experiments (**Publication IV**). The experiments were conducted on airborne cyanobacterial strains: *Nostoc* sp., (CCAA 03), *Synechococcus* sp., (CCAA 14), *Aphanothece* sp. (CCAA 48); green algae: *Oocystis* sp. (CCAA 20), *Kirchneriella* sp. (CCAA 38), *Coccomyxa* sp. (CCAA 21); and diatoms: *Amphora* sp. (CCAA 34), *Halamphora* sp. (CCAA 47); *Nitzschia* sp. (CCAA 17). A total of 300 isolated monoculture samples were

analysed. The criterion for the selection of organisms for the experiment was the frequency of occurrence in the atmosphere in the southern Baltic region. Among the most common are cyanobacteria, green algae, and diatoms. In addition, the taxa used were the most numerous among the other isolated ones that occur in the study area. The exact same strains have been used in the study focusing on the effect of abiotic factors on the abundance and photosynthetic performance of airborne cyanobacteria and microalgae (Wiśniewska et al., 2021).

8.3.2 THE EXPERIMENT ON B(A)P EFFECTS ON CYANOBACTERIA AND MICROALGAE

The selected cyanobacteria and microalgae were cultured in 25 mL glass Erlenmeyer flasks containing sterile F/2 medium prepared using sea water with a salinity at 8 PSU, which is representative of the average salinity in the Baltic Sea. The lighting cycle was set at a 16:8 hours light:dark, with a photosynthetically active radiation (PAR) intensity of 100 µmol photon m^{-2} s^{-1}. The strains were cultured at various temperatures: 10℃, 15℃, 20℃, 25℃, and 30℃ (**Publication IV**). The temperature range was chosen to correspond to the temperature conducive to the presence of cyanobacteria and microalgae in the air, while also reflecting the temperature variability in the Gulf of Gdansk region throughout the year. The experimental setup was performed within thermostatic chambers (Biogenet, fitotron chamber, Józefów, Poland) that provided the necessary control over temperature conditions (±1ºC). After acclimatization, proper cultures of 20 mL volume were prepared. These cultures were in the logarithmic growth phase and contained a known number of initial cells. To prepare the cultures, a specific volume of inoculum (between 0.6 and 1.2 mL) was taken from the actively growing acclimatization culture (V = 20 mL) and added to a sterile F/2 medium. The number of initial cells in the proper culture was set at 10^5 cells in 1 mL of the medium.

Based on the review of scientific literature, the ranges of B(a)P concentrations in atmospheric aerosols in the coastal zone of the Gulf of Gdansk were determined between 0.5 and 40 ng m^{-3} (Staniszewska et al., 2013; Gaffke et al., 2015; Lewandowska et al., 2018; Skalska et al., 2019; Wiśniewska et al., 2019b). Considering the knowledge of benzo(a)pyrene concentrations in the air, average sampling time of aerosols, and flow rate during the sampling, it was possible to determine the concentrations of B(a)P used in the experiment. To all strains, a specific concentration of benzo(a)pyrene prepared from Sigma-Aldrich standard solution (1000 µg mL^{-1}) was added, with concentrations of: 7.8; 15,0; 78,0; 312,0 and 624,0 ng mL^{-1}. This was equivalent to atmospheric concentrations of 0.5, 1, 5, 20, 40 ng m^{-3}, respectively. The test involved conducting three replicates. The concentrations chosen encompassed a spectrum from low values to levels significantly exceeding the permissible annual average value for B(a)P in PM10 in EU countries, which is 1 ng m^{-3} as stipulated in Directive 2004/107/WE.

To prepare the cultures of isolated airborne cyanobacteria and microalgae for the experiments, they were initially acclimatized to the new incubation conditions, aligning with the appropriate culture requirements. The test cultures were incubated for 7 days until they reached the exponential growth phase. After that the cell concentration, chlorophyll *a* content, photosynthesis efficiency and B(a)P concentration were measured. Additionally, numerous blank samples were included, including analysis of a clean filter, a filter exposed only to B(a)P, and a filter exposed only to cyanobacteria and microalgae without the addition of B(a)P. The seven-day experimental time was chosen based on data from the literature, confirming that highly toxic B(a)P can be transformed by phytoplankton into diols and quinones within 5-6 days (Alegbeleye et al., 2017).

8.3.3 Determination of the number of cells

The number of cells was determined using two different methods (**Publication IV**). The first method, described by Śliwińska-Wilczewska et al. (2018), utilized the BD Accuri C6 Plus flow cytometer (BD Biosciences, San Jose, CA, USA) and was based on a linear correlation between the cell concentration (N mL^{-1}) and the optical density (OD_{750}). The second method, described by Śliwińska-Wilczewska and Latała (2018), involved using a light microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) and a Burkard counting chamber to count filamentous cyanobacteria. Both methods allowed for the determination of correlation coefficients between cell number and optical density.

8.3.4 Cell-specific chlorophyll content analysis

To determine cell-specific chlorophyll *a* content, after 7 days of incubation, a 5 mL culture sample was filtered using a 0.45 µm filter (Macherey-Nagel MN GF-5, Dueren, Germany) and extracted using cold 90% acetone p.a. in the dark for 2 h at −20ºC. After centrifugation at 12,000×g rpm for 2 min (Sigma 2-16P, Osterode am Harz, Germany) to remove cell debris and filter particles, the extinction was measured using a UV-VIS Multiskan GO spectrophotometer (Thermo Scientific, Waltham, MA, USA) at 750 nm, 665 nm, and 480 nm with a 1 cm glass cuvette (**Publication IV**).

8.3.5 Determination of chlorophyll fluorescence

The measurement of chlorophyll fluorescence was conducted using a pulse amplitude modulated (PAM) fluorometer (FMS1, Hansatech, King's Lynn, United Kingdom). After 7 days of the experiment, the fluorescence parameter F_v/F_m was analyzed. The 594 nm amber modulating beam with 4-step frequency control was used to provide illumination. The analyzed material was placed in the leaf clip on the 13 mm glass fiber filter (Whatman GF/C, Saint Louis, MO, USA). Saturating pulses of 0.7 s duration with an intensity of 4500 µmol photons m^{-2} s^{-1} were used to test airborne cyanobacterial and microalgal species. All samples were dark-adapted before the measurements (**Publication IV**).

8.3.6 B(a)P analysis using high-performance liquid chromatography

The concentration of benzo(a)pyrene was determined using high-performance liquid chromatography (Dionex UltiMate 3000) with a fluorescence detector (benzo(a)pyrene λex. = 296 nm, λem. = 408 nm) (**Publication IV**). All samples were filtered through preheated (6h, 560ºC) quartz filters. B(a)P was determined by solvent extraction with acetonitrile:dichloromethane (3:1 v/v) using an ultrasonic bath. The obtained solution was evaporated to dryness using a rotary evaporator, then enriched with 1 cm^3 of acetonitrile. The chromatographic separation process was carried out under gradient conditions using a mobile phase (water:acetonitrile) with a chromatographic column Thermo Scientific HYPERSIL GOLD C18 PAH (250 × 4,6 mm; 5 µm).

High-performance liquid chromatography (HPLC) grade solvents from Merc were employed for the analyses. The calibration curves, spanning concentrations ranging from 0.1 to 10 ng cm^{-3}, were prepared using the benzo(a)pyrene standard sourced from Sigma-Aldrich (1000 µg mL^{-1}). The standard solutions for calibration curves were prepared in methanol. The method exhibited linearity greater than 0.999%, and the precision, expressed as the coefficient of variation, was less than 15%. The method limit

of quantification (LoQ) was defined as the 10-fold signal-to-noise ratio for sample with very low (close to the detection limit) content of B(a)P and was 0.01 ng cm^{-3}. The recovery rate, when compared to the reference material (SRM-2585), was found to be 83%.

8.4 Statistical analysis

Spearman correlation coefficients were calculated between the number of microalgae and cyanobacteria cells and the daily rainfall amount (mm), mean temperature (°C), relative humidity (%), atmospheric pressure (hPa), wind speed (m s^{-1}), NO_3^- concentration in seawater (mg m^{-3}), PO_4^{3-} concentration in seawater (mg m^{-3}), cyanobacterial biomass (mg m^{-3}) and primary production (mg m^{-3}) in the Baltic Sea (**Publication II**, **Publication III**). The non-parametric Mann-Whitney U test was used to test the differences between two sets of independent data - number of microalgae and cyanobacteria in the vegetative and non-vegetative season, as well as the number of microalgae and cyanobacteria during night and day (**Publication II**). In **Publication II**, Kruskal-Wallis test was also used to compare more than two groups of independent variables, such as the amount of microalgae and cyanobacteria in individual months and the amount of microalgae and cyanobacteria in each particle size fraction. In turn, to investigate the effect of B(a)P concentration and temperature on the growth, chlorophyll *a* concentration, and fluorescence of isolated cyanobacteria and microalgae from the atmosphere, the factorial ANOVA was used. Significant differences between control and experimental levels were determined using Tukey's HSD post-hoc test. The statistical significance was determined at the level of $p < 0.05$ for **Publications II** and **IV** while at the level of $*p < 0.05$: $**p < 0.01$ $***p < 0.001$ for **Publication III**. To increase the scientific value of the studies, both statistical analysis and data visualization were performed using various programming languages such as R (**Publication II**, **Publication III**) and Python (**Publication IV**), as well as data visualization programs such as Origin (2021b) (**Publication II**). Geostatistical analysis and graphical presentation of results were carried out using ArcMap 10.6.1 software (**Publication I**).

9 PUBLICATIONS CONSTITUTING THE DOCTORAL DISSERTATION

9 PUBLIKACJE STANOWIĄCE ROZPRAWĘ DOKTORSKĄ

9.1 PUBLICATION I

9.1 PUBLIKACJA I

Wiśniewska K., Lewandowska A., Śliwińska-Wilczewska S. 2019. *The importance of cyanobacteria and microalgae present in aerosols to human health and the environment – Review study.* Environment International, 131,104964. DOI: 10.1016/j.envint.2019.104964

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Review article

The importance of cyanobacteria and microalgae present in aerosols to human health and the environment – Review study

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ABSTRACT

Airborne microalgae and cyanobacteria are among the least studied organisms in aerobiology. While those of them living in freshwater and seawater are well recognized, those constituting the components of aerosols are rarely the focus of research. However, their presence has been noted by scientists from all over the world. The presence of these organisms is not indifferent to the environment as they participate in the formation of clouds and influence both the hydrological cycle and Earth's climate. Recent studies have concentrated mostly on the negative impact of airborne cyanobacteria and microalgae, as well as the toxic compounds they produce, on human health. This review focuses on measurement results published on those bioaerosols, combining the achievements of scientists from the last century with the latest reports and trends. Within it gaps in current knowledge are discussed, including the role of airborne organisms in the transport of harmful chemicals like PAHs and heavy metals. The current studies on which it is based emphasize the advantages and disadvantages of the measurement methods used in sampling and analysing. It also visualizes, in the form of maps, where research on bioaerosols has so far been conducted, while at the same time determining the share of organisms potentially dangerous to human health. In addition, we have also tried to recommend future research directions for both environmental and laboratory-based studies.

1. Introduction

Bioaerosols are organisms or organisms excrements and by-products emitted from the sea surface or originating from the terrestrial environment, which occur in the atmosphere (Urbano et al., 2011). Their size ranges from 0.2 to 100 μm in diameter and they are subject to the same laws of physics as atmospheric particulate matter (Burge and Rogers, 2000; Sharma et al., 2007). Bioaerosols are a diverse group among which plant cell debris, pollen, fungi, microalgae, cyanobacteria, bacteria and viruses may all be distinguished (Urbano et al., 2011; Genitsaris et al., 2011). However, the numbers of cyanobacteria and microalgae in the atmosphere may be less than other particles, e.g., bacteria or viruses, hence the insufficient level of scientific knowledge regarding their presence in the atmosphere (Sharma et al., 2006a, 2006b; Després et al., 2012). According to a review by Després et al. (2012), airborne microalgae number concentration constitutes from $10^1\,m^{-3}$ up to $10^3\,m^{-3}$, whereas bacteria or viral particles number concentration is estimated at $10^4\,m^{-3}$, respectively. Furthermore, some scientists have claimed that airborne microalgae and cyanobacteria are the least-studied organisms in aerobiology and phycology (Sharma et al., 2007; Sahu and Tangutur, 2014). In the present study authors focus on the airborne cyanobacteria (also called blue-green algae) and microalgae. Further in the publication they are interchangeably called bioaerosols, because in the air they are part of them. A comprehensive taxonomy on microalgae and cyanobacteria can be found at http://www.algaebase.org and http://www.cyanodb.cz/, where it is updated on a current basis. This is due to the continuous development of science about cyanobacteria and microalgae in the air. Guiry (2012) admitted that number of algae species in the air are underestimated and many thousands of them are still undescribed.

The presence of microalgae and cyanobacteria in atmospheric aerosols has been known since about 1844, when Ehrenberg was the first to discover those organisms in aerosols collected by Darwin while travelling in the Atlantic Ocean (Sharma et al., 2007; Genitsaris et al., 2011). In Darwin's samples 18 species of freshwater diatoms were noted. The first studies of bioaerosols focused on the determination of species and identifying the proportion of certain taxa (Meier and Lindbergh, 1935; Schlichting, 1969). Using different methods scientists began to trap and culture microalgae and cyanobacteria to improve their identification in the air (Lee and Eggleston, 1989).

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Scientists have sampled microalgae and cyanobacteria at various heights, as well as in various matrices including snow, rain or soil (van Overeem, 1937; Carson et al., 1976; Sharma et al., 2007; Genitsaris et al., 2011). Airborne microalgae have been discovered in different parts of the world, sometimes distant from their potential source of origin, e.g., Antarctica, which has led to a broadening of biogeographical knowledge (Broady, 1996; Kristiansen, 1996). Moreover, a number of scientists have studied bioaerosol distribution patterns and dispersal methods (Messikommer, 1943; Bullock et al., 2003; Sharma et al., 2006a, 2006b; Sharma and Singh, 2010; May et al., 2018).

Hitherto research results showed the role of bioaerosols in atmospheric processes and global climate change (Tesson et al., 2016). Airborne microalgae and cyanobacteria can form ice nucleating particles and cloud condensation nuclei (Hoose and Möhler, 2012; Tesson and Šantl-Temkiv, 2018), which was briefly described by Després et al. (2012). Additionally, similar to particulate matter, bioaerosols influence the Earth's radiation budget by absorbing and scattering solar radiation (Després et al., 2012).

Contemporary studies in the field of bioaerosols focus on cyanobacteria and microalgae or the harmful toxins produced by these organisms, which after the aerosolization process invade human respiratory tract and constitute a serious health risk (Murby and Haney, 2015; Facciponte et al., 2018; NOAA, 2019). Among other things, scientists consider the ability of microalgae and cyanobacteria present in aerosols to transport other toxins like heavy metals, pesticides and carcinogens into the human body (Sharma et al., 2007; Lewandowska et al., 2017; Singh et al., 2018; May et al., 2018).

This review provides information on the significance of airborne microalgae research. It describes the state of global knowledge on the processes of their emission into the atmosphere, their size distribution, methods of collection, distribution over land, the share of individual species of cyanobacteria and microalgae in aerosols in various regions of the world, etc. Above all, however, it focuses on the impact of bioaerosols on human health, showing the latest measurement methods, accomplishments and issues. Additionally, the authors would like to suggest what further research ought to include.

2. Mechanisms of emission into the atmosphere

Microalgae and cyanobacteria are emitted into the atmosphere mainly from water surfaces, but also from soil. Additionally, microalgae may occur on buildings, trees or roofs and according to Schlichting (1969) this may constitute a significant airborne microalgae load. Emission of bioaerosols from freshwater is similar to the emission of marine aerosols. Phytoplankton and products of microbiological metabolism are concentrated at the sea surface and its transfer from seawater to the air occurs through the bursting of bubbles and the breaking of waves (Blanchard, 1989). A rough waterbody forms three types of droplets that take part in emitting bioaerosols, namely spume drops, film drops and jet drops. It is assumed that when the wind speed exceeds 7–11 $m\,s^{-1}$ spume drops are effectively torn off the waves (Löndahl, 2014). During marine wave breaking up to 7 $ng\,m^{-3}$ of the toxic metabolic algal products may be emitted into the atmosphere (Cheng et al., 2005; Backer et al., 2010). In turn, Murby and Haney (2015) determined the effectiveness of aerosolization of the freshwater picocyanobacteria up to $1.6{\cdot}10^5$ cells m^{-3}. A lower impact of aerosolization was noted in the case of waves and water turbulence as well as horizontal air flow. The efficiency of picocyanobacteria emission under laboratory conditions was higher, reaching up to $3.6{\cdot}10^5$ cells m^{-3}. Other laboratory experiments, conducted by Marks et al. (2019) in brackish and oceanic saline water on diatoms: *Nanofrustulum* and *Cyclotella* cells, indicated that their incorporation into jet droplets compared to their original concentrations in the waterbody may range from 8 to 307. The enrichment factor varies with the changing concentration of suspended diatoms in water and higher salinity was found to enhance scavenge and aerosolization (Marks et al., 2019). Another study concerning the transfer of bioaerosols from freshwater to the atmosphere was conducted by May et al. (2018). These authors suggested that even in a freshwater environment, wave breaking plays an important role in the emission of aquatic toxins into the atmosphere. Scientists demonstrated that cyanobacteria, the primary component of harmful algae blooms in freshwater sources, release toxic products that can be aerosolized when lake aerosol spray is formed, resulting in human exposure to algae toxins. Furthermore, increasing numbers of biological particles in the lake spray aerosols were noted when cyanobacterial concentration increased in the waterbody (May et al., 2018).

Bioaerosols are also emitted during the splashing of raindrops on the water surface (Burge and Rogers, 2000; Huffman et al., 2013). Löndahl (2014) emphasized that the production of bioaerosols originating from a waterbody depends on season and can be influenced by ice cover and wind speed. Emission rates for the natural bioaerosols mentioned may hinge on their concentration in the source area. It is worth adding that emission from waterbodies can occur as the consequence of human activity such as water sports or irrigation (Benson et al., 2005; Backer et al., 2010).

3. The role of meteorological factors on the airborne microalgae and cyanobacteria

Several factors have a significant impact on the presence of cyanobacteria and micromicroalgae in the air, their emission effectiveness, transport and deposition. Aside from the concentration of phytoplankton in the waterbody or other surface, which is related to physical and chemical processes, the size distribution of the bioaerosol and changing weather conditions are also crucial. Sahu and Tangutur (2014) noted in their study that phytoplankton of smaller size has greater potential to become aerosolized, and may thus stay longer in the atmosphere. In the case of particulate matter it is obvious that larger particles tend to settle to the ground by gravity and because of that travel over shorter distances. However, Sharma and Singh (2010) indicated that the size of microalgae is not necessarily linked to its ubiquitous dispersal. This thesis was supported by the fact that bioaerosols in similar size ranges differ in atmospheric abundancy. Statistical analysis carried out by the authors of the publication suggests that microalgae presence in the atmosphere is a consequence of the overlap of many meteorological factors, such as wind speed, temperature and humidity of air, rainfall and sunshine hours (Tormo et al., 2001; Sharma and Singh, 2010). Lewandowska et al. (2017) also highlight the role of air mass advection, which may transport marine organisms over the land. Likewise, wind direction was established to be a significant factor for airborne microalgae frequency in a study conducted by Rosas et al. (1989). Fröhlich-Nowoisky et al. (2016) also pointed out that such organisms as algae and (cyano)bacteria can be transported passively through the air.

Furthermore, it should be noted that during emission or dispersion microorganisms are exposed to stress associated with unfavourable conditions, such as desiccation, atmospheric pollutants and UV radiation. That makes them more sensitive to atmospheric stimulus (Sharma and Singh, 2010). According to Ehresmann and Hatch (1975) microalgae originating from soil tolerate atmospheric stress better that aquatic ones. There are also differences in humidity tolerance between bioaerosol taxa. Cyanobacteria have a wide spectrum of humidity tolerance, while microalgae prefer humidity as high as saturation (Ehresmann and Hatch, 1975). This is related to relative humidity variations – bioaerosols of soil origin are more acclimatized to dryness. However, other studies have shown that concentration of airborne microalgae increases when air humidity is low. Under high humidity conditions the hygroscopic wall of the microalgae absorbs water which increases its velocity of settling (Sharma et al., 2006a). As mentioned by Sharma et al. (2006a), hours of sunshine also have an influence on humidity as they add a drying effect. The next factor, rainfall, both

stimulates and reduces algal presence in the atmosphere. Heavy raindrops increase the emission of bioaerosols from waterbodies by splash effect and stimulate algal growth, but also emit microalgae deposited on leaf surfaces (Burge and Rogers, 2000; Zhu et al., 2018). On the other hand, similar to in the case of particulate matter, wet deposition constitutes the most effective process of removing contaminates from the atmosphere (Loosmore and Cederwall, 2004). Atmospheric pollutants, including bioaerosols, are removed by wet deposition in two processes – washout (below cloud) and rainout (in cloud). The role of wind is also similar both in bioaerosols and particulate matter. Increased wind speed affects the generation of bioaerosols and transports them over longer distances (Lewandowska et al., 2017). An interesting dependence was noted by Marshall and Chalmers (1997) that desiccation accentuates the chance of microalgae becoming airborne. Tormo et al. (2001) proved a statistically important negative correlation ($p < 0.001$) between relative humidity or rain and daily concentration of Chlorococcales coenobia and diatoms cells, while temperature ($p < 0.001$) and wind ($p < 0.01$) speed correlate positively. The importance of those factors was confirmed by Sharma et al. (2006a, 2006b). However, it should be remembered that all processes occurring in the atmosphere, both those related to bioaerosols and particulate matter (PM), are not influenced by a single factor but by several co-occurring factors (Carson and Brown, 1976; Rosas et al., 1989; Tormo et al., 2001; Sharma et al., 2006a, 2006b).

4. Measurement methods and related issues

The study of microalgae and cyanobacteria in atmospheric air is a difficult research task. A number of complications concern not only the sampling itself, but also both quantitative and qualitative analyses of the airborne algae and cyanobacteria. There are no standards regarding the way in which samples are collected, stored or analysed. These methods are often inspired by techniques used to study bacteria and fungi in the air or particulate matter. Below, the methods used for collecting, cultivating, quantitative and qualitative analysis of the airborne microalgae and cyanobacteria will be discussed.

The preparation of the medium on which the bioaerosols will be taken is the first step of the research. Microorganisms respond in a different way to stress, so choosing the right medium to culture will determine the survival of the airborne organism (Lee and Eggleston, 1989; Gupta and Agrawal, 2006). Several authors used sterile Bold basal medium (Rosas et al., 1989; Sharma et al., 2006a, 2006b; Ng et al., 2011; Chu et al., 2013) or sterile mineral f/2 culture medium (Lewandowska et al., 2017). Other authors used agarized medium, which according to Andersen and Kawachi (2005) is an old and common method. However, not every alga is able to grow on agar e.g., dinoflagellate *Heterosigma* sp. or green alga *Peridinium* sp. (Andersen and Kawachi, 2005). Andersen and Kawachi (2005) pointed out that the problem does not depend on agar concentration, but rather on the physical state of the medium. Furthermore, the use of agar is conducive to the development of bacteria and fungi that make it difficult to analyze the intended bioaerosols further under the microscope. Lee and Eggleston (1989) compared the growth of microalgae on different media including a liquid one, as well as other methods (soil seawater, Erdschreiber medium, Guilliard's F1 medium, Alga-Gro seawater medium), noting significant differences between them such as no growth of any species on the soil seawater. These authors also used three types of sampling methods for every medium – exposed Petri dish with agarized medium, bottles with 7 cm mouth full field with medium and Millipore prefilters dipped into media and mounted on a Staplex sampler (Lee and Eggleston, 1989). The most efficient method was found to be filters dipped into Erdschreiber medium, while regardless of the method, no microalgae grew on the soil seawater.

In addition to the preparation of medium on which cyanobacteria and microalgae will be taken, the sampling method is important. Early studies of bioaerosols were characterized by their huge variety. The early investigators took samples from an airplane or car travelling at a constant speed (Carson and Brown, 1976). Moving at high speed can translate into a greater number of taxa, however, it makes the assignment of biogeography difficult. Some researchers used filters for bioaerosol sampling, passing a certain volume of air through them, and then placing the filters in a suitable medium (Schlichting, 1969). In the last decade, in order to extract a large volume of air in a short time, researchers often use impactors with a known air flow such as the small and handy IDEAL3P (Chu et al., 2013) or a rotorod sampler with U-shape arms covered in sticky tape (Sharma et al., 2006a, 2006b). Another popular sampler is The Burkard trap used by Tormo et al. (2001) that has a clockwork rotating disc which is covered with adhesive tape. Others exposed a Petri dish with the medium, where only the deposited material was collected (Lee and Eggleston, 1989; Singh et al., 2018). It should be remembered that high-volume air flow causes stress to cyanobacteria and microalgae (Tesson et al., 2016). Therefore, the samplers used are usually characterized by having low air flows, in the order of $30\,L\,min^{-1}$. These include the Andersen cascade impactor sampler used by Lewandowska et al. (2017), which enables collection of material of certain sizes depending on the impactor cascade (from below 1.1 up to above 7 μm). To collect smaller particles on the Petri dishes located in subsequent cascades of the impactor, the air speed progressively increases. Due to their inertia, the particles hit the agar and remain held on it (https://tisch-env.com/).

Tormo et al. (2001) counted particles under 400 optical magnifications using four longitudinal scans to obtain number of particles per cubic meter of the air. To determine the amount of bioaerosols, scientists also count colonies after cultivation, as in the case of bacteria. Sharma et al. (2006a, 2006b) used light microscopes for this purpose and for smaller colonies a binocular microscope with magnification of 600. The collected samples of cyanobacteria and microalgae were usually grown for about 30 days under constant conditions of 14–28 °C with a photoperiod on a light:dark cycle at 42–50 μmol photons $m^{-2}\,s^{-1}$ (Carson and Brown, 1976; Lee and Eggleston, 1989; Sharma et al., 2006a, 2006b; Chu et al., 2013; Lewandowska et al., 2017). The growth time is long enough to observe a visible increase in microalgae. However, it should be borne in mind that prolonged storage of microalgae and cyanobacteria samples may lead to an increase in allelopathic relationships between antagonistic species and, consequently, the death of some species (Śliwińska-Wilczewska and Latała, 2018; Barreiro Felpeto et al., 2019). Determining the maximum incubation time seems impossible without knowing the microalgae and which allelopathic compounds they produce. However, based on literature data, incubation for 30 days or more is not recommended (Carson and Brown, 1976; Sharma et al., 2006a, 2006b; Chu et al., 2013; Lewandowska et al., 2017; Śliwińska-Wilczewska and Latała, 2018).

After their cultivation microalgae are examined under a light microscope (Ng et al., 2011; Chu et al., 2013; Lewandowska et al., 2017; Singh et al., 2018; Fig. 1). In order to verify the study material, an epifluorescence microscope with block filters is highly recommended. A qualitative analysis is made, often based only on taxonomic keys and phycological literature (Ng et al., 2011; Chu et al., 2013; Lewandowska et al., 2017; Singh et al., 2018).

According to Tesson et al. (2016) such identification can lead to underestimation of genetic diversity, especially in the case of rarely occurring species. Therefore, to achieve qualitative recognition in the most reliable way genetic analyses should be performed. For example metabarcoding can be used. It allows a choice of existing and widely used molecular markers (mitochondrial or nuclear). The success of DNA or RNA sequence analyses translates to a growing variety of genes deposited in the GenBank reference data base. Still advancing technology nowadays enables to assess the species composition of the environmental samples from water, soil and other environments with multiple species. To identify airborne microbes in rain and snow Caliz et al. (2018) used sequencing of 16S and 18S rRNA, while Evans et al. (2019) used this method for fog samples. Katra et al. (2014) and Polymenakou

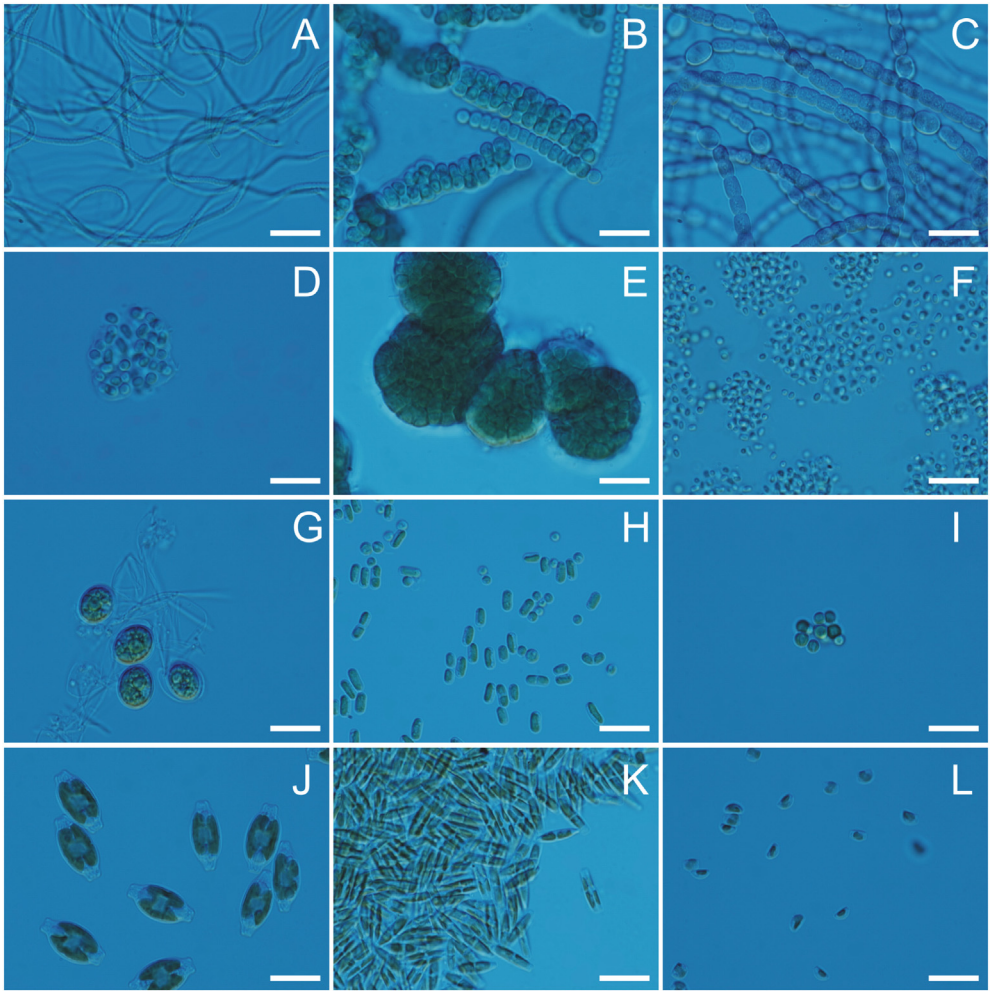

Fig. 1. Examples of airborne cyanobacteria and microalgae from the Baltic Sea region: *Pseudanabaena catenata* (A), Nostoc edaphicum (B), Nostoc sp. (C), Gloeocapsa sp. (D), Woronichinia sp. (E), Aphanothece sp. (F), Oocystis sp. (G), *Stichococcus bacillaris* (H), *Chlorella vulgaris* (I), Amphora sp. (J), Nitzschia sp. (K), Halamphora sp. (L). Scale bars = 10 μm.

et al. (2008) used the sequencing method to determine microorganisms from dust samples. The advantages and disadvantages of molecular markers for plants, including algae, were discussed in detail in the research of Chase et al. (2007). The biggest disadvantage is greater costs than in the case of identification using taxonomic keys. According to Singh et al. (2018) molecular identification of airborne microalgae and cyanobacteria is a rare used method. However, to obtain satisfactory results, both microscopic and molecular methods should be used.

5. Size distribution of airborne cyanobacteria and microalgae

With bioaerosols, size is a crucial factor when it comes to being transported over long distances and penetrating the human respiratory tract. The size distribution of bioaerosols can be assessed by using a multiple-stage sampler to collect particles in several size ranges. The typical sizes of airborne microalgae range between 0.3 μm and 15 μm in diameter (Schlichting, 1969). Research conducted by Lewandowska et al. (2017) revealed that the size of bioaerosols depends on the studied area. In Poland, over the land microalgae and cyanobacteria occurred frequently in particles not exceeding diameter of 3.3 μm, while over the sea bioaerosol particles had a diameter of > 3.3 μm. This may be due to the fact that in the atmosphere fine particles are able to be transported on further distance than coarse ones (Marshall and Chalmers, 1997). However, in the case of fungi, which are also important bioaerosols, their fragments and spores are wind-driven (Fröhlich-Nowoisky et al., 2016; Elbert et al., 2007). Airborne microalgae and cyanobacteria can be emitted from the surface as a particle fragments or temporary cysts or/and akinetes, but also they undergo fragmentation and lysis in the atmospheric environment (Fröhlich-Nowoisky et al., 2016). Thus, above mentioned processes can be responsible for increased frequency in smaller particles.

Qualitative identification of cyanobacteria and microalgae under the microscope allows for estimation of the length and width, in the case of filamentous organisms, or the diameter for the coenobial and unicellular morphology of the organism. Information on the dimensions of bioaerosols was determined by Ng et al. (2011) from cyanobacteria collected in Malaysia. The airborne cynanobacteria *Phormidium anomala* was the longest with a length of 8.6–10.0 μm, while *Nostoc spongiaeforme* was the widest at 2.9–8.6 μm. Based on their studies, it can be concluded that in terms of size, the observed species of cyanobacteria are not less than the cyanobacteria present in soil and water. However, it can also be stated that species recorded in water or soil, not in the air, achieve a longer length and width, e.g., *Monoraphidium griffithii* with a length of 22.9–28.6 μm, or *Navicula* sp. with a width of 26.7–53.3 μm. It is possible, therefore, that larger microalgae are more difficult to emit into the atmosphere. Nevertheless, according to Fröhlich-Nowoisky et al. (2016) bioaerosols including airborne microalgae and cyanobacteria suspended in the air can become fragmented, which yielding smaller particles, or they may be emitted as a bioparticles fragments. This process can be especially significant in the

case of filamentous organisms.

6. Daily and seasonal cycle of microalgae and cyanobacteria concentration variation

Knowledge of bioaerosol distribution is important for understanding biogeography and biodiversity. However, there is still a lack of comprehensive information on the diversity, evolution, abundancy or ecological role of bioaerosol taxa. The quantitative measurements on the airborne microalgae and cyanobacteria are scant. It was estimated by Reisser (2001) that during sunny summer days 300–500 cells m^{-3} of microalgae are present in the atmosphere. In another study, conducted by Mayol et al. (2014), although bioaerosols were only dissociated into airborne prokaryotic or eukaryotic cells, their abundance reached from $2{\cdot}10^3$ to $1.9{\cdot}10^4$ prokaryotes m^{-3} and from $2{\cdot}10^2$ to $1.2{\cdot}10^4$ eukaryotes m^{-3}. Differences in both abundancy and taxonomical diversity depend on location or atmospheric conditions.

The quantities and taxa of bioaerosols have been shown to exhibit daily and seasonal cycles (Schlichting, 1961; Burge and Rogers, 2000; Sharma et al., 2006a, 2006b; Singh et al., 2018). Early work by Schlichting (1961, 1964) reported that diurnal variation occurs, but for some locations (e.g. Michigan) it is marginal. However, in studies conducted in Texas (Schlichting, 1964) the author found there to be twice as many airborne microalgae from 12:00 to 24:00 as from 24:00–12:00. However, Schlichting (1964) admitted that there was no diurnal pattern of airborne microalgae occurrence. The significance of microalgae diurnal pattern has also been noted in Spain (Tormo et al., 2001). The maximum distribution of airborne microalgae was noted in the late afternoon (17:00–20:00) and ranged from 3 to 85 microalgae cells m^{-3}. The minimum values were obtained at night (01:00–7:00) and ranged from 0 to 18 cells m^{-3}. The hourly distribution showed that hours of still air correlate negatively with microalgae concentration on a significant level. The authors admitted that microalgae concentration was roughly constant throughout the year, but particularly high in spring and early summer, which correlated positively with temperature and wind speed. The maximum abundance of airborne diatoms was noted at the end of May, reaching 72 cell m^{-3} (Tormo et al., 2001), and had been caused by the end of spring rains which intensify atmospheric wet deposition of bioaerosols. On the island of Hawaii diurnal pattern was reported by Singh et al. (2018), not only in terms of diversity, but also abundance of airborne microalgae. Collected samples were divided into daytime (6:00–18:00) and night time (18:00–6:00). In total, the researchers identified 192 colonies of bioaerosols, among which 127 colonies were collected during the night time and 65 during the daytime hours. The observed pattern may have occurred due to breeze activity, which brings sea breezes to the leeward side of the islands in Hawaii during the day and reverses to bring a land breeze at night (Singh et al., 2018). Moreover, the authors noted a significant trend, that cyanobacteria constituted 95% of the daytime collection, while the opposite was observed during the night when 87% of the collection was green algae. This may be associated with individual temperature preferences among the microalgae. However, this pattern still requires more detailed investigation.

Seasonal variability was noted in India by Sharma et al. (2006a, 2006b). Researchers reported high diversity of airborne microalgae and large particle concentration from May to July due to high temperature, low humidity, strong wind speed and long periods of sunshine. High diversity of airborne microalgae was noted during October and November, caused by the combined effect of a marginal decline in cyanobacterial genera together with a simultaneous increase in green algal genera (Sharma et al., 2006a, 2006b). The maximum number of microalgae genera identified from May to July and in October was equal to 18–23, while a minimum of 9–10 genera were noted from January to February. During the warm season the authors recorded that cyanobacteria dominated, while during colder months green algae was in greater abundance. The third genera, diatoms, were constant throughout the entire sampling period and demonstrated only a slightly minimal number in early winter (Sharma et al., 2006a, 2006b).

Lewandowska et al. (2017) conducted analysis of airborne microalgae and cyanobacteria from April to November in the region of the southern Baltic Sea (Poland) and identified 41 taxa overall. The authors reported that the highest number of identified taxa occurred in April (equal to 25), the diversity stayed high until June (19 taxa) and after that dropped successively. In the last month of measurements, only one taxon was identified. The authors suggest that the observed variability was due to the vegetation period of algae and cyanobacteria in the Baltic Sea acting as the source of bioaerosols. In the case of diatoms, results were similar to Sharma et al. (2006a, 2006b). However, in the Polish coastal zone during a warm spring, phylum Cyanobacteria dominated significantly over green algae, while from June to November Chlorophyta was the most abundant group.

7. Presence of microalgae and cyanobacteria over the land

The presence of cyanobacteria and microalgae in the air over land should be of special scientist interest due to the fact that it is here that they represent the potentially greatest threat to human health. These organisms have been observed over the land (Fig. 2, Appendix 1) by a number of scientists (van Overeem, 1937; Brown et al., 1964; Chang 1967; Luty and Hoshaw, 1967; Schlichting, 1969; Brown, 1971; Carson and Brown, 1976; Lee and Eggleston, 1989; Rosas et al., 1989; Broady, 1996; Sharma et al., 2006a, 2006b; Genitsaris et al., 2011; Ng et al., 2011; Chu et al., 2013; Sahu and Tangutur, 2014; Lewandowska et al., 2017; Singh et al., 2018). Composition and amount of bioaerosols were different depending on the location of the sampling place, which was caused by different factors. The origin of microalgae and cyanobacteria in aerosols over land is also varied. These bioaerosols are often studied in coastal areas as they are mainly emitted from the sea or the ocean (Genitsaris et al., 2011; Lewandowska et al., 2017). However, there are inland studies confirming that freshwater reservoirs can also be a source of bioaerosols (May et al., 2018). In turn, research conducted by Ng et al. (2011) in Malaysia proved that waterbodies are not the only source of bioaerosols, but also the soil. However, from these studies it does not appear that a specific source was responsible for the emissions of a given species of microalgae, nor does it indicate that any of the sources was more related to the emission of toxic microalgae.

Studies on bioaerosols have been carried out in various parts of the world. Locations in which cyanobacteria and microalgae were detected in the air are shown in Fig. 2, confirming the rather low-level knowledge we have about the biogeography of these aerosol components. So far, no research has been carried out in Australia or South America. China, which excels in publications regarding air pollution and particulate matter, also does not offer information on the mentioned microorganisms. It would be tempting to say that there is a prevalence of areas, in which research of this type has not yet been conducted.

Not all of the bioaerosol studies shown in Fig. 2 focused on taxonomic analysis. Some of them described the effect of microalgae and cyanobacterial emissions into the air, while others concentrated on the emission effect of toxins such as microcystins on human health (Facciponte et al., 2018; May et al., 2018).

The composition of bioaerosols varies depending on the location of the measurement stations (Fig. 3). However, cyanobacteria have been observed in all air samples. In the studies conducted by Ng et al. (2011) and El-Gamal (2008) only the presence of cyanobacteria was recorded. Research conducted by Ng et al. (2011) in Malaysia documented phylum Cyanobacteria belonging to such taxa as *Gloeothece* sp., *Raphidiopsis* sp., *Nostmethoc* sp., *Leptolyngbya* sp., and *Phormidium* sp. in bioaerosol samples. A similar situation was noted in Egypt (El-Gamal, 2008), where 15 phylum Cyanobacteria taxa were noted (*Calothrix* sp., *Chroococcus* sp., *Gloeocapsa* sp., *Hydrocoleum* sp., *Lyngbya* sp., *Microcoleus* sp., *Mixosarcina* sp., *Nodularia* sp., *Nostoc* sp., *Phormidium* sp., *Plectonema* sp., *Pseudanabaena* sp., *Schizothrix* sp., *Trichodesmus* sp., and

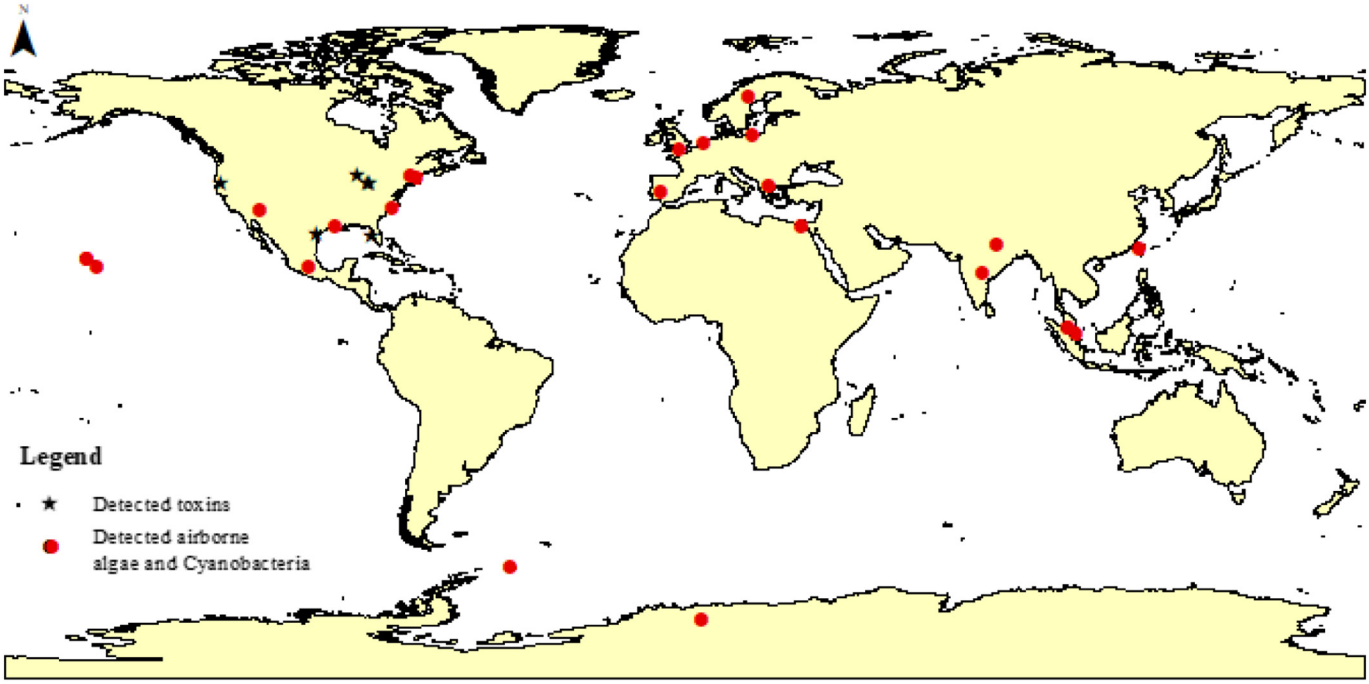

Fig. 2. Area where airborne microalgae and cyanobacteria or their toxic metabolites have been detected based on taxa found in bioaerosol studies using ArcMap 10.6.1 (Literature sources are described in Appendix 2).

Xenococcus sp.). On the other hand, samples collected in New England (USA) by Lee and Eggleston (1989) contained similar numbers of different microalgae taxa, the authors noting 5 taxa of Chlorophyta (*Oocystis* sp., *Stichococcus* sp., *Chlorella* sp., *Chlorococcum* sp., *Chlorosarcinopsis* sp.), 4 Bacillariophyta (*Chaetoceros* sp., *Cocconeis* sp., *Navicula* sp., *Nitzschia* sp.) and 2 phylum Cyanobacteria (*Lyngbya* sp., *Schizothrix* sp.).

A high degree of biodiversity among bioaerosols was recorded by Brown et al. (1964) in Texas (USA), where there were 62 species of cyanobacteria and microalgae: 17 phylum Cyanobacteria, 38 Chlorophyta and 7 Bacillariophyta. The success of the research lay in the varied methodology used by the authors as sampling was conducted from vehicles, airplanes and normally-exposed Petri dishes in several locations. In these bioaerosol studies, the most common Chlorophyta was *Chlorella* sp. (detected in 13 of 17 studies), while another which occurred frequently was *Chlorococcum* sp. (12 of 17 studies). Among phylum Cyanobacteria *Phormidium* sp. occurred the most frequently (12 of 17 studies), and *Lyngbya* sp., *Nostoc* sp. and *Anabaena* sp. both

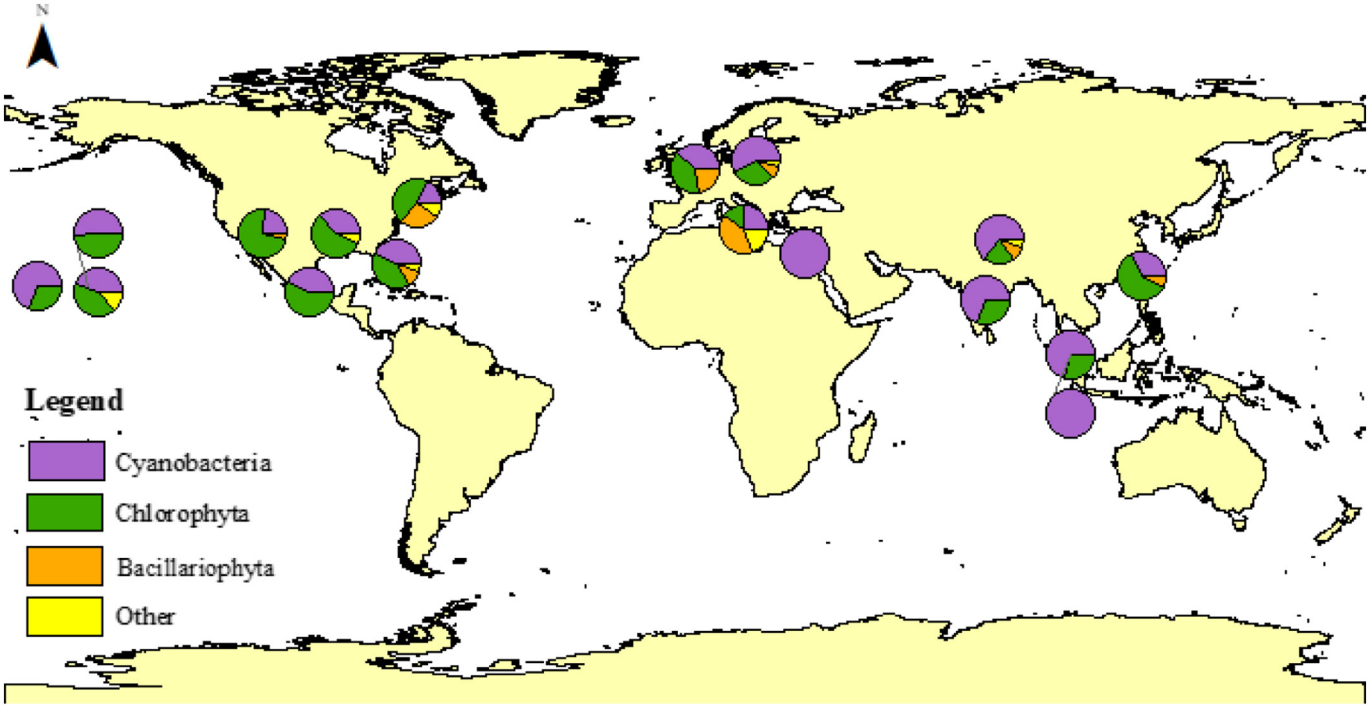

Fig. 3. Share of airborne microalgae and cyanobacteria in selected area based on taxa found in bioaerosol studies using ArcMap 10.6.1 (Literature sources are described in Appendix 2).

featured in 10 of the 17 studies. In turn, *Navicula* sp. (7 of 17 studies) and *Nitzschia* sp. (5 of 17 studies) were the most often identified Bacillariophyta. Several authors (Schlichting, 1961; Brown et al., 1964; Sharma et al., 2006a, 2006b) noted that cyanobacteria dominated in the tropical regions due to high temperature, which was confirmed by more recent research conducted by Ng et al. (2011) and Sahu and Tangutur (2014). Meanwhile green algae dominated in temperate areas, as proven by Luty and Hoshaw (1967) and Schlichting (1961). However recent studies from Poland conducted by Lewandowska et al. (2017) have shown that the domination between cyanobacteria and green algae depends on season, and that cyanobacteria was dominant for a few months. This suggests that more studies from Europe are needed.

Airborne microalgae have been also noted in the polar region of Antarctica; however, their presence was shown as dispersal method without much focus on identification of the airborne microalgae (Marshall and Chalmers, 1997; Broady, 1996).

8. The role of cyanobacteria and microalgae present in aerosols in the transport of harmful substances

Recent studies suggest that bioaerosols are able to actively accumulate harmful non- biodegradable pollutants and may play an important role in transporting hazardous substances in the atmosphere (Lunceford, 1968; McGovern et al., 1965; Mittal et al., 1979; Schlichting, 1970; Tiberg, 1986; Sahu and Tangutur, 2014; Lewandowska et al., 2017). However, this topic still poses an open question and presents a challenge for future research. Authors suggest that microalgae and cyanobacteria may transport radionuclides, heavy metals, pesticides, herbicides, and cancerogenic, as well as mutagenic agents (Sahu and Tangutur, 2014; Lewandowska et al., 2017). For instance, phytoplankton, which is one of the bioaerosols pioneer, demonstrates the ability to absorb and adsorb polycyclic aromatic hydrocarbons (PAHs), which are toxic pollutants both in the marine and atmospheric environment. Furthermore, the most environmentally significant fraction of PAHs is bioavailable and can be accumulated in organisms (Thuy et al., 2018). Several studies reported accumulation of PAHs in phytoplankton (Kirso et al., 1990; Kowalewska and Konat, 1997; Wan et al., 2007). An especially dangerous situation took place in the Baltic Sea (North Europe) where PAH concentrations in phytoplankton, particularly in blue – green algae and diatoms, reached an extraordinary value of $16{\cdot}10^3$ ng g^{-1} dry weight (Kowalewska and Konat, 1997). Thuy et al. (2018) highlighted diffusive sorption as a main mechanism of bioaccumulation. Researchers also reported phytoplankton uptake and bioaccumulation of other dangerous chemicals such as methylmercury, PCBs and organochlorine pesticides (Joiris and Overloop, 1991; Kim et al., 2014; Tiano et al., 2014; Lee and Fisher, 2016). As bioaccumulation by phytoplankton is often the first step for chemicals entering into the aquatic food web, thus toxic substances accumulated in the aquatic environment may be transported into the atmosphere with bioaerosols, from which they can then penetrate into e.g. the human respiratory tract.

Tiano et al. (2014) confirmed the key role of phytoplankton in the fate of polychlorinated biphenyls and gave examples of its ability to control air – sea exchanges of contaminants. The results presented by Kowalewska and Konat (1997) indicated that transport of polycyclic aromatic hydrocarbons may occur via both absorption and adsorption. Furthermore, this process was found to involve not only living phytoplankton cells, but also dead cells and phytoplanktonic detritus. Phytoplankton bioaccumulation depends on the cell's area to volume ratio, and lipid content.

In the atmosphere chemicals like PAHs adsorb on particulate matter. As Burge and Rogers (2000) suggested, bioaerosols may be subject to the same laws of physics as atmospheric particulate matter, and dangerous chemicals may therefore also adsorb on airborne microalgae and cyanobacteria. However, this process requires further research.

9. Airborne microalgae and cyanobacteria in the indoor environment

The seemingly harmless home environment is a habitat of substances hazardous to human health that are emitted as a result of domestic activities (Cao et al., 2005; Hussei et al., 2006; Jung et al., 2011; Wiśniewska et al., 2017). The issue becomes even more important when you consider that humans spend as much as 80% of their time indoors. For the sake of air quality at home or work environment, microbes, mostly bacteria and fungi are often taken into consideration (Genitsaris et al., 2011; Ng et al., 2011; Urbano et al., 2011; Nazaroff, 2014). Airborne microalgae and cyanobacteria in the indoor environment are rarely investigated. However, research literature reports the presence of several microalgae genera in the indoor environment (McGovern et al., 1965; Bernstein and Safferman, 1973; Tiberg et al., 1983; Chu et al., 2013; Tesson et al., 2016). Although bioaerosols are of course more abundant in the natural environment, they can easily be introduced into indoor environments through open windows/doors or via ventilation systems, and can also be carried in by humans or animals from the external environment (Chu et al., 2013). Household dust is a source of bioaerosols, which could induce additional allergy problems (Bernstein and Safferman, 1973; Holland et al., 1973). Tiberg et al. (1983) confirmed that the presence of airborne microalgae in an indoor environment may lead to sensitization, thus increasing the health risk of a reaction to outdoor airborne microalgae.

Early studies of bioaerosols showed that both microalgae and cyanobacteria were detected in household dust (Bernstein and Safferman, 1973; Holland et al., 1973). Holland et al. (1973) noted 40 algal genera in house dust, while Bernstein and Safferman (1973) reported multilocular cyanobacteria such as *Anabaena* sp. or *Schizothrix* sp., as well as *Chlorella* sp., *Chlorococcum* sp., *Chlamydomonas* sp., and *Planktosphaeria* sp. which represent green algae. It was found by Tiberg et al. (1983) that indoor bioaerosol genera were mostly the same as those collected in the outdoor environment, but with a non-significant higher abundance of green algae.

In the most recent study of indoor air made by Chu et al. (2013), 26 bioaerosol taxa were determined within the indoor environment of an office building. Among them, the authors distinguished 12 taxa of phylum Cyanobacteria, 9 taxa of Chlorophyta and 5 taxa of Bacillariophyta. Moreover, in addition to air samples, soil and wall scrapings were sampled and 14, 17 and 10 taxa were identified respectively. The dominant airborne cyanobacterium was *Phormidium angustissima* and microalgae recorded from the exposed culture medium were mainly green algae, especially *Chlorella vulgaris* and *Chlorococcum humicola*. The main factor responsible for the transport of bioaerosols into the building was heavy human movement. Chu et al. (2013) included meteorological factors in the study and reported that only temperature and relative humidity were relevant, despite its far more constant state in comparison with outdoors.

10. Health effect of airborne microalgae and cyanobacteria

The presence of pollution in the atmosphere is particularly important for human health. Particles suspended in the air, both aerosols and bioaerosols, penetrate into the human respiratory tract and can settle in the air sacs and bronchi, leading to many illnesses (Franck et al., 2003). Schlichting et al. (1969) estimated that during the day humans inhale approximately 1500 algal cells. Thus for humans, inhalation is a significant mechanism of exposure to airborne microalgae, cyanobacteria and their toxins (Backer et al., 2010; Lewandowska et al., 2017; May et al., 2018; Facciponte et al., 2018). Another route of exposure is related to recreation activity in water which leads to dermatological contact with and the swallowing of contaminated water. Scientists have discovered several acute health issues linked to bioaerosols

including allergy, inflammatory response, hay fever, skin irritation, burning of the eyes, rhinitis, sclerosis and respiratory irritation (Genitsaris et al., 2011). These authors based on far identified bioaerosols, reported that 15% of airborne microalgae and cyanobacteria induce health negative effects. Of particular importance is the fact that among the microalgae listed as dangerous, there are ubiquitous taxa such as *Chlorella* sp., which were found in almost every aquatic airborne environment and represented 7.8% of Chlorophyta in collected bioaerosols studies (Fig. 4, Appendix 1). *Chlorella* sp. was found to induce allergy and rhinitis (Bernstein and Safferman, 1966; Genitsaris et al., 2011).

Another widespread green alga is *Chlorococcum* sp., which constituted 7.2% of Chlorophyta in the collected bioaerosol studies (Fig. 4, Appendix 1) and is linked to lead allergy. Among phylum Cyanobacteria the most commonly determined was *Phormidium* sp. (6.9% of cyanobacteria in collected bioaerosol studies - Fig. 4), which may cause allergies (Sharma and Rai, 2008). Genitsaris et al. (2011) enumerated *Chlorella* sp., *Chloroccocum* sp., *Scenedesmus* sp., *Hormidium* sp. and *Lyngbya* sp. as being the most frequent taxa causing negative effects to human health. Bacillariophyta, among which *Navicula* sp. dominates, are less abundant (Fig. 4, Appendix 1).

According to the collected bioaerosol studies, the highest number of harmful airborne microalgae was recorded by Brown et al. (1964) in the US. In that study, the authors collected 20 taxa of phylum Cyanobacteria, 22 taxa of Chlorophyta and 2 taxa of Bacillariophyta, which were found to induce negative effects on human health e.g. allergy, dermatitis, swelling of the eye membrane, rhinitis (Genitsaris et al., 2011). Unfortunately, in every study found for the purposes of this publication, at least one harmful alga was found (Fig. 4). Among airborne microalgae and cyanobacteria from the collected studies, harmful taxa constituted from 13% to 71%. However, location of sampling was not found to hold significance in terms of the presence of harmful microalgae and cyanobacteria in the air. In presented datasets (Appendix 1) harmful phylum Cyanobacteria occurred more frequently than Chlorophyta or Bacillariophyta (Fig. 5). Another important finding was that proximity to a waterbody wasn't a significant factor in terms of exposure.

It is well known from particulate matter research, that the smallest particles (< 3 μm) have greater ability to penetrate deeper into the human respiratory tract and can reach the alveoli. It has been proven by Lewandowska et al. (2017) that toxic cyanobacteria and microalgae frequently occur in aerosols with diameter not exceeding 3.3 μm. This may suggest that bioaerosols and related toxins of relatively small size can also penetrate the respiratory tract. Moreover, as cyanobacteria and microalgae are less regular in shape than particulate matter, this may result in the transport of particles with a larger surface area – minor width and major length, which is typical for filamentous microalgae like the harmful *Anabaena* sp. or *Lyngbya* sp. Recently, Facciponte et al. (2018) reported high frequencies of cyanobacteria in the human upper respiratory tract and central airway. The authors suggested no seasonal pattern in exposure to cyanobacteria, indeed cyanobacterial frequency was found to be on a similar level during warm and cold months. In addition to microalgal and cyanobacterial aerosolization, several authors have noted the presence of harmful metabolic products of microalgae in the atmospheric air (Backer et al., 2003; Cheng et al., 2005; Backer et al., 2010; Kirkpatrick et al., 2010; Kirkpatrick et al., 2011; Murby and Haney, 2015). Thus, the presence of cyanobacteria in the environment is closely related to the toxins they produce. One should draw particular attention here to the nodularin produced by *Nodularia spumigena*, which is classed as a strong hepatotoxin showing carcinogenic properties. Another group of compounds with similar structure and toxicity to nodularin are microcystins, produced by e.g. *Microcystis* sp., *Anabaena* sp. and picoplanktonic *Synechococcus* sp. and *Synechocystis* sp. (Mazur-Marzec et al., 2008; Śliwińska-Wilczewska et al., 2018). Scientists from Florida Gulf Coast University found that such toxins were found in particles of all sizes, however their concentrations were relatively low (NOAA, 2019). Other studies showed that the concentration of brevetoxins in the air can range from 0.01 up to 80 ng m^{-3} (Kirkpatrick et al., 2010; Cheng et al., 2005; Backer et al., 2010). Backer et al. (2010) measured the concentration of aerosolized microcystins produced during bloom involving *Microcystis aeruginosa*. Other studies showed that microcystin concentrations in personal samples can range from 0.1 ng m^{-3}, to 0.4 ng m^{-3}. However, those values did not correlate with the concentrations of *Microcystis* sp. cells, dissolved microcystis, or total microcystins concentration in the water. Notwithstanding the above, when inhaled microcystins can have toxic effects on different target organs even at lower doses (Sahu and Tangutur, 2014). Harmful toxins produced by cyanobacteria are associated with several acute conditions and chronic human diseases including gastroenteritis, non-alcoholic liver disease, and amyotrophic lateral sclerosis (Cheng et al., 2005; Backer et al., 2010).

Both bioaerosols and the toxins produced and emitted by them are a

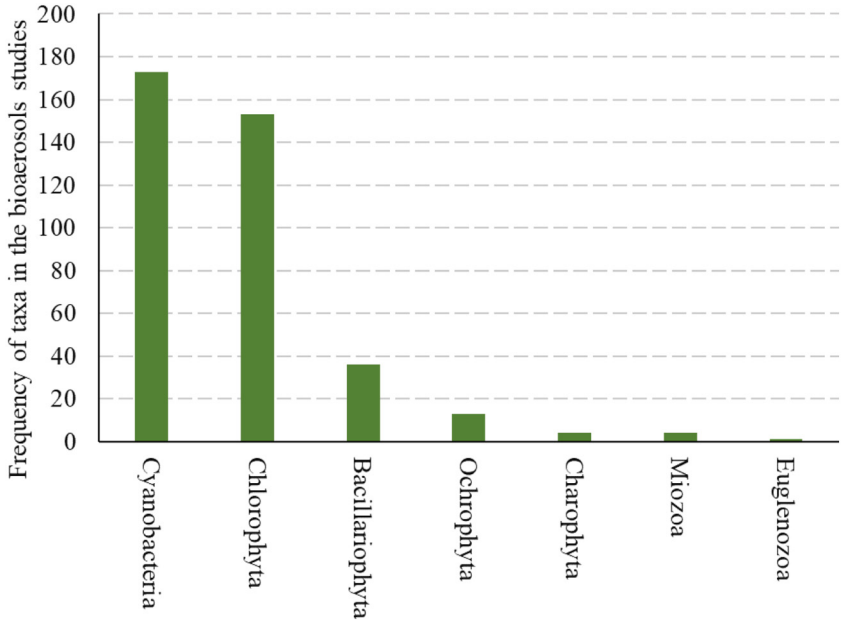

Fig. 4. Frequency of occurrence of airborne microalgae and cyanobacteria based on studied bioaerosol literature (Literature sources are described in Appendix 2).

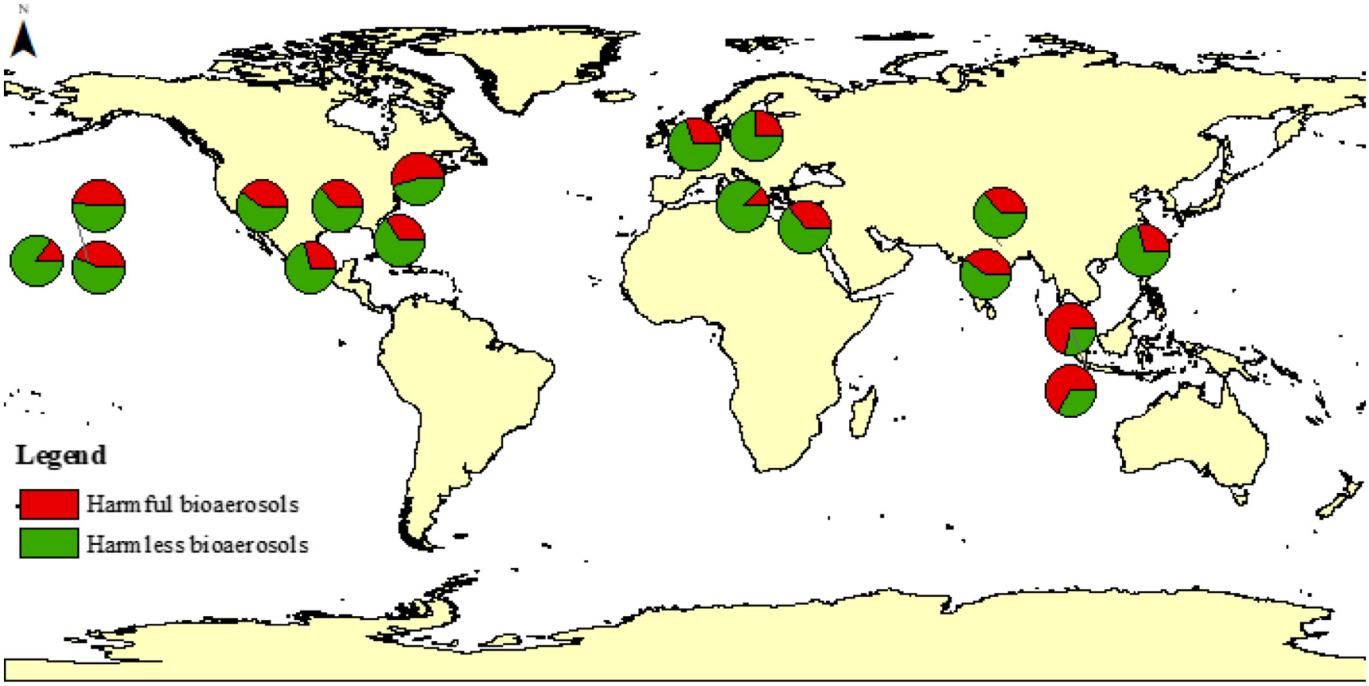

Fig. 5. The share of potentially harmful airborne microalgae and cyanobacteria based on taxa found in bioaerosol studies using ArcMap 10.6.1 (Literature sources are described in Appendix 2).

threat not only in the immediate vicinity of the emission source. Kirkpatrick et al. (2010) detected brevetoxins produced by the toxic marine dinoflagellate *Karenia brevis*, in aerosol samples as far as 4 km from the beach. Murby and Haney (2015) detected microcystins of fresh water origin in the air. The authors' results suggest that aerosolized microcystins deposit in the human upper respiratory tract especially when emitted during recreation activity in water. Cheng et al. (2005) estimated deposition of brevetoxins in the respiratory tract to be as high as 3.5–5 ng h^{-1} in the nasal, oral, and pharyngeal regions, 0.10–0.12 ng h^{-1} in the tracheobronchial region, and 0.06–0.07 ng h^{-1} in the alveolar region. The authors pointed out that such concentrations were high enough to cause irritation of the upper respiratory tract.

Nowadays during toxic algal blooms it is prohibited to bathe in the contaminated water. The above-mentioned studies suggest that the respiratory route is an important mechanism of exposure to microalgae and their toxic products. It should be pointed out, however, that the mere avoidance of recreational use of water basins, especially during blooms, will not protect against the dangerous effects of microorganisms.

According to Anderson (2009) every coastal region is affected by harmful algae blooms. The blooms can occur in fresh, marine, and brackish water bodies. Regardless of whether the bloom is toxic or not, it has negative consequences for the environment e.g. damage to ecosystem and recreational facilities (Anderson, 2009; Backer et al., 2010; Hallegraeff 2010; Lewitus et al., 2012; Backer et al., 2015). Harmful algae bloom is an worldwide problem e.g. blooms of *Ostreopsis ovata* that occur in the Mediterranean is associated with respiratory problems and skin irritation (Totti et al., 2009). The toxic blooms of *Alexandrium fundyense* in Gulf of Maine (USA) induce paralytic shellfish poisoning in humans but also have a negative effect on copepod (Roncalli et al., 2016). In the Gulf of Gdansk (Poland) toxic blooms of cyanobacteria prevent recreational use during summer. Only in 2018 up to 50 bathing sites have been closed due to toxic scums. Moreover, tropical cyanobacterial blooms are prominent issue for freshwater systems in Australia and Asia (Mowem et al., 2015). As a result of climate change, dangerous blooms may become more frequent and extent the range, which is a significant threat for human health. Therefore, it is important to monitor harmful algae blooms, and in consequence to study the presence of algae and cyanobacteria in the air.

11. Recommendations for future research

Research on bioaerosols is conducted by scientists from around the world and recent works have focused not only on taxonomic composition, but also combine the knowledge in the field of phycology, aerobiology and medicine, by analysing the presence of cyanobacteria and microalgae in humans organs and tissues. Research conducted by Facciponte et al. (2018), for example, focused on the detection of cyanobacteria in the human respiratory system in a very foresighted manner. In this, they proved that such organisms can penetrate the human respiratory system and that this is associated with adverse effects. In this era of climate change, in which toxic blooms are frequently prolonged until early winter, the participation of medical units in bioaerosol research is most desirable (Pearl, 2018; Flombaum et al., 2013).

In addition, it seems to be necessary to investigate more extensively just how far bioaerosols including microalgae and cyanobacteria are transported inland and whether they pose a danger to people in confined spaces. Furthermore, it would be advisable to investigate whether microalgae and cyanobacteria present in the atmospheric air are also abundantly present in the home environment in which the people spend the most time during the day. Besides, in the era of changing climate and the growing human impact on the environment, eutrophication of water reservoirs occurs more intensively. Coastal areas are willingly used for recreation, therefore, it would be worth researching such places in terms of bioaerosols, especially during the occurrence of toxic phytoplankton blooms.

We recommend that future research on the airborne cyanobacteria and microalgae concerned their transport in the atmosphere over the land and the chemical and physical processes that occur during their travel. There is also not enough literature data on the fragmentation or lysis of cells of these organisms. Moreover, little is known about the mechanism of absorption or adsorption of chemicals during the emission of cyanobacteria and microalgae from the sea surface into the air

and later on during their transport over land.

Last but not least, the size of the transported particles is of great importance, thus it would be necessary to investigate what is the qualitative and quantitative composition of cyanobacteria and microalgae in aerosols of various size under the influence of changeable weather conditions, time of day or interaction with chemical compounds present in the air.

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Declaration of Competing Interest

The work described in this publication has not been published previously and it is not under consideration for publication elsewhere. Its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

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AUTHORS CONTRIBUTION STATEMENT

We hereby confirm that the specific contribution to the publication:

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were as follows:

Wiśniewska Kinga Areta – 60%:
Preparation of the work concept, collection and analysis of literary data, graphical and statistical processing of results, preparation of the manuscript.

Lewandowska Anita –20%:
Preparation of the work concept, supervision over the work progress, preparation of the manuscript, proofreading of the manuscript.

Śliwińska-Wilczewska Sylwia –20%:
Supervision over the work progress, preparation of the manuscript, proofreading the manuscript.

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Wiśniewska Kinga Areta Lewandowska Anita

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Śliwińska-Wilczewska Sylwia

1.1 PUBLICATION II

9.2 PUBLIKACJA II

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Quantitative and qualitative variability of airborne cyanobacteria and microalgae and their toxins in the coastal zone of the Baltic Sea

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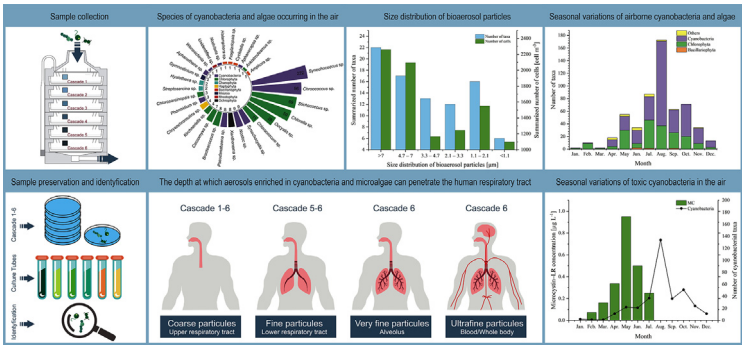
HIGHLIGHTS

- The number of airborne cyanobacteria and microalgae ranged from zero to 1685 cells m^{-3}.
- Nearly 30% of detected taxa were small enough to reach human secondary bronchi.
- Picocyanobacteria *Synechococcus* sp. was the dominant species in the air.
- *Synechococcus* sp. showed the highest concentration of microcystin-LR.

GRAPHICAL ABSTRACT



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ABSTRACT

Apart from viruses and bacteria, cyanobacteria and microalgae present in the atmosphere may pose a threat to the health of humans by inducing illnesses and diseases. Yet, they play an important role in the environment, influencing the Earth's radiation budget by absorbing and scattering solar radiation. The present study determined the daily and seasonal qualitative and qualitative variabilities of airborne cyanobacteria and microalgae during both vegetative and non-vegetative seasons in the coastal zone of the Baltic Sea. Samples were collected from January to December 2020 with a Tisch six-stage microbiological impactor which was used as a substitute for the respiratory tract. The stage levels of the impactor represented the respiratory tract and reproduced lung penetration by airborne particles, which allowed us to assess penetration of cyanobacteria and microalgae to the deepest parts of the human respiratory system. A total of 296 samples of cyanobacteria and microalgae were collected during the day and 276 samples during the night. The results showed that cyanobacteria and microalgae were present in the air all year, and their maximum abundance was 1685 cells m^{-3} in July. Furthermore, the ability of these microorganisms to produce the toxin microcystin-LR (MC-LR) was confirmed, which has a high potential negative impact on human health. MC-LR has been found in *Nostoc* sp., *Pseudanabaena* sp., *Leptolyngbya* sp., *Synechococcus* sp., *Gloeocapsa* sp., *Aphanothece* sp., and *Rivularia* sp. maintained at our Culture Collection of Airborne Algae (CCAA), as well as from air samples. The highest concentrations of MC-LR were recorded in airborne *Synechococcus* sp. CCAA 46 and amounted to as much as 420 fg $cell^{-1}$. In turn, the highest mean concentration of 0.95 μg L^{-1} for MC-LR was recorded in an air sample taken in May. This research expands the knowledge on cyanobacteria and microalgae present in the atmosphere in the coastal zone of the southern Baltic Sea. We propose these microorganisms be used as indicators for further research on bioaerosols, which are potentially dangerous to human health.

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1. Introduction

In urbanized coastal areas, the composition of aerosols, excluding the influence of anthropogenic factors, is closely related to biological processes occurring in seawater (O'Dowd et al., 2004). Bioaerosols are organisms in the atmosphere that were emitted from the sea surface or originated from the terrestrial environment (Urbano et al., 2011; Fröhlich-Nowoisky et al., 2016). They are a diverse group, including plant cell debris, pollen, fungi, microalgae, bacteria, and viruses (Urbano et al., 2011; Genitsaris et al., 2011; Fröhlich-Nowoisky et al., 2016; Wiśniewska et al., 2019). However, the airborne microalgae and cyanobacteria that are the subject of this study are the least studied organisms in aerobiology. Their presence has been noted by several scientists from all over the world, including the United States, Spain, Greece, Italy and India (Tormo et al., 2001; Sharma et al., 2007; Genitsaris et al., 2011; Facciponte et al., 2018; Singh et al., 2018; Wiśniewska et al., 2020). Airborne microalgae have even been discovered near Antarctica, indicating long-range transport from their source of origin (Broady, 1996). However, there is still a need for global quantitative and qualitative analysis, including the Baltic coastal area, to broaden biogeographical knowledge on airborne microalgae and cyanobacteria. Knowledge about their presence in aerosols and their impact on human health is particularly scant (Urbano et al., 2011; Lewandowska et al., 2017; Facciponte et al., 2018; Wiśniewska et al., 2020).

In 1844, Ehrenberg first reports the presence of microalgae and cyanobacteria in atmospheric aerosols collected by Darwin while traveling in the Atlantic Ocean (Sharma et al., 2007; Genitsaris et al., 2011). Since then, bioaerosols studies have focused on the identification of species and determination of the proportion of each taxa (Meier and Lindbergh, 1935; Schlichting, 1969; El-Gamal, 2008; Lewandowska et al., 2017). Using different methods, scientists began to isolate and culture airborne microalgae and cyanobacteria to improve their identification methods (Després et al., 2012; Fröhlich-Nowoisky et al., 2016; Wiśniewska et al., 2019); however, quantitative determination of microalgae in air is still problematic due to the limitations of research techniques applied (Wiśniewska et al., 2020). Many researchers are simply counting colonies grown on agar (Lee and Eggleston, 1989; Sharma et al., 2006a, 2006b; Singh et al., 2018); the disadvantages of this method were discussed in detail by Andersen and Kawachi (2005). Nevertheless, the development of a reliable technique for counting any microorganisms, including microalgae, is important to assess their impact on organisms that breathe in the bioaerosols and the environment and in which they are deposited. According to a review by Després et al. (2012), airborne microalgae concentrations range from 10^2 cells m^{-3} up to 10^3 cells m^{-3}. Tesson et al. (2016) noted that humans inhale approximately 1000 algal cells m^{-3} and assumed that about 300 microalgae cells per hour are deposited in the human respiratory tract posing a significant health risk (Tesson et al., 2016). This can lead to diseases and conditions such as allergies, inflammatory responses, hay fever, skin irritation, burning of the eyes, rhinitis, and respiratory irritation. Therefore, contemporary studies in the field of bioaerosols focus on cyanobacteria and microalgae and the harmful toxins they produce (Murby and Haney, 2015), which invade the human respiratory tract by means of aerosolization and constitute a serious health risk (Murby and Haney, 2015; Tesson et al., 2016; Facciponte et al., 2018). However, the role of bioaerosols in atmospheric processes and global climate change cannot be overlooked (Tesson et al., 2016). Airborne microalgae and cyanobacteria can form ice nuclei and cloud condensation nuclei (Després et al., 2012; Hoose and Möhler, 2012). Additionally, bioaerosols influence the Earth's radiation budget by absorbing and scattering solar radiation, as do other particles (Després et al., 2012).

To assess the impact of microalgae on the quality of human life, a qualitative assessment is required first. Individual microalgae are responsible for allergies, skin irritations, and several other ailments (Genitsaris et al., 2011). Bartra et al. (2007) and D'Amato et al. (2013) claimed that human sensitivity can increase when coupled with high pollutant concentration and high temperature. The size of the microalgae is also important since smaller aerosols (below 3 μm) penetrate deeper into the human respiratory tract and can settle in the air sacs and bronchi, leading to many illnesses (Fröhlich-Nowoisky et al., 2016; Lewandowska et al., 2017; Facciponte et al., 2018). This study assessed how many cyanobacteria and microalgae present in the air could be deposited in each part of the respiratory system. We used a six-stage Tisch impactor to simulate the respiratory tract and reproduce lung penetration by airborne particles of varying sizes. Depending on the taxa, the analyzed microorganisms are often capable of producing toxins hazardous to human health (Puschner, 2018; Śliwińska-Wilczewska et al., 2021). These studies were the first to measure the amount of microcystin in individual cyanobacteria that have been isolated and maintained in the Culture Collection of Airborne Algae (CCAA) at the University of Gdańsk in Poland (more of CCAA in Wiśniewska et al., 2021).

The main goals of this work were to determine the taxonomic composition of airborne cyanobacteria and microalgae in the coastal zone of the Baltic Sea, investigate the diurnal changes in the composition of the aeroalgal community, examine their variability during the vegetative and non-vegetative seasons from January to December 2020, compare their size fractions and importance for human health, and determine whether cyanobacteria present in the air are capable of producing toxins; specifically, microcystin-LR (MC-LR) and in what quantities. Such comprehensive research on algae has never been done; therefore, this work is a compendium for the coastal part of the Baltic Sea and indicates a strong need for such research in other regions, especially those with highly developed tourism.

2. Material and methods

2.1. Sampling location

The sample collection station was set up 20 m above sea level on the roof of the Institute of Oceanography building in Gdynia, Poland (54°31′ N, 18°48′E), situated approximately 1 km from the Gulf of Gdansk coastal zone but within the city center. The height of the building enabled samples of airborne microalgae and cyanobacteria to be taken above the level of neighboring tree canopies and buildings thus sampling the mixed air masses of both land and sea. Moreover, this height reduced the possibility of sampling direct suspensions of microorganisms from disturbed soil around the air sampler, it allowed easy access for lab personnel and a secure location away from public access. The station has been previously used for sampling bioaerosols, particulate matter, and rainfall (e.g., Lewandowska et al., 2017; Buch et al., 2021; Wiśniewska et al., 2021).

2.2. Sample collection

A pilot study was carried out in 2018 and 2019 to refine the sampling method (Lewandowska et al., 2017). Samples for this study were collected from January to December 2020. Samples were collected, at minimum, four times per month during the night and day. During the summer months, especially during algal blooms, additional samples were taken. Samples collected during rainfall were excluded in this study. The pilot study served to provide mainly qualitative data on airborne cyanobacteria and microalgae and therefore was not included in this study.

Prior to collecting the bioaerosol samples, a sterile mineral F/2 culture medium was prepared (Guillard, 1975) and calibrated using seawater with a salinity of 8 PSU. The salinity was verified by salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany). A modified bioaerosol sample collection method was implemented from a combination of methods used by Lewandowska et al. (2017) and Wiśniewska et al. (2020). Petri dishes with liquid medium were placed in a biological impactor (Tisch Environmental, Inc) consisting of six cascades that allowed particles of various diameters to be collected depending on the impactor cascade: >7 μm (1), 4.7–7 μm (2), 3.3–4.7 μm (3), 2.1–3.3 μm (4), 1.1–2.1 μm (5), or ≤1.1 μm (6) while maintaining an air flow-through rate of 28.3 L min^{-1}. The impactor, containing the Petri dishes with liquid F/2 medium (6 mL) in each cascade, were exposed for 6 h with precise sampling time recorded in order to calculate the volume of air sampled.

Separate samples were collected in a 24 h period representing daytime and nighttime samples.

Strains isolated from the air over the Baltic Sea region are maintained as unialgal cultures in the Culture Collection of Baltic Algae (as part of Airborne Algae – CCAA) (Table S1) at the Institute of Oceanography, University of Gdańsk, Poland (Latała et al., 2006; Wiśniewska et al., 2021).

2.3. Sample preservation until analysis

The bioaerosol samples were incubated in F/2 medium for 30 days in an incubator set at a constant temperature of 20 °C (±1 °C) and a light regime of 16:8 h light:dark cycle at 10 μmol photons $m^{-2} s^{-1}$. The intensity of photosynthetically active radiation (PAR) was measured using a quantum meter (LI-189, LI-COR Inc., Nebraska, USA) with a cosine collector. Fluorescent lamps (Cool White 40 W, Sylvania, OH, USA) were used as a source of irradiance. This method was used previously by Lewandowska et al. (2017) and Wiśniewska et al. (2020).

2.4. Meteorological data and other parameters

Supplementary meteorological data (Table S2) were obtained using a Vaisala WXT520 (Vaisala Inc., Woburn, MA) weather sensor and data from ARMAAG (Fundacja "Agencja Regionalnego Monitoringu Atmosfery Gdańsk-Gdynia-Sopot" https://armaag.gda.pl/). Weather data were collected in parallel with the air samples and includes; air temperature, wind velocity, relative humidity, pressure, and rainfall amounts.

The ecohydrological model (http://model.ocean.univ.gda.pl) was used to estimate blue green algae biomass and total primary production, as well as NO_3^- and PO_4^{3-} concentrations in Baltic Sea seawater (Table S3).

2.5. Taxonomic composition and number of identified taxa in the bioaerosol samples

The taxonomic composition and number of identified taxa were determined using a light microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) equipped with a camera (Nikon DSU2, Plan Apo VC 100 objective; magnification of ×1000). An epifluorescence microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) with UV-2A, B-2A, and G-2A block filters was used to verify microscope results and the presence of photosynthetic autofluorescent chlorophyll *a* content in identified taxa. Autofluorescence is widely used in algal physiology as an indicator of pigment compositions and the condition of the chloroplasts (Boluda et al., 2014; Barreiro Felpeto et al., 2018).

The analyzed material was collected from Petri dishes and later transferred in triplicate into 5-mL sterile plastic culture tubes (Fisherbrand™, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Phytoplankton were identified at the species or genus level under the light and epifluorescence microscopes using keys and relevant literature. The number of vegetative cyanobacterial and microalgal cells in bioaerosols were determined by flow cytometery (BD Accuri™ C6 Plus; BD Biosciences, San Jose, California, USA) according to the method described by Śliwińska-Wilczewska et al. (2018). Samples were run at a flow rate of 14 μL min^{-1} and each event was recorded in list mode. The flow cytometer was calibrated with Spherotech 6- and 8-Peak Validation Beads and SPHERO Rainbow Calibration Particles (BD Biosciences). Fluorescence emissions excited by the blue laser (480 nm) are measured with the FL1, FL2 and FL3 detectors, whereas the FL4 detector reads emissions excited by the red laser (640 nm). In bioaerosol samples, the use of these different detectors allows for complete differentiation of cyanobacteria and algae from other heterotrophic organisms.

2.6. Microcystin-LR analysis

MC-LR assays were performed on selected CCAA strains as well as the air samples. Quantitation of MC-LR equivalents were performed using a colorimetric MC-LR, Enzyme-linked immunosorbent assay (ELISA) kit (Abnova, Taipei, Taiwan) as per the manufacturer's specifications (Perez and Chu, 2020). The detection limit of the kit was 0.1 μg L^{-1} is and described in more detail by Kumar et al. (2020). Final absorbances were read at 450 nm in triplicate using a Thermo Scientific Multiscan Go microplate reader (Thermo Scientific, Waltham, MA, USA).

2.7. Statistical analysis

Spearman correlation coefficients were calculated between the number of microalgae and cyanobacteria cells and the daily rainfall amount (mm), mean temperature (°C), relative humidity (%), atmospheric pressure (hPa), wind speed (m s^{-1}), NO_3^- concentration in seawater (mg m^{-3}), PO_4^{3-} concentration in seawater (mg m^{-3}), cyanobacterial biomass (mg m^{-3}) and primary production (mg m^{-3}) in the Baltic Sea. The non-parametric Mann-Whitney U (M-W U) test was applied to test differences between two sets of independent data, the number of microalgae and cyanobacteria in the spring-summer and autumn-winter seasons and the number of microalgae and cyanobacteria during the night and day. Additionally, the Kruskal-Wallis test was used to compare more than two groups of independent variables, the amount of microalgae and cyanobacteria in individual months and the amount of microalgae and cyanobacteria in each size fractions. The threshold for statistical significance in above mentioned tests was $^*p < 0.05$.

3. Results and discussion

This study focuses on the quantitative and qualitative analysis of cyanobacteria and microalgae present in the air from January to December 2020. Samples were taken several times each month during the day and night with the particulate matter collected into six size fractions depending on the cutoff for each stage of impactor. Each of these 6 size fractions are considered as 6 replications of the experiment. However, to better illustrate the quantitative and qualitative variability of cyanobacteria and microalgae in the air, the total sum of collected organisms were determined. In future studies, we plan to deploy more biological impactors enabling the simultaneous collection of replicate samples.

During the 2020 sampling year, a total of 296 and 276 samples were collected during the day and night, respectively. In addition, samples of cyanobacteria and microalgae were collected during 2018 and 2019, but due to a lack of quantitative analysis, were not included in this study. However, due to their rich taxonomic composition, these microorganisms were isolated and collected as cultures at the CCAA (Wiśniewska et al., 2021). Thus, our measurement campaign was one of the longest in the history of airborne cyanobacteria and microalgae measurements. Previously, Sharma et al. (2006a) conducted all-season research campaigns of similar length.

3.1. Community structure – taxonomical composition and quantity

During the sampling period from January to December 2020, cyanobacteria and microalgae belonging to eight phyla were detected. The most abundant phylum was Cyanobacteria, which constituted 63% of all detected phyla. The results showed the presence of the following taxa: *Aphanothece* sp., *Aphanocapsa* sp., *Chroococcus* sp., *Nodularia* sp., *Nostoc* sp., *Phormidium* sp., *Pseudanabaena* sp., *Synechococcus* sp., *Synechocystis* sp., and *Woronichinia* sp. Among these species, *Synechococcus* sp. were the most frequently reported (62% of cyanobacteria) (Fig. 1). Cyanobacteria were also the dominant species in the air in many studies worldwide, e.g., Malaysia (Ng et al., 2011), Egypt (El-Gamal, 2008), and India (Sharma et al., 2006b). Furthermore, cyanobacteria were the only organisms identified at all stations during the research on airborne cyanobacteria and microalgae by Wiśniewska et al. (2019). However, little is currently known about the biogeography of airborne microbes. In the case of waters, both environmental and historical variables clearly have a role in structuring cyanobacteria diversity through time and location. Furthermore, the distribution patterns of various species may differ significantly, which is

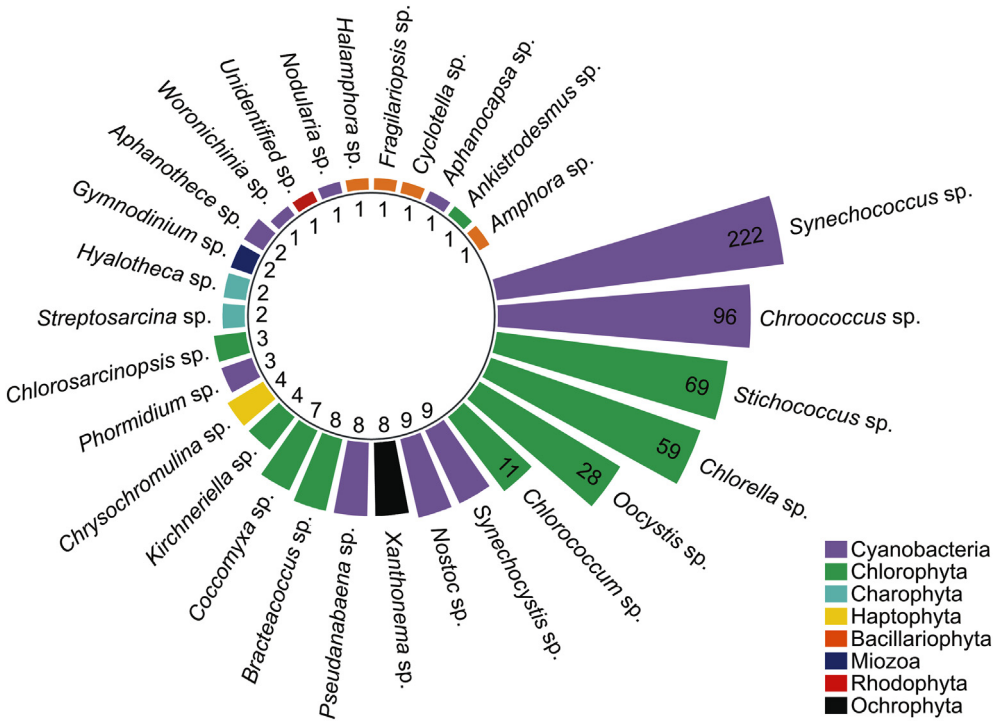

Fig. 1. The detected taxa and their frequency of occurrence [summarized number of taxa] in the air from January to December 2020 in the coastal zone of the Baltic Sea.

the focus of research by Ribeiro et al. (2018). On the other hand, the domination of *Synechococcus* sp. among airborne cyanobacteria and microalgae is not a surprising phenomenon, as this taxon is one of the most common photo-oxygenic microorganisms on the Earth (Whitton and Potts, 2000). This taxon widely occurs in the Baltic Sea as well (Ribeiro et al., 2018). *Synechococcus* is a marine cosmopolitan genus of cyanobacteria and one of the major primary producers in the oceans (Li, 1994). Its frequent occurrence in seawater, in combination with its microscopic size and relatively low sensitivity to changes in environmental conditions between water and air, favors its emission into the air and abundant occurrence in the atmosphere (Wiśniewska et al., 2021). According to worldwide studies (reviewed by Wiśniewska et al., 2019) *Phormidium* sp. occurred the most frequently among cyanobacteria (71% of reviewed studies). Cyanobacteria of *Nostoc* sp., *Anabaena* sp., and *Lyngbya* sp. were slightly less frequent but still numerous (58% of reviewed studies).

Although the samples were dominated by cyanobacteria, the significant percentage of green algae in the taxonomic composition of the bioaerosols should also be emphasized (Table S4). In this study, Chlorophyta constituted as much as 33.6% of all analyzed organisms. Among this group, the following taxa were noted: *Bracteacoccus* sp., *Chlorella* sp., *Chlorococcum* sp., *Chlorosarcinopsis* sp., *Chroococcum* sp., *Coccomyxa* sp., *Kirchneriella* sp., *Oocystis* sp., and *Stichococcus* sp. (Fig. 1). The most abundant were *Stichococcus* sp., which constituted 36.3% of the detected green algae. Ettl and Gärtner (1995) described it as an "extremely common terrestrial algae with global range." It may be found on a variety of surfaces, including stone walls, roofing tiles, tree trunks, and glass, but it can also be seen floating in water bodies (John, 2011). Although this organism was noted in many studies on bioaerosols (Sharma et al., 2006b; Tesson et al., 2016; Broady, 1996; Lewandowska et al., 2017; Genitsaris et al., 2011; Wiśniewska et al., 2020), the dominant organism among green algae is *Chlorella* sp. (Schlichting, 1964; Rosas et al., 1989). The alternating dominance between cyanobacteria and green algae in the air was explained by Sharma et al. (2007); tropical aeroalgal floras are dominated by cyanobacteria, whereas the temperate ones are dominated by chlorophytes.

There are studies showing the dominance of other phyla; for example, Tormo et al. (2001) indicated the dominance of diatoms after green algae. In our study, the remaining microalgae were Bacillariophyta (0.7%), Charophyta (0.7%), Haptophyta (0.7%), Miozoa (0.4%), Ochrophyta (1.4%), and Rhodophyta (0.2%) (one unidentified taxa belonging to Rhodophyta is presented in Fig. S1). Overall, 29 taxa were found to be present in the air during 2020 (Fig. 1).

Determining the amount of cyanobacteria and microalgae in the atmosphere is still quite a challenge for researchers (Wiśniewska et al., 2019). Due to different sampling techniques, among which using agar is the most common, it is very difficult to determine the number of cells immediately after collection. Contamination of air samples with fungi, bacteria, pollen, etc. poses additional challenges for quantitative analysis. These factors may cause differences in the amounts of airborne cyanobacteria and microalgae obtained by scientists in different research areas. Reisser (2001) detected 100 to 1000 algal cells m^{-3} in the atmosphere, yet Tormo et al. (2001) reported a range of 0.18 to 3.85 cells m^{-3}. In the present study, the number of cyanobacteria and microalgae in the air ranged from zero to 1685.3 cells m^{-3}. The amount of cyanobacterial and microalgae cells in the air was not closely related to the number of taxa isolated from the samples. Thus, the greatest taxonomic diversity does not necessarily occur when the greatest number of microalgae and cyanobacteria cells are present in the air. Therefore, it is particularly important that future research on airborne cyanobacteria and microalgae also focus on determining the quantitative composition immediately after sampling and the use of agar for growing colonies should be avoided if they are destined to be counted. Additionally, future research should focus on isolating cyanobacteria and microalgae from the air. The creation of a collection of cyanobacteria and microalgae from the air favors the capturing of new taxa, sometimes brought with the wind from distant areas. In addition, air samples are less abundant in algae and cyanobacteria than a similar volume of seawater, which promotes isolation. These cultures can be used in the future for numerous laboratory experiments (Chiu et al., 2020; Wiśniewska et al., 2021).

3.2. Diurnal variation

Studies show a differing amount of cyanobacteria and microalgae during the night versus the day (Tormo et al., 2001; Fröhlich-Nowoisky et al., 2016; Singh et al., 2018). However, these results do not clearly

demonstrate whether more bioaerosols will occur during the night or day. Singh et al. (2018) showed that 66% of airborne algae colonies were recorded at night. Conversely, the opposite conclusions were drawn by Tormo et al. (2001) who found the lowest values of cyanobacteria and microalgae were recorded at night in Spain. Similarly, in our study, the difference between the amount of microalgae and cyanobacteria during the day and night varied throughout the year. In the winter, spring, and autumn months, the difference between the amount of these microorganisms during the day and night was not statistically significant (M-W U test, $p > 0.05$) (Fig. 2).

Differences in the diurnal amounts of cyanobacteria and microalgae in the air were more pronounced in the summer than in other seasons, especially in July and August (Fig. 3). Surprisingly, a much greater number of cyanobacteria and microalgae occurred at night in July (48% on average), conversely the opposite occurred in August (13% on average).

According to Singh et al. (2018), cyanobacteria accounted for 95% of all organisms tested during the day, while at night they accounted for less than 13%. Our results show that in the months when more organisms were recorded during the day, cyanobacteria dominated (representing from 57% to 77.5%). In July, when more airborne organisms were recorded at night, green algae dominated (up to 87%), as was observed by Singh et al. (2018). So far, scientific research has not shown why cyanobacteria dominate during the day and green algae dominate at night. Sharma and Singh (2010) suggested that aerosolized cyanobacteria become heavier by absorbing moisture from the atmosphere due to their hygroscopic mucilage coating and fall onto exposed culture plates at increased rates. In August, after cyanobacterial blooms in the southern Baltic Sea, episodes of intense rain occurred (Wiśniewska et al., 2022, unpublished). The rain more effectively washed out the microalgae and cyanobacteria during the night, and consequently, the relative humidity was high during the day (RH from 66% to 79%). This may explain why cyanobacteria dominated during the day; however, this topic requires further consideration.

3.3. Seasonal variability

Seasonal variability in airborne cyanobacteria and microalgae has been demonstrated in some studies (Sharma et al., 2006a, 2006b; Lewandowska et al., 2017; Wiśniewska et al., 2019). The average monthly number of taxa identified in aerosols collected in the atmosphere of the coastal zone of the southern Baltic Sea (Poland) ranged from 2 to 15. Research has shown that cyanobacteria and microalgae occurred in the atmosphere during all months of the year. According to the review study conducted by Wiśniewska et al. (2019), cyanobacteria are the only phylum that have been identified in all studies worldwide. In some places, such as Egypt (El-Gamal, 2008), only cyanobacteria were recorded. Cyanobacteria dominated in tropical regions; however, in Europe, cyanobacteria dominated alternately with other phyla depending on the season (Lewandowska et al., 2017).

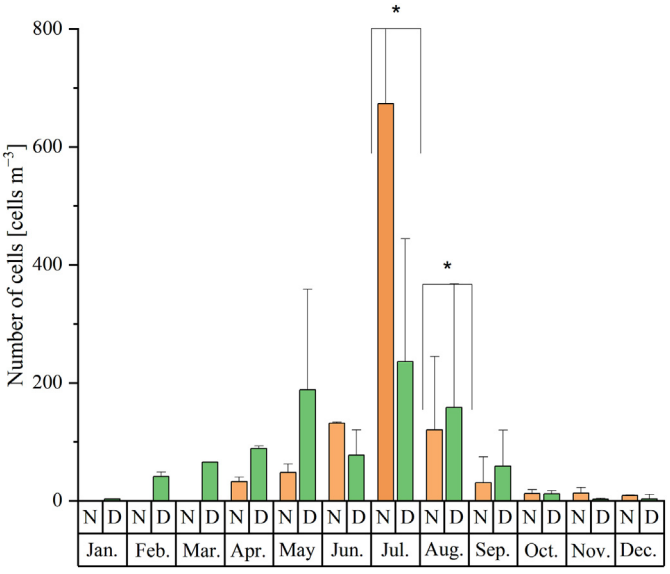

Fig. 2. Monthly cell counts of cyanobacteria and microalgae found in 2020 depicting night (N) and day (D) surveys. The asterisk (*) shows the statistically significant differences in diurnal values obtained in July and August 2020.

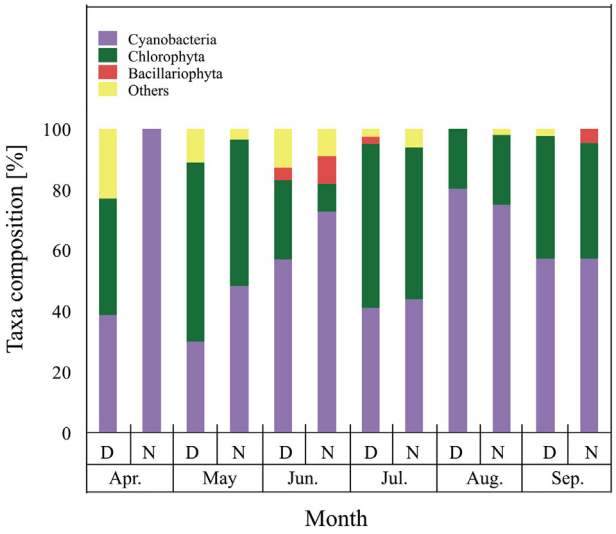

Fig. 3. Day (D) and night (N) taxonomical compositions expressed as a percent of the total number of taxa of airborne cyanobacteria and microalgae during the spring and summer months of 2020. Taxa surveyed: Cyanobacteria, Chlorophyta, Bacillariophyta, and Others (Charophyta, Haptophyta, Miozoa, Ochrophyta, and Rhodophyta).

In this study, the greatest diversity in terms of taxonomic composition was recorded in July (15 taxa), and the smallest in January (two taxa). In India, Sharma et al. (2006a and 2006b) recorded the highest number of taxa at the end of May, at the beginning of June, and in October; it was approximately 18–23 taxa of cyanobacteria and microalgae (Sharma et al., 2006a, 2006b). An increased number of cyanobacteria and microalgae in the air is usually associated with higher temperatures and more sunny hours (Sharma et al., 2006a, 2006b). Such conditions also favor cyanobacteria and algae blooms in the Baltic Sea and hence could be responsible for the higher number of microorganisms in the atmosphere. Lewandowska et al. (2017) recorded the highest number of taxa in the air of the coastal zone of the Baltic Sea from April to July; afterwards, this number began to decline. In the present study, the maximum number of green algae taxa was reached in July and subsequently decreased, as did the total amount of cyanobacteria and microalgae in the air (Fig. 4). Additionally, Sharma et al. (2006a and 2006b) discovered that cyanobacteria dominated during the warm season, whereas green algae was more abundant during the cooler months. Our measurements showed that in the winter months (December to March), there was a negligible amount of cyanobacteria and microalgae in the air (Fig. 4). Both single cyanobacteria and green algae appeared in the samples, but it is difficult to clearly conclude that any taxa were particularly dominant. However, in the winter months in the Polish coastal zone, no other phyla than cyanobacteria and green algae were found in the air (Fig. 4). Moreover, the percentage of cyanobacteria in aerosols ranged from 10% of the measured taxa in February to 77.5% in August. From August to December, the amount of cyanobacteria and microalgae in the air was high, but their taxonomic diversity declined.

However, the highest percentage for total number of recorded green algae taxa occurred in February and amounted to 90%, while a significantly lower value of 21% was observed in June. Among the remaining phyla, Ochrophyta had the largest share in April, when it amounted to 16% of the total recognized taxa.

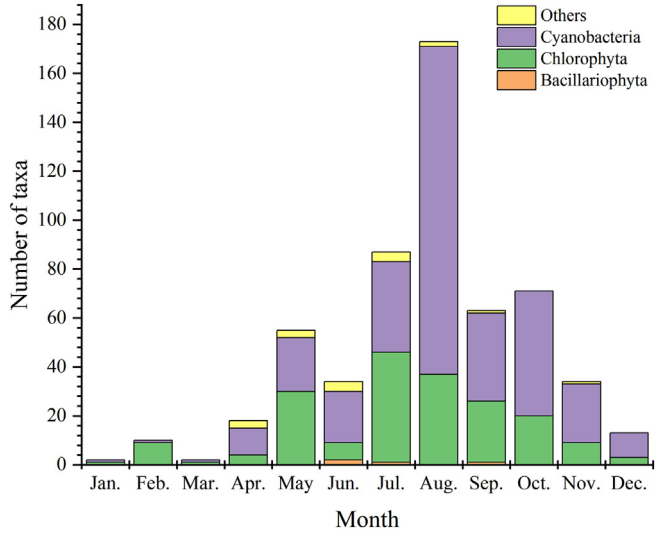

Fig. 4. The total number of collected airborne cyanobacteria and microalgae. Taxa divided into Cyanobacteria, Chlorophyta, Bacillariophyta, and Others (Charophyta, Haptophyta, Miozoa, Ochrophyta, and Rhodophyta) recorded in the air in individual months during 2020.

When analyzing the meteorological data for 2020, August had highest average air temperature (19.6 °C) and the lowest average wind speed (2.3 m s^{-1}). Such conditions favor blue-green algae blooms in the sea, which could also contribute to the high proportion of cyanobacteria in the air in August. This is consistent with the results of Sharma et al. (2006a and 2006b). The intensive growth of cyanobacteria in the Baltic Sea in August was documented by the ecohydrological model (http://model.ocean.univ.gda.pl/). In August 2020, during a toxic cyanobacteria bloom in the Baltic Sea, cyanosis of *Nodularia* sp., which is particularly dangerous to human health, was recorded in the aerosol samples. In recent years, *Nodularia* sp. appeared in the air during toxic blooms of cyanobacteria and microalgae in the Baltic Sea; therefore, it was isolated and included in the CCAA collection (Wiśniewska et al., 2021). Thus, during toxic blooms in the Baltic Sea, there is an increased risk of inhalation of toxic organisms. Therefore, we recommended that people avoid remaining in areas near the water for long periods of time. To understand how far toxins produced by cyanobacteria are transported inland, it would be necessary to increase the number of sampling stations at regular distances from the water.

Our results also show that cyanobacteria and microalgae can stay airborne year-round, especially under favorable weather conditions (Fig. 5). Significantly more cyanobacteria and microalgae occurred in the spring and summer than in the autumn and winter (M-W U test, $p < 0.05$). The highest average number of cyanobacteria and microalgae cells was recorded in July 2020 at 479 cells m^{-3}. The recorded amount of cyanobacteria and microalgae in the air was consistent with Reisser (2001), where 300–500 cells m^{-3} of microalgae in the atmosphere were detected during sunny summer days. The average amount of cyanobacteria and microalgae increased from March to October, peaking in July, and then decreased sharply in October, with the lowest values in January (Fig. 5). The highest value of 1658 cells m^{-3} was recorded on July 22, 2020. There are statistically significant differences among the quantities of algae in individual months (KW test, $p < 0.05$).

Seasonal variability in the amount of cyanobacteria and microalgae in the air is strongly related to meteorological parameters (Sharma et al., 2006a). On a yearly scale, in the Gulf of Gdańsk region, the wind speed is higher in winter than in summer (an average of 5.83 m s^{-1} in December compared to 2.65 m s^{-1} in July). Cyanobacteria blooms in the Gulf of Gdańsk are favorable during the summer months due to higher seasonal temperatures and lower wind speeds. Although wind is necessary for drying, fragmentation, and airborne transportation of algae, our results showed a decrease of airborne algae as wind speed increased, as confirmed by a Spearman rank correlation of $r = -0.825$. The lack of high waves and stable, almost windless conditions could favor algae blooms on the surface of the water reservoir, and thus more organisms could be emitted from the sea to the air. On the other hand, a positive correlation was found between the amount of cyanobacteria and microalgae and the air temperature (Spearman rank correlation $r = 0.755$). This is mainly due to the intensive primary production during the summer. Additionally, as noted by Sharma et al. (2006a), during dry weather, algal crusts flake off, and air blows microorganisms from the surface to the atmosphere.

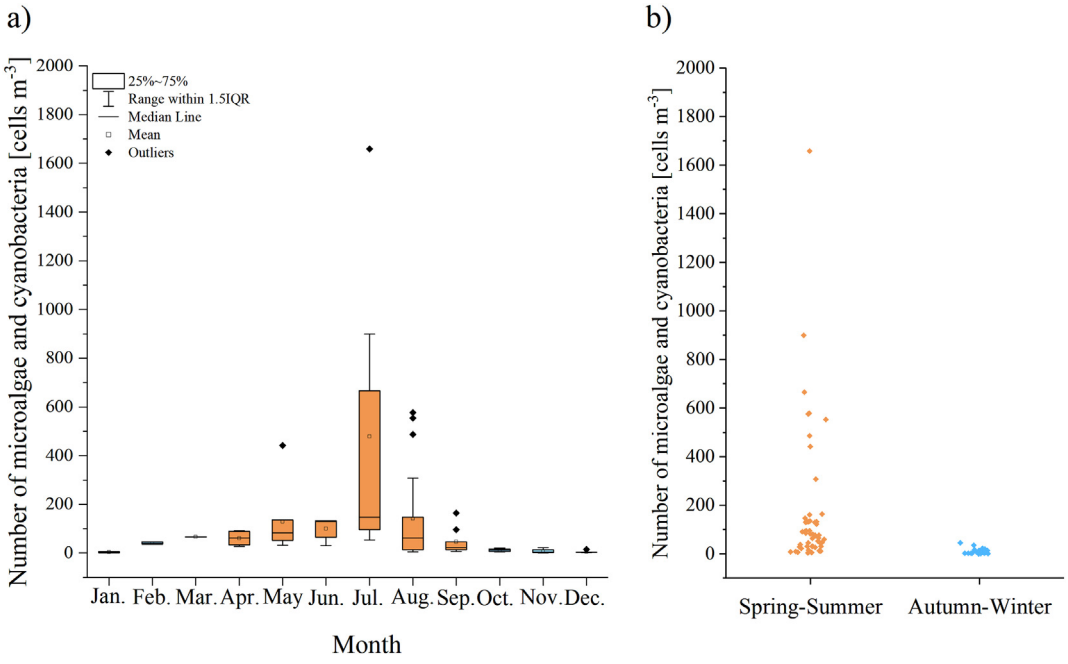

Fig. 5. The number of airborne cyanobacteria and microalgae summarized together in individual months of 2020 (a), and the data distribution during spring-summer and autumn-winter months of 2020 (b).

3.4. Size fractions and importance for human health

The size of the particles in the air we breathe is of particular importance for human health. Tesson et al. (2016) assumed that about 300 algal cells are deposited in the human respiratory tract per hour, which means they pose a significant health risk. Based on the size of the particle, we can determine how deeply it penetrates the human respiratory system. Smaller aerosols can penetrate deeper into the human respiratory tract and can settle in the air sacs and bronchi, leading to many illnesses (Fröhlich-Nowoisky et al., 2016; Lewandowska et al., 2017; Facciponte et al., 2018).

To carry out quantitative analysis of the airborne algae and cyanobacteria, we used the six-cascade impactor (sizes: >7 μm; 4.7–7 μm; 3.3–4.7 μm; 2.1–3.3 μm; 1.1–2.1 μm; and ≤1.1 μm in diameter), allowing the collection of particles of appropriate diameter. According to the assumptions of the manufacturer, Tisch Inc., the impactor was calibrated in such a way that all collected particles, regardless of their physical size, shape, or density, were classified aerodynamically and could be deposited in different parts of the human respiratory tract (Fig. 6) (Lewandowska et al., 2017). In this study, we analyzed organisms both in terms of coarse (1–4 cascade) and fine particles (5–6 cascade), and separately in each size distribution range.

The total number of cyanobacteria and microalgae cells was the highest among the coarse particles (>2.1 μm) and was 6901 cells m^{-3} of air (Fig. 7). This constituted 61% of all cells detected in all particle sizes. Particles of this size can settle in the upper respiratory tract and do not penetrate deeper than secondary bronchi. Cyanobacteria and microalgae were most abundant in aerosols >7 μm in diameter (2260 cells m^{-3}) and consisted of 22 taxa. Moreover, the lowest number was detected in the finest particles, with a diameter <1.1 μm (1100 cells m^{-3}; 6 taxa). In other bioaerosol sizes, the differences in the number of recorded taxa were relatively similar, from 12 in aerosols between 3.3 and 4.7 μm in diameter to 17 in aerosols between 4.7 and 7.0 μm (Fig. 7). However, no significant statistical differences were found in the amount of cyanobacteria and microalgae in the individual cascades (KW test, $p > 0.05$). This means that individual organisms cannot be uniquely attributed to only one size fraction. This can also be explained in the following way. The coccoid algae ranged from a few to several dozen μm in diameter, which defines what size organisms will pass through the impeller nozzles of a given diameter. A more complicated matter is the case of filamentous organisms, the length and width of which vary from a few to several μm, which means that an organism arranged in a shorter plane can pass through nozzles with a smaller diameter and be deposited deeper in the human respiratory tract.

A particularly important issue is whether the organisms with a negative impact on human health, which Genitsaris et al. (2011) described, are among the smallest fraction of particles. During our study, harmful organisms such as *Amphora* sp. *Bracteacoccus* sp., *Chlorococcum* sp., *Chlorosarcinopsis* sp., *Oocystis* sp., *Stichococcus* sp., *Nodularia* sp., *Nostoc* sp., *Synechocystis* sp., *Chrysochromulina* sp., and *Gymnodinium* sp.

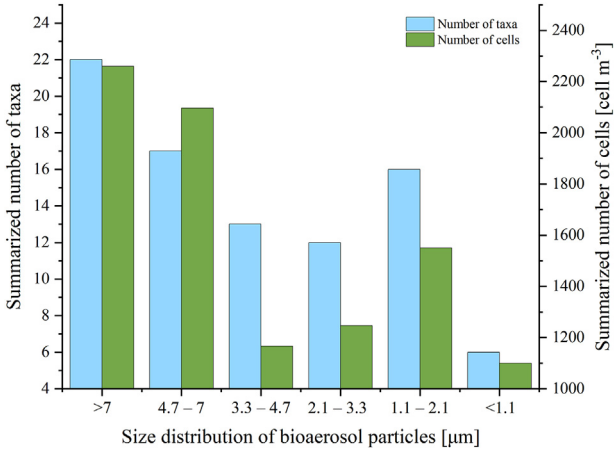

Fig. 7. Summarized number of taxa and number of collected airborne cyanobacterial and microalgal cells [cells m^{-3}] as a function of size distribution of bioaerosol particles [μm] from January to December 2020 in the coastal zone of the Baltic Sea.

(Genitsaris et al., 2011) were noted in aerosols. All these organisms are harmful and can cause allergies or produce toxins when inhaled (Genitsaris et al., 2011). The current study indicated that harmful airborne microalgae and cyanobacteria were more frequently present in coarse particles (>2.1 μm) at 71.3%. Thus, 28.7% of harmful organisms were recorded in small particles. The majority of harmful microorganisms in the larger fraction, which do not reach the deeper parts of the human respiratory system, is good news in the context of human health. However, it has not been shown that each taxon only had one given size range. Therefore, in the case of significant emissions of toxic cyanobacteria and microalgae, such as during toxic cyanobacterial blooms, it should be considered that these organisms can also get into human alveoli.

3.5. Presence of microcystin-LR

Secondary metabolites, such as the microcystins produced by cyanobacteria, pose a threat to both environmental and human health (Brózman et al., 2020). Cyanobacterial toxins have been linked to a variety of acute and chronic human illnesses, including non-alcoholic liver disease, gastroenteritis, and amyotrophic lateral sclerosis (Cheng et al., 2005; Backer et al., 2010). Sahu and Tangutur (2014) confirmed that, even at lower doses, microcystins have toxic effects on organisms when inhaled. Although little is known regarding acute toxicity in humans, according to the World Health Organization, pulmonary absorption of MC-LR (purified from a cyanobacterial bloom sample) was demonstrated by intratracheal

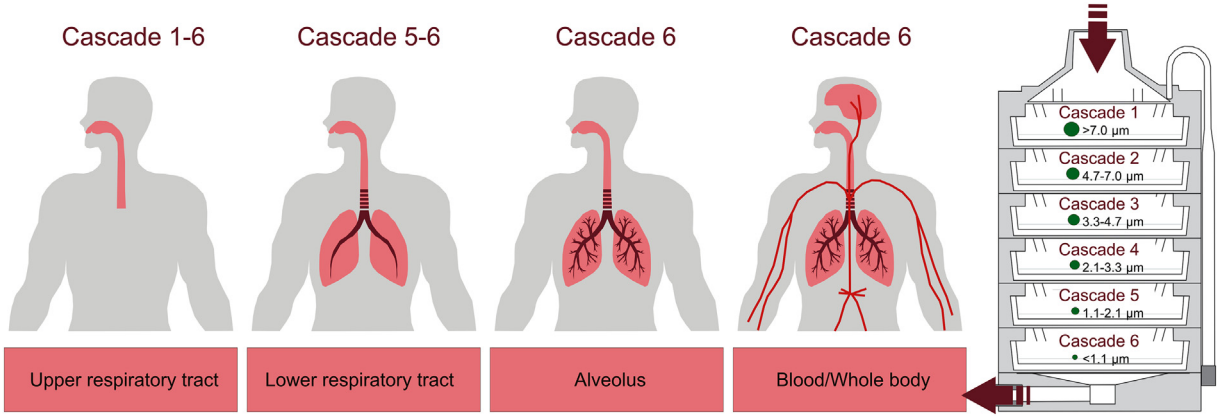

Fig. 6. The depth at which aerosols enriched in cyanobacteria and microalgae can penetrate the human respiratory tract as a function of particle size distribution collected with the microbiological impactor.

instillation of a sublethal dose of 50 μg kg^{-1} body weight or a lethal dose of 100 μg kg^{-1} body weight in mice (Ito et al., 2001). Many studies confirm that the microcystin produced in the water can be aerosolized and then inhaled by humans (Backer et al., 2010; Murby and Haney, 2015). Backer et al. (2010) found from <0.1 ng m^{-3} to 2.89 ng m^{-3} of microcystin in personal air samplers near blooming lakes.

Recently, it has been popular to research airborne cyanobacteria and microalgae using laboratory experiments (Kumari and Singh, 2021; Wiśniewska et al., 2021; Chiu et al., 2020). We tested whether cyanobacteria and microalgae isolated from the air behave in the same way as those present in other environments. In this study, the MC-LR content in cyanobacteria isolated from the air (belonging to CCAA collection) was determined (Table S5). The ability of individual strains to produce microcystin has been demonstrated, which confirms that cyanobacteria present in the air can be potentially harmful to humans. MC-LR concentrations obtained in our measurements ranged from below the detection limit to 420 fg $cell^{-1}$ (Fig. 8).

Several strains isolated as monocultures of cyanobacteria, specifically *Aphanothece* sp., *Pseudanabaena* sp., *Leptolyngbya* sp., *Synechococcus* sp., *Gloeocapsa* sp., *Nostoc* sp., and *Rivularia* sp., were tested. The presence of MC-LR has been found in *Nostoc* sp., *Nostoc edaphicum, Pseudanabaena galeata*, *Pseudanabaena catenata*, *Leptolyngbya* sp., *Synechococcus* sp., *Gloeocapsa* sp., and *Rivularia* sp. from the CCAA collection (Fig. 8). The picocyanobacterium *Synechococcus* sp. CCAA 46 produced the highest concentration of this toxin (420 fg $cell^{-1}$). However, not all strains belonging to the same phylum produced MC-LR (Fig. 8). In the case of *Synechococcus* sp., all strains had relatively high concentrations of MC-LR, but in the case of *Nostoc* sp., toxin concentration ranged from below the detection limit to 21 fg $cell^{-1}$. Mohamed and Al-Shehri (2015) also showed that marine cyanobacteria were characterized by a different production of MC. In this work, the authors showed that *Oscillatoria tenuis* produced about 718 μg g^{-1} d·wt MC, while in the case of *Oscillatoria accuminata* MC production was below the detection limit. It is worth noting here that it is currently unknown why cyanobacteria strains produce different amounts of MCs (Christiansen et al., 2008). Our research suggests that airborne cyanobacteria can produce toxins that are harmful to humans however, the presence of a specific phylum in the air does not necessarily mean a threat to human health.

As not all cyanobacteria and microalgae present in the air were isolated, the mean concentration of MC-LR for samples abundant in cyanobacteria was also determined for each month (Fig. 9). In this study cyanobacteria and microalgae were only counted in total; therefore, only the relationship between the amount of all tested bioaerosols and the concentration of MC-LR can be shown. A positive relationship was found between the total amount of cyanobacteria and microalgae and the concentration of MC-LR (Spearman rank correlation equal to $r = 0.623, p < 0.05$). This dependence,

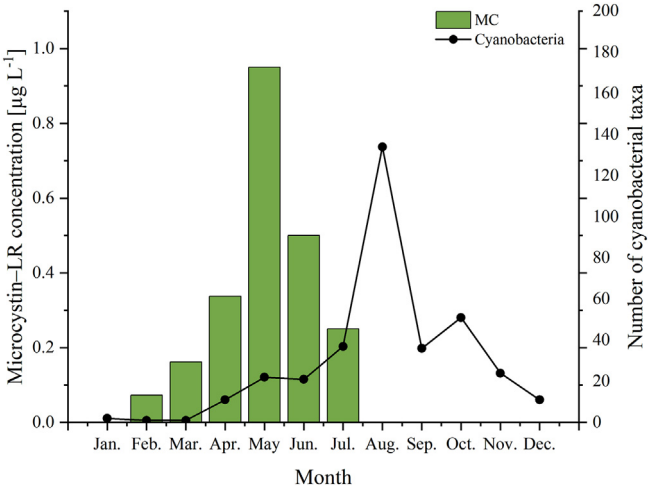

Fig. 9. Number of airborne cyanobacterial taxa in individual months along with the average concentration of microcystin in aerosols samples collected in 2020.

despite the use of the total count of cyanobacteria and microalgae, may result from the fact that cyanobacteria accounted for over 60% of all recorded phyla. The highest concentration of MC-LR occurred in May, while the highest amounts of cyanobacteria and microalgae in the air were recorded in later months. Since airborne cyanobacteria and microalgae were only counted in total, it is worth examining the taxonomic variability during the analyzed period (Fig. 9). The high concentration of MC-LR in May may be related to the presence of only *Synechococcus* sp. and *Chroococcus* sp., both of which can produce MC-LR. The lack of MC-LR in August is also surprising, considering the significant increase in cyanobacteria compared to other taxa. However, in addition to *Synechococcus* sp. and *Chroococcus* sp., *Nodularia* sp., *Phormidium* sp., and *Pseudanabaena* sp. were present; therefore, future research should also include nodularin or anatoxin. In addition, the information shown above on the different toxicity of individual strains, including *Synechococcus* sp., indicates that future toxicity studies should be enriched with genetic analysis of individual strains.

Facciponte et al. (2018) confirmed the presence of cyanobacteria in the upper respiratory tracts and central airways of humans; however, more research is needed on the survival of cyanobacteria and the production of toxins in such an environment. These are the first studies of this type; thus, future studies on the presence of airborne cyanobacteria and microalgae should collect the microorganisms on a liquid medium, so that it is possible to determine the concentration of toxins immediately after taking the sample.

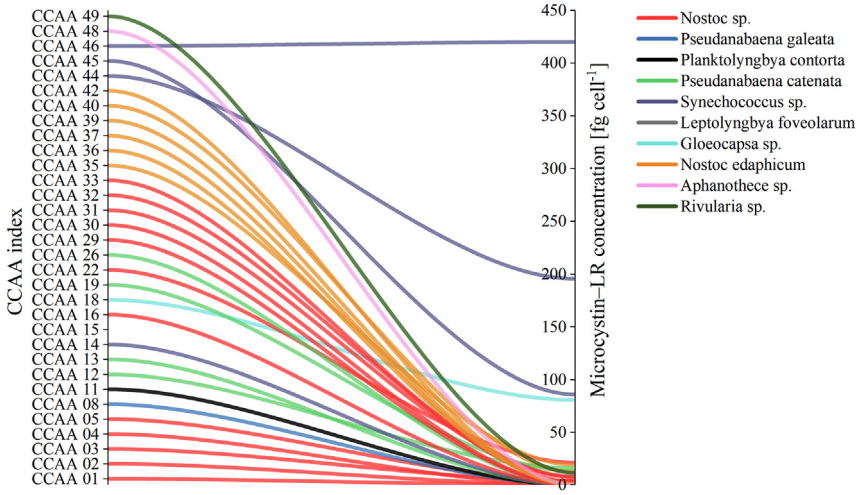

Fig. 8. MC-LR concentrations [fg $cell^{-1}$] produced by individual strains of airborne cyanobacteria. The MC-LR concentration values show arithmetic means ($n = 3$).

4. Conclusions

This study was innovative in terms of its liquid medium sampling method and the six-cascade impactor. Thanks to this technique, it was possible to determine the number of cyanobacteria and microalgae, which ranged from zero to 1685 cells m^{-3}. Additionally, cyanobacteria and microalgae were present in the air throughout the entire year. The highest number was recorded in July, at the peak of the tourist season. Their increased numbers during summer were mainly related to an increase in temperature and a decrease in wind speed. Moreover, differences in the amount of cyanobacteria and microalgae in the air during the day and night were recorded in July and August. In general, the samples showed the presence of 29 taxa; cyanobacteria constituted over 60% of detected organisms, while picocyanobacteria *Synechococcus* sp. dominated. Cyanobacteria and microalgae have been shown to have negative effects on human health; nearly 30% of potentially harmful cyanobacteria and microalgae were small enough to reach secondary bronchi. Moreover, *Synechococcus* sp. CCAA46 showed the highest concentration of toxic MC-LR. Depending on the strain, cyanobacteria and microalgae isolated from the air may produce different concentrations of toxins. In addition, high concentrations of MC-LR were recorded in May, before the cyanobacterial bloom period, which means that throughout the growing season, to a greater or lesser extent, people living in the coastal area of the Baltic Sea were exposed to cyanobacteria and microalgae, negatively impacting their health. However, most airborne cyanobacteria and microalgae are present as large aerosols and if inhaled, will likely remain in the human nostrils, thus less dangerous to human health. It is therefore important to consider that in coastal tourist regions, especially during the cyanobacteria and microalgae blooms, these organisms are also present airborne and can be inhaled by humans even at a considerable distance from the water.

CRediT authorship contribution statement

Kinga Wiśniewska: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Visualization, Roles/Writing - original draft. **Sylwia Śliwińska-Wilczewska:** Conceptualization, Supervision, Visualization, Roles/Writing - original draft. **Mireille Savoie:** Roles/Writing - original draft. **Anita U. Lewandowska:** Conceptualization, Supervision, Validation, Roles/Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.154152.

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AUTHORS CONTRIBUTION STATEMENT

We hereby confirm that the specific contribution to the publication:

Wiśniewska K., Śliwińska-Wilczewska S., Savoie M., Lewandowska A. 2022. *Quantitative and qualitative variability of airborne cyanobacteria and microalgae and their toxins in the coastal zone of the Baltic Sea.* Science of The Total Environment, 826, 154152. DOI: 10.1016/j.scitotenv.2022.154152

were as follows:

Wiśniewska Kinga Areta – 60%:
Preparation of the research concept, development of methods, acquisition of funds, sample collection, gathering and analysis of literature data, laboratory experiments, analysis of results, graphical and statistical processing of results, preparation of the manuscript.

Śliwińska-Wilczewska Sylwia –15%:
Preparation of the research concept, development of methods, supervising, conducting analyses, revision the manuscript.

Savoie Mireille – 5%:
Graphical processing, language correction.

Lewandowska Anita –20%:
Preparation of the research concept, development of methods, supervising and revision of the manuscript, and preparation of the manuscript.

…… ……
Wiśniewska Kinga Areta Śliwińska-Wilczewska Sylwia

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Savoie Mireille Lewandowska Anita

1.1 PUBLICATION III

9.3 PUBLIKACJA III

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Airborne microalgal and cyanobacterial diversity and composition during rain events in the southern Baltic Sea region

Kinga A. Wiśniewska[1], Sylwia Śliwińska-Wilczewska[2]✉ & Anita U. Lewandowska[1]

Airborne cyanobacteria and microalgae are commonly found in the atmosphere and may pose a serious human health risk. This study presents an innovative investigation of the washout efficiency of airborne cyanobacteria and microalgae in the Gulf of Gdańsk (southern Baltic Sea). For the first time, the number and type of cyanobacteria and microalgae were determined in rainwater samples and in air before and after rainfall events. The number of cyanobacteria and microalgae cells in the rainwater samples ranged, depending on, e.g., weather conditions, from 100 cells L^{-1} to 342.2×10^3 cells L^{-1}. Several harmful taxa, such as *Chlorococcum* sp., *Oocystis* sp., *Anabaena* sp., *Leptolyngbya* sp., *Nodularia* sp., *Pseudanabaena* sp., *Synechococcus* sp., *Synechocystis* sp., and *Gymnodinium* sp., were noted in our study. Washing out by rain is extremely relevant to human health and decreases the chance that people inhale these species and their toxic metabolic products. The greatest diversity of airborne microalgae and cyanobacteria was recorded in July 2019, despite this being the period with the lowest number of cells in rainwater samples. Research conducted in the southern Baltic Sea region confirmed the relationship between the occurrence of cyanobacteria and microalgae in the air and blooms in the sea. It is worth emphasizing that the number of microalgae and cyanobacteria cells decreased by up to 87% after a rainfall event relative to that before the rainfall event. The obtained results significantly increase the level of knowledge about cyanobacteria and microalgae present in the air. By demonstrating the washout efficiencies of cyanobacteria and microalgae, the results indicate the potential of individual taxa to be removed from the atmosphere with rainfall. The findings of this study are helpful for further research on airborne microorganisms and air quality.

The atmosphere contains diverse living microbes called bioaerosols. Among them, bacteria, viruses, fungi, pollen, microalgae, and cyanobacteria can be distinguished[1,2]. However, autotrophic organisms in the atmosphere are still poorly studied in comparison with heterotrophic organisms[3–8]. Cyanobacteria and microalgae present in the atmosphere are involved in cloud formation and influence the hydrological cycle and Earth's climate[6,8,9]. Recent studies have demonstrated the negative health impacts of airborne cyanobacteria and microalgae, as well as the toxic compounds they produce[4,10,11]. The importance of these organisms in the atmosphere is described in detail elsewhere[3,4,8,9]. The present study focuses exclusively on the presence of cyanobacteria and microalgae in atmospheric aerosols and their wet deposition.

Depending on the prevailing weather conditions (e.g., wind speed, wind direction, temperature, air humidity)[12–14], microorganisms, including cyanobacteria and microalgae, are emitted from water reservoirs or re-emitted from other surfaces to the atmosphere. The process is most effective during a period of high primary productivity in the oceans. According to Marshall and Chalmers[15], air humidity is an important meteorological parameter in the cyanobacteria and microalgae emission process to the atmosphere. Marshall and Chalmers[15] found that desiccation could increase the possibility of algae becoming airborne. Airborne microorganisms can subsequently be transported over long distances and/or incorporated into clouds before undergoing wet and/or dry deposition[6,8,9]. The first reports on this topic were reported during the 1970s, when it was suggested that heavy rainfall could lead to the intensive washout of airborne microalgae[16]. Sharma et al.[13] noted that although

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rainfall removes airborne algae through the effects of rainout and washout, rainfall also releases algae through splash, tap, and puff mechanisms, thus emitting them into the air[17].

Rain can efficiently remove airborne microbes. The microbial community in rain includes the microbes in the associated cloud as well as the air column below it. However, the diversity of microorganisms in rain is still poorly understood[18]. To date, scientists have detected cyanobacteria in clouds at an abundance ranging from ~ 1 to 50% of the total microbial community[19]. The size of the particles removed during atmospheric deposition determines their activity as cloud condensation nuclei, so determining the size of deposited particles is necessary to assess the effective removal from the atmosphere[20].

Both wet and dry deposition are responsible for removing particles, including carbon and iron, from the atmosphere; however, wet deposition is more effective and can remove up to 80% of aerosols by mass[21–26]. Regarding the wet deposition of all atmospheric particles, both washout and rainout can remove cyanobacteria and microalgae from the atmosphere. Washout is a below-cloud process whereby aerosols are collected by falling hydrometeors. In contrast, rainout involves in-cloud scavenging, whereby particles act as cloud condensation nuclei in supersaturation conditions above the cloud[27]. Few studies have investigated the ability of cyanobacteria and microalgae to remain in the atmosphere and colonize new regions as a result of atmospheric deposition[28,29]. Airborne organisms may have an important impact on atmospheric processes and could also have an impact on ecosystems after their deposition. However, very few studies have investigated the presence of these organisms in clouds and rain.

In consideration of the aforementioned processes and dependencies, this study investigates the washout efficiency of airborne cyanobacteria and microalgae that may pose a human health risk. Many acute health problems related to bioaerosols have been identified, including asthma, allergic reactions, hay fever, skin inflammation, burning of the eyes, rhinitis, and respiratory irritation[2]. Accordingly, this study assesses whether cyanobacteria and microalgae are effectively removed from the atmosphere by determining the number and type of cyanobacteria and microalgae in rainwater samples and in air samples before and after the corresponding rainfall event.

Results and discussion

This research focuses on the quantitative and qualitative analyses of cyanobacteria and microalgae present in rainfall during the summer phytoplankton bloom season of August–September 2019. In addition, a continuous episode of rainfall over several days was selected to demonstrate the washout process of microorganisms from the air with rain.

Quantity of cyanobacteria and microalgae washed out with rain during the growing season. Currently, there is a growing number of scientific articles on cyanobacteria and microalgae in the atmosphere[8]. Unfortunately, there is a reference methodology for efficiently counting the microorganisms present in the air or in rainfall. A popular method for quantifying cyanobacteria and microalgae in the air is to show the number of taxa found in the collected samples after growth[6,31,42–46]. In this study, a total of 16 taxa of airborne cyanobacteria and microalgae were found in the samples. In the rainwater samples obtained during the summer of 2019, 11 taxa of cyanobacteria and microalgae were distinguished. The green algae in the rainwater samples included *Bracteacoccus* sp., *Oocystis* sp., *Coenochloris* sp., *Chlorella* sp., and *Chlorococcum* sp., while the cyanobacteria included *Leptolyngbya* sp., *Pseudanabaena* sp., *Synechococcus* sp., and *Synechocystis* sp. In addition, *Chrysochromulina* sp., which belongs to Haptophyta, was observed.

Other studies recorded the presence of several to several dozen taxa in the air[6,31,42–46]. Certainly, a number of factors, starting with atmospheric conditions and ending with physical and chemical parameters of the surrounding waters, influence the diversity of cyanobacteria and microalgae in the atmospheric air. Analyzing global trends, only cyanobacteria have been found in the atmosphere of every region of the world[31]. However, according to Dillon et al.[47], cyanobacteria have been detected in clouds at variable abundances between ~ 1% and 50% of the total microbial community. Xu et al.[48] found that cyanobacteria constituted only 1.1% of the total bacterial community in clouds. It needs to be highlighted that there is still a lack of research available to provide this type of information for rainfall samples.

For the period from July to September 2019, the results showed that the number of cyanobacteria and microalgae cells present in rainfall varied over time (Fig. 1) and ranged between 100 cells L^{-1} and 342.2×10^3 cells L^{-1}. From July to the end of August, the cell number was relatively low, ranging from 100 cells L^{-1} to 28.6×10^3 cells L^{-1}. This variability was related to the change in the biomass of blue green algae in the Gulf of Gdańsk (Table S2; Fig. 1). Therefore, this research also shows the close relationship between the processes taking place in the Baltic Sea and the presence of cyanobacteria and microalgae in the atmosphere. As the biomass of cyanobacteria in the Baltic Sea increased, the number of cyanobacteria and microalgae cells in the rainfall samples also increased ($^{***}p < 0.001$). This result may be representative of the dominant number of cyanobacteria cells in the rainfall over the Bay of Gdańsk. Based on the data from the hydrodynamic model (http://model.ocean.univ.gda.pl/) for the Bay of Gdańsk, intense increases in the biomasses of cyanobacteria and total phytoplankton in seawater were recorded at the beginning of September 2019 (Fig. 1). Moreover, when analyzing the meteorological conditions, the sudden increase in the biomass of cyanobacteria and microalgae in seawater could have been related to the relatively low wind speeds (mean of 1.3 m s^{-1} over a few days) and the highest air temperature (up to 31.2 °C on September 1) in the analyzed period ($^{*}p < 0.05$ for air temperature). The influence of atmospheric pressure was also an important factor ($^{***}p < 0.001$). It is known that the presence of cyanobacteria and microalgae in air, and subsequently in rainfall, is strongly related to the changes occurring in nearby seawater[8]. Moreover, the results of the present study revealed a high Spearman correlation between the number of cyanobacteria and microalgae cells in the rainwater samples and the NO_3^- concentration of seawater ($^{*}p < 0.05$) (Table S3). Therefore, these studies indirectly indicated that the processes leading to increased blooms in water bodies, with

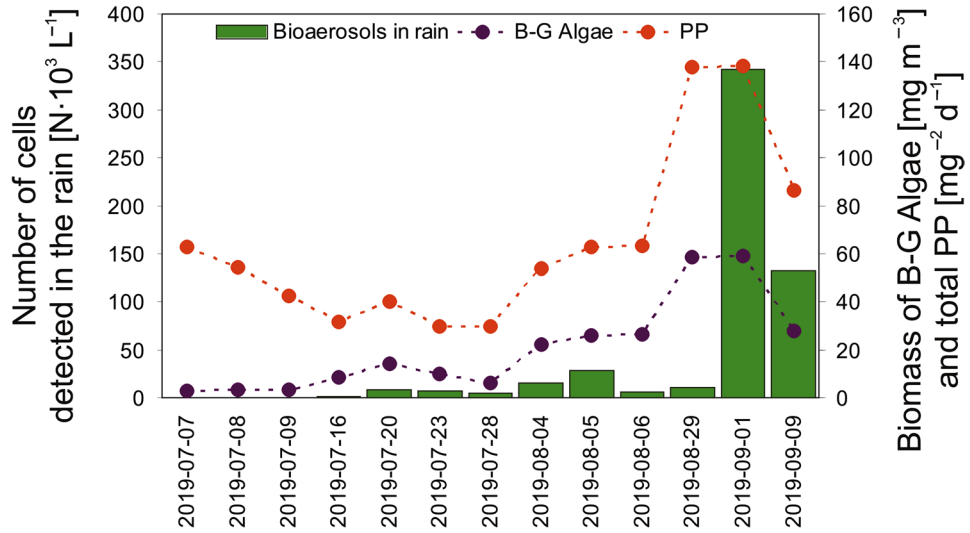

Figure 1. Number of cyanobacteria and microalgae cells present in the rainfall samples and the corresponding changes in their primary production (PP) and the biomass of cyanobacteria (B–G Algae) in the Gulf of Gdańsk (http://model.ocean.univ.gda.pl).

particular emphasis on blooms of toxic organisms, significantly affected the air quality in this region and could also influence the health of its citizens.

Quality of cyanobacteria and microalgae washed out with rain during the growing season. Many studies have described the species composition of cyanobacteria and microalgae present in the atmosphere[2,4,6,12–14,16,42,43,46,49,50]. However, research on how these organisms are removed with precipitation from the atmosphere is still lacking. To the best of our knowledge, only Dillon et al.[47] have reported on the number of microorganism taxa present in rain. Green algae of Trebouxiophyceae and cyanobacteria of Xenococcaceae were predominant in rainwater samples taken at the Opme meteorological station in France[47]. The authors classified the cyanobacteria in their rainwater samples as Phormidiaceae, Rivulariaceae, and Nostocaceae and the orders Pseudanabaenales and Synechococcales. Among the genre of green microalgae, *Chlorella* sp. was often observed by Dillon et al.[47]. Regardless of being in aerosols or rainwater, cyanobacteria and green algae have been found to be the dominant organisms[8,47]. Similar conclusions can be drawn from the results of the present study. In addition to cyanobacteria and green algae, *Chrysochromulina* sp. and *Gymnodinium* sp. were observed in the air aerosol samples during dry periods; however, they were not present in the subsequent rainfall samples. Differences in taxonomic composition between clouds and rainfall were reported by Dillon et al.[47]. Accordingly, we concluded that differences may exist between the taxonomic composition of aerosol and rain samples. Thus, in our opinion, there is a need for future in-depth research on the physics of microalgal and cyanobacterial particles removed from the air and clouds that would explain the exact reason why some organisms are washed out faster than others.

Among the microalgae and cyanobacteria present in the air, Genitsaris et al.[2] distinguished those that have been shown to be harmful to human health once inhaled. These organisms can cause allergies, skin irritation, hay fever, rhinitis, and respiratory problems and may produce toxins. Several harmful taxa, such as *Chlorococcum* sp., *Oocystis* sp., *Anabaena* sp., *Leptolyngbya* sp., *Nodularia* sp., *Pseudanabaena* sp., *Synechococcus* sp., *Synechocystis* sp., and *Gymnodinium* sp., were observed in our study. However, on the one hand, presence in rainwater implies a successful purification process, but on the other hand, washout might result in the colonization of new regions. The origin of organisms in rainwater is related to their transport over marine waters, freshwater reservoirs, and terrestrial areas. According to Olenina[51], most of the detected microalgae and cyanobacteria in rainwater and aerosol samples are typical of those in the Baltic Sea. Among them, we distinguished *Chlorella* sp., *Coenochloris* sp., *Oocystis* sp., *Anabaena* sp., *Leptolyngbya* sp., *Nodularia* sp., *Pseudanabaena* sp., *Synechococcus* sp., *Synechocystis* sp., *Gymnodinium* sp., and *Chrysochromulina* sp. According to Guiry and Guiry[52], *Bracteacoccus* sp. and *Coccomyxa* sp. are freshwater and/or terrestrial taxa, while *Chlorococcum* sp. is a cosmopolitan taxon. *Coccomyxa* sp. has been previously found in air samples from the Baltic Sea region[29]. *Bracteacoccus* sp. and *Chlorococcum* sp. were isolated by Mikhailyuk et al.[53] from biological soil crusts of maritime sand dunes of the Baltic Sea. In many respects, the Baltic is similar to an inland lake or an estuary and is unique because there are areas where freshwater, brackish water, and marine species are all present. Hence, the cyanobacteria and microalgae that we collected at our sampling station may have different salinity preferences. Wiśniewska et al.[29] presented a detailed analysis of the salinity preferences of cyanobacteria and microalgae isolated from air samples.

Cyanobacteria and microalgae washed out from the air: a case study. Although bacteria have been well studied, research in the area of airborne cyanobacterial and microalgal washout appears to be limited. The particular difficulty of this research is that it is impossible to plan a period of rainfall in advance. As there

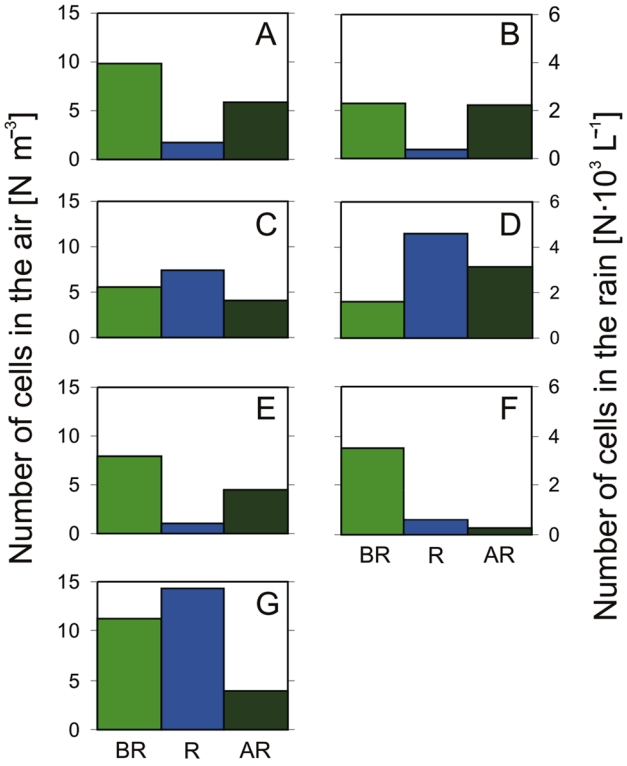

Figure 2. Number of microalgae and cyanobacteria cells in aerosol samples [cells m^{-3}] before (BR) and after rainfall (AR) and in rain samples (R) [cells L^{-1}] on the morning of August 25, 2020 (**A**), the afternoon of August 25, 2020 (**B**), on August 26, 2020 (**C**), at noon on August 27, 2020 (**D**), in the evening of August 27, 2020 (**E**), on August 28, 2020 (**F**), and from August 30 to September 1, 2020 (including 2 days of rainfall) (**G**).

was no such period during the seasonal sampling in 2019, we performed additional measurements from August 27 to September 2, 2020, when there was almost daily intermittent rainfall. Aerosol samples were collected before and after each rainfall episode, and the qualitative and quantitative compositions of cyanobacteria and microalgae were determined in both sets of samples. In the rainwater samples, the observed cyanobacteria included *Anabaena* sp., *Synechococcus* sp., *Leptolyngbya* sp., and *Nodularia* sp., while the observed green algae included *Ankistrodesmus* sp., *Oocystis* sp., and *Stichococcus* sp. In the aerosol samples, the representative cyanobacteria were *Nodularia* sp. and *Synechococcus* sp., while the observed green algae included *Ankistrodesmus* sp., *Chlorella* sp., *Chlorococcum* sp., *Oocystis* sp., and *Stichococcus* sp. *Gymnodimium* sp. (Miozoa) and *Chrysochromulina* sp. (Haptophyta) were also observed in the aerosol samples. In the rain samples, 400–5000 cells L^{-1} were recorded during this period, whereas only 0.6–11.2 cells m^{-3} were measured in the aerosol samples (i.e., three orders of magnitude lower). The number of cyanobacteria and microalgae cells in the aerosols was comparable to that reported by Tormo et al.[54] for samples collected in southwest Spain (0.18–3.85 cells m^{-3}). The authors also found that the daily concentrations of microalgae and cyanobacteria in their air samples were positively correlated with temperature and wind speed and negatively correlated with rainfall and relative humidity.

The present research primarily aims to determine whether the presence of rainfall, as well as the number of microalgae and cyanobacteria cells recorded in it, influenced the number of cyanobacteria and microalgae cells in the air (Fig. 2). The results showed that the number of cyanobacteria and microalgae cells in the aerosol samples decreased by 21–87% after each rainfall event (relative to that prior to rainfall). The only exception was on August 27, when the number of microalgae cells increased significantly in the aerosol samples despite previous rainfall (Fig. 2D). On this day, sea air masses from the central Baltic Sea were transported over the measurement station (Fig. S1). The influx of air masses above the sea surface could have been associated with an increase in the microalgae and cyanobacteria taxa in the aerosol samples[6]. With the exception of this case, the largest decrease was 87% on August 29 (Fig. 2F), when the air mass trajectory after the period of rainfall changed from the north (carrying sea air masses) to the south (carrying inland air masses). A significant decrease (64%) in the number of microalgae and cyanobacteria cells in the aerosol samples was also observed after a period of rainfall lasting more than a day (Fig. 2G). This study is the first to discuss the effectiveness of the washing out of cyanobacteria and microalgae from the atmosphere with rain. It would be interesting to conduct similar types of research in other regions of the world, where the presence of cyanobacteria and microalgae, especially those that are harmful to human health, has also been demonstrated.

To date, the results obtained in this study can be compared only to the washing out of bacteria from the atmosphere. Research on washout conducted by Ouyang et al.[55] showed that rainfall could remove up to 40% of bacteria from the atmosphere. However, we are not aware of any data in the literature regarding the washout

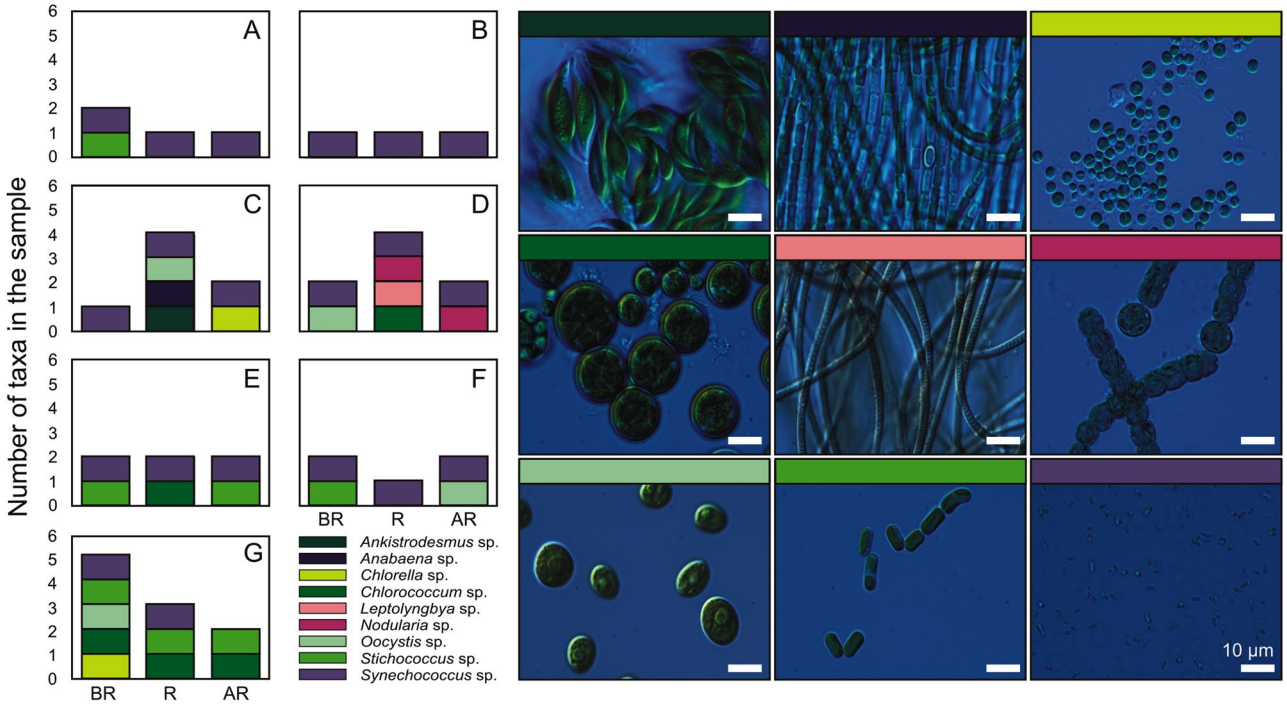

Figure 3. Number of microalgae and cyanobacteria cells in air samples before (BR) and after rainfall (AR) and in rainwater samples (R) on the morning of August 25, 2020 (**A**), the afternoon of August 25, 2020 (**B**), on August 26, 2020 (**C**), at noon on August 27, 2020 (**D**), in the evening on August 27, 2020 (**E**), on August 28, 2020 (**F**), from August 30 to September 1, 2020 (including 2 d of rainfall) (**G**) (left panel), and examples of microalgae and cyanobacteria collected from dry and wet deposition samples (right panel).

efficiency of microalgae from the atmosphere. It should be noted that the number of microalgae and cyanobacteria cells present in rainwater does not necessarily mean that the cyanobacteria and microalgae that were in the air before the rainfall event were effectively removed. There was a case where a significant number of microalgae cells was found in a rainfall sample, but no decrease in the microalgae content of the aerosol sample was observed (Fig. 2D). This result may have been due to the continuous supply of cyanobacteria and microalgae from the sea, especially during strong phytoplankton blooms.

Dillon et al.[47] found that cyanobacteria and microalgae were also present in clouds; thus, the microorganisms present in rainwater not only came from the aerosols present in the surrounding air but also could be washed out from clouds. Therefore, the taxonomic composition of rainwater and clouds[47] may differ from that of aerosols. Most of the research on the washout of particles in air with rain has focused on bacteria. Joung et al.[56] found that the amount of bacteria in the air after rainfall may significantly change. As a result of raindrops colliding with a substrate, bioaerosols can be re-emitted from the substrate to the air[56]. In the present study, an analysis of the taxonomic composition before and after periods of rainfall was also performed. Only on one occasion did the composition of the rainwater sample fully reflect the composition of the aerosol sample taken before the rainfall event, when *Synechococcus* sp. was observed in both samples (Fig. 3). There were no cases of a specific taxon being completely removed from the air by the rainfall event; however, for rainfall events lasting more than 24 h, *Synechococcus* sp. was completely washed out with the rain. This could have been related to the almost daily change in the direction of the air mass trajectory, whereby other taxa of microorganisms may have been supplied from slightly different source regions. Other studies have confirmed that the presence of new microalgae in a sample can be associated with a change in the air mass flowing over the measurement station[6,31].

An interesting case was recorded after the rainfall event on August 27 (Fig. 3D), when the highest number of algae cells and the highest number of taxa were recorded in the rainwater sample. It is particularly interesting that *Nodularia* cf. *harveyana* was found in the rainwater sample because it was not observed in the aerosol samples before the rain, but it was found in the aerosol sample after the rainfall event. This result may suggest that, as in the case of bacteria, the re-emission of previously deposited particles could occur during intense rainfall[57]. Joung et al.[56] found that when raindrops collided with soil, 0.01% of the total bacteria were emitted back into the air. Therefore, in the case of an increase in the amount of cyanobacteria and microalgae in the air, the re-emission of particles from the soil after rain should also be taken into consideration. However, this topic requires further detailed investigation. Additionally, after the rainfall event on August 27, two different species of *Nodularia* were recorded, as shown in Fig. 3D.

This research on washing out cyanobacteria and microalgae from the atmosphere by rain is pioneering and, therefore, definitely needs to be continued. We hope that our measurements will significantly influence the development of research on these organisms. In addition, it seems to be necessary to more extensively investigate the presence of cyanobacteria and microalgae in rain in different parts of the world. It would be advisable to learn more about the spatial variability and temporal variability of cyanobacteria and microalgae in rain. Our

measurements were conducted for a relatively long time but only at one station. We would recommend further research on airborne cyanobacteria and microalgae regarding how they are washed out from the air at different kinds of research stations and at varying distances from the sea, both during the growing and the nonvegetative seasons. Information on airborne cyanobacteria, microalgae, and bacteria is summarized in Table S4.

Conclusions

The results presented in the publication for the first time demonstrate the numbers of cyanobacteria and microalgae in rain. The number of cyanobacteria and microalgae cells in rainwater samples ranged from 100 cells L^{-1} to 342×10^3 cells L^{-1}. The taxonomic diversity as well as the numbers of airborne cyanobacteria and microalgae during changing meteorological conditions were thoroughly analyzed. The greatest diversity of airborne microalgae and cyanobacteria was recorded in July 2019, despite this being the period with the lowest number of cells in the rainwater samples. The highest number of cells for airborne microalgae and cyanobacteria corresponded to the highest concentration of phytoplankton in seawater, especially with respect to blue green algae. Thus, research conducted in the South Baltic Sea region confirmed the relationship between the occurrence of cyanobacteria and microalgae in the air and biochemical processes in the sea. Moreover, days of intensive rainfall favored the washing out of airborne microalgae and cyanobacteria. However, even a short dry period was sufficient to increase the number of cells again. Organisms were washed out of the atmosphere efficiently. The number of microalgae and cyanobacteria cells in aerosol samples decreased by up to 87% after a rainfall event with respect to that before the rainfall event. Rainfall had no significant effect on the taxonomic composition of cyanobacteria and microalgae, except when rainfall lasted more than 24 h. It is recommended that future research focus on developing methods to count cyanobacterial and microalgae particles in rain as well as in the atmosphere. In addition, it is particularly important to expand the research area on cyanobacteria and microalgae in rain. Increasing the emphasis to understanding the spatial variability and temporal variability of microalgae and cyanobacteria in rain and air, respectively, can also be crucial or fulfill existing gaps in the area of bioaerosol research.

Methods

Sampling location. Samples of airborne microalgae and cyanobacteria were collected at an observation station (20 m above sea level) on the roof of the Institute of Oceanography building in Gdynia (54° 31′ N, 18° 48′ E). The height of the building enables measurements to be taken from above the levels of neighboring tree canopies and buildings. The station is situated approximately 1 km from the Gulf of Gdansk coastal zone but is still in the city center and has been previously used for sampling bioaerosols, particulate matter, and rainfall[6,22,25,26].

Sample collection. In this study, two measurement campaigns were conducted during the period of highest primary production (PP) in the Baltic Sea. The first campaign was from May to September 2019, when rainwater samples were collected during periods of rainfall and air samples were collected. The second 1-week measuring campaign was from August 27 to September 2, when rainfall occurred almost every day, and aerosol samples were always collected before and after each rainfall event. In total, 20 rainwater samples and 11 samples of cyanobacteria and microalgae in aerosols were collected. The exposure time of the sample ranged from 30 min to 48 h depending on the rainfall duration. The collector was retracted as much as possible when it stopped raining.

The bulk rainfall collector consisted of a 1 dm^3 polyethylene bottle with a small vent and a Teflon funnel with an area of 0.314 m^2 for collecting rainfall. The bottle was tightly joined with the funnel and sealed by a Teflon ring. Before sampling, each bottle was treated with 1.0 M hydrochloric acid for 24 h and then rinsed three times with distilled and deionized water before being dried.

Prior to collecting the aerosol samples, a sterile mineral f/2 culture medium was prepared[30] and calibrated using seawater with a salinity of 8 PSU. A combination of the methods used by Lewandowska et al.[6] and Wiśniewska et al.[31] was applied to collect bioaerosol samples. The samples in the liquid medium were placed in a biological impactor (Tisch Environmental, Inc.) consisting of six cascades that allowed particles of various diameters to be collected depending on the impactor cascade (1) > 7 µm; (2) 4.7–7 µm; (3) 3.3–4.7 µm; (4) 2.1–3.3 µm; (5) 1.1–2.1 µm; (6) < 1.1 µm). The impactor containing Petri dishes with liquid f/2 medium (6 mL) was exposed for between 30 min and 6 h depending on the rainfall duration. Samples were taken during the day and night. The sampler air flow was 28.3 L min^{-1}.

Sample preservation until analysis. To cultivate the microalgae and cyanobacteria present in the rainwater samples, the components of the f/2 medium were added to 20 mL of rainwater in at least one repetition depending on the sample volume. The rainwater and bioaerosol samples were grown for 30 d under a constant temperature of 20 °C on a 16:8 h light:dark cycle at 10 µmol photons m^{-2} s^{-1}. The intensity of photosynthetically active radiation (PAR) was measured using a quantum meter (LI-189, LI-COR Inc., Nebraska, USA) with a cosine collector.

Identification of taxonomic composition and number of identified taxa in the collected material. The taxonomic composition and number of identified taxa were determined using a light microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) equipped with a camera (Nikon DSU2, Plan Apo VC 100 objective; magnification of × 1000). In addition, to verify the studied material, an epifluorescence microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) with UV-2A, B-2A, and G-2A block filters was used. The latter proves the chlorophyll *a* content in the identified taxa and thus the ability to conduct photosynthesis processes. This fluorescence is also widely used in plant physiology as an indicator of the condition of chloroplasts and algal cells[32,33].

Date	Air temp [°C]	Relative humidity [%]	Wind velocity [m s^{-1}]	Atmospheric pressure [hPa]	Amount of precipitation [mm]
07.07.2019	16.52	64.23	1.67	1004.15	5.97
08.07.2019	16.15	80.13	1.92	1007.20	21.85
09.07.2019	16.31	79.99	1.55	1008.65	3.01
16.07.2019	22.70	67.21	1.12	1010.38	6.33
20.07.2019	18.78	68.79	1.15	1010.04	2.18
23.07.2019	20.55	77.92	0.89	1013.14	0.13
28.07.2019	22.10	75.20	2.11	1007.99	3.44
04.08.2019	19.17	66.38	0.52	1012.05	1.32
05.08.2019	19.10	77.81	1.30	1013.20	7.80
07.08.2019	20.72	70.41	1.93	1012.69	14.91
29.08.2019	22.39	68.45	1.27	1012.81	4.58
01.09.2019	24.67	56.85	1.33	1016.75	6.70
09.09.2019	22.77	81.44	1.86	1018.85	5.71
25.08.2020	16.31	72.39	1.72	1009.14	5.29
26.08.2020	16.70	73.15	1.68	1001.29	4.66
27.08.2020	17.27	75.15	2.28	1003.25	13.35
28.08.2020	16.60	67.78	1.48	1007.66	2.37
29.08.2020	17.84	75.15	2.28	1003.25	–
30.08.2020	17.89	73.20	0.95	1007.98	–
31.08.2020	17.46	72.99	2.14	1010.48	3.16
01.09.2020	15.36	63.22	2.05	1016.49	5.91
02.09.2020	16.13	78.17	2.52	1012.76	–
03.09.2020	15.34	78.49	1.35	1013.20	–

Table 1. The average of the meteorological parameters during the sampling days and the summed amount of precipitation. – means no rain event.

The analyzed material was collected from Petri dishes and later transferred (in triplicate) into 5-mL plastic tubes. It was then checked under a light and epifluorescence microscope. Phytoplankton organisms were identified at the species level or, if this was impossible, at the genus level. Taxa were identified using keys and relevant literature[34–37]. A 20-mL sample was used to determine the number of microorganism cells in each rainwater sample. The number of cyanobacterial and microalgal cells (N) in bioaerosols was counted by a flow cytometer (BD Accuri™ C6 Plus; BD Biosciences, San Jose, California, USA). Detectors FL1, FL2, and FL3 read the fluorescence emissions excited by the blue laser (480 nm), while detector FL4 read the emissions excited by the red laser (640 nm)[38]. In the bioaerosol samples, the populations of cyanobacteria and microalgae were examined using flow cytometry and an epifluorescence microscope. In the case of filamentous cyanobacteria, the individual cells in the filaments were counted separately according to the method proposed by Śliwińska-Wilczewska et al.[39].

Meteorological data and other parameters. Meteorological data supplied by ARMAAG (https://armaag.gda.pl/) were used to supplement the results (Table 1). Additionally, 48 h backward trajectories were determined using the Hybrid Single-Particle Lagrangian Integrated Trajectory (HYSPLIT)[40,41] model to approximate the air mass source (Fig. S1). The results were also supplemented with chemical analysis data (e.g., NO_3^{2-} and PO_4^{3-}) of the rainwater and seawater samples[39] (Table S1). The ecohydrodynamic model http://model.ocean.univ.gda.pl) was used to estimate data for the blue green algae biomass and total primary production in the Baltic Sea (Table S2).

Statistical analysis. Spearman correlation coefficients were calculated between the number of microalgae and cyanobacteria cells in the rainwater samples (cells L^{-1}) and the daily rainfall amount (mm), mean temperature (°C), relative humidity (%), atmospheric pressure (hPa), wind speed (m s^{-1}), NO_3^- concentration in seawater (mg m^{-3}), PO_4^{3-} concentration in seawater (mg m^{-3}), blue green algae biomass in the Baltic Sea (mg m^{-3}), and primary production (mg m^{-3}) in the Baltic Sea (Table S3). Asterisks are used to indicate a significant difference compared with the control as follows: $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Data availability

All data generated or analyzed during this study are included in this article (and its Supplementary Information files).

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Author contributions

K.W. participated in field sample collection and laboratory analysis of the airborne cyanobacteria and microalgae. S.Ś.-W. assisted in the field sampling and data collection. K.W. wrote the main manuscript text and S.Ś.-W. prepared Figs. 1–3. A.L. proposed this study and contributed to major revisions of the manuscript. All authors contributed to writing the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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AUTHORS CONTRIBUTION STATEMENT

We hereby confirm that the specific contribution to the publication:

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were as follows:

Wiśniewska Kinga Areta – 60%:
Preparing the concept of work, developing methods, acquiring funds, collecting samples, gathering, and analyzing literature data, conducting laboratory research, analyzing results, graphically and statistically processing data, preparing the manuscript.

Śliwińska-Wilczewska Sylwia –20%:
Preparing the concept of work, developing methods, supervising, graphic revision of results, proofreading the manuscript.

Lewandowska Anita –20%:
Preparing the concept of work, developing methods, supervising, preparing the manuscript. revision the manuscript.

…… ……

Wiśniewska Kinga Areta Śliwińska-Wilczewska Sylwia

……

Lewandowska Anita

1.1 PUBLICATION IV

9.4 PUBLIKACJA IV

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Article

The Ability of Airborne Microalgae and Cyanobacteria to Survive and Transfer the Carcinogenic Benzo(a)pyrene in Coastal Regions

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Abstract: Air pollution has been a significant problem threatening human health for years. One commonly reported air pollutant is benzo(a)pyrene, a dangerous compound with carcinogenic properties. Values which exceed normative values for benzo(a)pyrene concentration in the air are often noted in many regions of the world. Studies on the worldwide spread of COVID-19 since 2020, as well as avian flu, measles, and SARS, have proven that viruses and bacteria are more dangerous to human health when they occur in polluted air. Regarding cyanobacteria and microalgae, little is known about their relationship with benzo(a)pyrene. The question is whether these microorganisms can pose a threat when present in poor quality air. We initially assessed whether cyanobacteria and microalgae isolated from the atmosphere are sensitive to changes in PAH concentrations and whether they can accumulate or degrade PAHs. The presence of B(a)P has significantly affected both the quantity of cyanobacteria and microalgae cells as well as their chlorophyll *a* (chl *a*) content and their ability to fluorescence. For many cyanobacteria and microalgae, an increase in cell numbers was observed after the addition of B(a)P. Therefore, even slight air pollution with benzo(a)pyrene is likely to facilitate the growth of airborne cyanobacteria and microalgae. The results provided an assessment of the organisms that are most susceptible to cellular stress following exposure to benzo(a)pyrene, as well as the potential consequences for the environment. Additionally, the results indicated that green algae have the greatest potential for degrading PAHs, making their use a promising bioremediation approach. *Kirchneriella* sp. demonstrated the highest average degradation of B(a)P, with the above-mentioned research indicating it can even degrade up to 80% of B(a)P. The other studied green algae exhibited a lower, yet still significant, B(a)P degradation rate exceeding 50% when compared to cyanobacteria and diatoms.

Keywords: bioaerosols; airborne cyanobacteria; airborne microalgae; benzo(a)pyrene; PAHs

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1. Introduction

Nowadays, air quality is one of the most critical issues concerning human health. The reduction of air pollution, caused by both chemical substances and microbial particles, represents a significant challenge today. Dealing with smog and viruses has become a particularly important issue in recent years, both in Europe and worldwide. A plethora of the literature indicates that in addition to chemical pollutants, bioaerosols, which encompass bacteria, viruses, fungi, pollen, cyanobacteria, and microalgae, negatively affect public health [1–4]. Studies on COVID-19's spread since 2020, as well as previous reports on avian flu, measles, and SARS, have further demonstrated that viruses and bacteria pose a more significant threat to human health when present in polluted air [5–11].

The question arises whether cyanobacteria and microalgae can also pose a threat when present in poor-quality air. Compared to other bioaerosols, they are still insufficiently understood. In our present research, we focused on cyanobacteria and microalgae present in the air of an urbanized coastal zone. Cyanobacteria and microalgae were present in the air throughout the year, and their average abundance was similar to that of pollen in the atmosphere [12]. Among this group of bioaerosols, representatives of cyanobacteria were most frequently found in the atmosphere, and numerous green algae were also detected. Additionally, the presence of species belonging to Bacillariophyta, Charophyta, Haptophyta, Miozoa, Rhodophyta, and Ochrophyta phyla was observed [12]. Other researchers have also highlighted the abundance of cyanobacteria and microalgae in the atmosphere [13–20], which is extensively described in the work of Wiśniewska and colleagues [3].

In an urbanized coastal zone, cyanobacteria and microalgae coexist with chemical pollutants, including polycyclic aromatic hydrocarbons (PAHs), which are known to be human carcinogens and mutagens and toxic to other living organisms [21].

PAHs mainly originate from anthropogenic sources, including domestic, mobile, industrial, and agricultural activities [22]. The major sources of PAH emissions are coal and wood burning, petrol and diesel oil combustion, and industrial processes [22–24]. The European Community and the U.S. Environmental Protection Agency have identified PAHs as priority pollutants due to their negative impact on human health. Exposure to PAHs can lead to respiratory problems, such as impaired pulmonary function and bronchitis [25]. However, the health effects of individual PAHs vary depending on the structure of the molecule and the number of aromatic rings [22].

The most well-known PAH is benzo(a)pyrene (B(a)P), which is listed as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) [26]. B(a)P serves as a major indicator of PAH pollution, and its concentration in the air is regulated by the World Health Organization (WHO) and air quality agencies in Europe, North America, China, and several other countries [22,26]. The acceptable annual concentration for B(a)P in PM10 in European countries is 1 ng m^{-3} (Directive 2004/107/WE); however, this value is significantly exceeded, and the highest values are noted in the winter season [3].

It has been well established that microorganisms can transform polycyclic aromatic hydrocarbons (PAHs) [27,28]. According to Ghosal et al. [27], PAHs can be neutralized through various processes such as adsorption, volatilization, photolysis, and chemical oxidation. Among these methods, biotransformation by microorganisms is considered the most environmentally friendly approach to neutralizing high concentrations of PAHs in the atmosphere. Although the role of cyanobacteria and microalgae in PAH transformation is less understood compared to that of bacteria and fungi, recent scientific studies suggest that algae may play a crucial role in the degradation of PAHs. Warshawsky and his co-authors [29] demonstrated that the rate of degradation depends on several factors, including the amount of light energy emitted and absorbed, the number of PAHs exposed to the algae, the phototoxicity of PAHs and their metabolites, as well as the species and strain of algae. A review by Alegbeleye et al. [28] highlights the ability of different algae to transform various PAHs. The literature reports demonstrate that certain species of algae are capable of transforming PAHs such as naphthalene, phenanthrene, acenaphthene, pyrene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenzo(a, h)anthracene, indeno(1, 2, 3-c, d)pyrene, benzo(g, h, i)perylene, and benzo(a)pyrene [29–36]. A review also notes that even the highly toxic B(a)P can be transformed by marine algae into diols and quinones within 5–6 days [28]. However, it is important to consider whether the newly formed compounds are also toxic. According to Warshawsky et al. [29], several quinones, such as menadione, danthron, phenanthrene-quinone, and hydroquinone, were found to be highly phototoxic, whereas others, such as menadione, danthron, phenanthrene-quinone, and hydroquinone, were not.

The objective of this study was to investigate the interaction between airborne cyanobacteria and microalgae with polycyclic aromatic hydrocarbons (PAHs) using benzo(a)pyrene (B(a)P) as a model compound. In addition, we aimed to determine whether B(a)P has any

effect on the life functions of cyanobacteria and microalgae and to identify the conditions that are conducive to such interactions. As this is the first scientific article devoted to the relationship between B(a)P and microalgae and cyanobacteria present in the air, we also suggest further necessary research directions.

2. Materials and Methods

2.1. Criteria for Selecting Experimental Organisms from Airborne Microalgae and Cyanobacteria

Microalgae and cyanobacteria were collected from a research station located 1 km from the coastal zone of the Gulf of Gdańsk in Gdynia, a region in the southern Baltic Sea, between 2018 and 2020 [12,19,37]. During 2018 and 2019, sampling was conducted during selected seasons, whereas in 2020, samples were collected throughout the year. The cyanobacteria and microalgae that were cultured, identified, and isolated for monocultures have been included in the Culture Collection of Baltic Algae (Airborne Algae—AA) at the Institute of Oceanography, University of Gdańsk, Poland [12,38]. A comprehensive list of the species isolated during the study period can be found in Wiśniewska et al. [38]. It is worth noting that not every detected taxon may have been isolated into a monoculture.

A study conducted in 2020 [12] revealed that cyanobacteria accounted for over 60% of all strains detected in the air, and Chlorophyta represented approximately 34% of them. The experiments were conducted on airborne cyanobacterial strains: *Nostoc* sp., (CCAA 03), *Synechococcus* sp., (CCAA 14), *Aphanothece* sp. (CCAA 48); green algae strains: *Oocystis* sp. (CCAA 20), *Kirchneriella* sp. (CCAA 38), *Coccomyxa* sp. (CCAA 21); and diatom strains: *Amphora* sp. (CCAA 34), *Halamphora* sp. (CCAA 47); *Nitzschia* sp. (CCAA 17). We utilized organisms from identical strains for our previous study, which investigated the influence of abiotic factors on the abundance and photosynthetic performance of these organisms [38]. The criteria for choosing these organisms included their prevalence in the air over the Baltic Sea area. A global analysis of bioaerosol availability reveals that cyanobacteria, green algae, and diatoms are among the most commonly found. Additionally, the chosen taxa were the most abundant among the other isolated taxa present in the coastal region of the Polish Baltic Sea [38].

2.2. Experimental Investigation of B(a)P Effects on Cyanobacteria and Microalgae in Laboratory Cultures

For the batch cultures, we utilized 25 mL glass Erlenmeyer flasks that contained sterilized F/2 medium [39]. These strains were cultured under a 16:8 h light: dark cycle, with photosynthetically active radiation (PAR) irradiance of 100 µmol photons m^{-2} s^{-1}, and at various temperatures (10 °C, 15 °C, 20 °C, 25 °C, and 30 °C) while maintaining salinity at 8 PSU, which is representative of the average salinity in the Baltic Sea. We determined PAR levels using a Li-Cor (Lincoln, NE, USA) LI-189 model with a cosine collector, and salinity was determined using a salinometer (inoLab Cond Level 1, Weilheim in Oberbayern, Germany). The experimental setup was performed within thermostatic chambers (Biogenet, fitotron chamber, Józefów, Poland) that provided the necessary control over temperature conditions (±1 °C). The chosen temperatures were determined based on the varying temperature conditions observed within the Gdańsk Bay region. Specifically, the warm winters or early springs were favorable for the year-round presence of airborne algae, whereas the hot summers provide ideal conditions for algae blooms in the sea [12,37].

To prepare the cultures of isolated airborne cyanobacteria and microalgae for the experiments, they were first acclimatized to the new incubation conditions that corresponded to the proper culture conditions. The acclimatization period lasted for 7 days. After acclimatization, proper cultures of 20 mL volume were prepared. These cultures were in the logarithmic growth phase and contained a known number of initial cells. To prepare the cultures, a specific volume of inoculum (between 0.6 and 1.2 mL) was taken from the actively growing acclimatization culture (V = 20 mL) and added to a sterile F/2 medium. The number of initial cells in the proper culture was set at 10^5 cells in 1 mL of the medium.

In this experiment, a specific concentration of benzo[a]pyrene (B(a)P) from Sigma-Aldrich was added to all strains, with concentrations in the sample ranging from 7.8 to 624 ng mL^{-1} (7.8, 15, 78, 312, and 624 ng mL^{-1}). The test was performed with 3 replicates. These values were chosen to correspond to B(a)P concentrations in atmospheric particulate matter over the Gulf of Gdansk, ranging from 0.5 to 40 ng m^{-3} (0.5, 1, 5, 20, 40 ng m^{-3}), based on previous monitoring of B(a)P concentration in the air over Gdynia (Gulf of Gdansk, Poland) [40–44]. The selected concentrations covered a range from low values to values well above the permissible annual average value for B(a)P in PM10 in EU countries, which is 1 ng m^{-3} (Directive 2004/107/WE). The test cultures were grown in incubator for one week until they reached the exponential growth phase, at which point the cell concentration, chlorophyll *a* content, photosynthesis performance, and B(a)P concentration were measured. Additionally, numerous blank samples were included, including analysis of a clean filter, a filter exposed only to B(a)P, and a filter exposed only to cyanobacteria and microalgae without the addition of B(a)P.

2.3. Calculation of Cell Density for Cyanobacteria and Microalgae

The quantification of cell numbers was performed using two different methods. The first method, described by Śliwińska-Wilczewska et al. [45], utilized a BD Accuri C6 Plus flow cytometer (BD Biosciences, San Jose, CA, USA) and was based on the linear correlation between cell concentration (N mL^{-1}) and optical density (OD_{750}). The second method, described by Śliwińska-Wilczewska and Latała [46], involved using a light microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan) and the Bürker counting chamber to quantify filamentous cyanobacteria. Both methods allowed for the determination of correlation coefficients and linear correlations between cell number and optical density.

2.4. Determination of Chlorophyll a

The concentration of chlorophyll *a* (chl *a*) in the tested cyanobacterial and microalgal strains was quantified according to established protocols [47,48]. Briefly, after 7 days of incubation, a 5 mL culture sample was filtered using a 0.45 µm filter (Macherey-Nagel MN GF-5, Dueren, Germany) and extracted using cold 90% acetone in the dark for 2 h at −20 °C. After centrifugation at 12,000× *g* rpm for 2 min (Sigma 2-16P, Osterode am Harz, Germany) to remove cell debris and filter particles, the extinction was measured using a UV-VIS Multiskan GO spectrophotometer (Thermo Scientific, Waltham, MA, USA) at 750, 665, and 480 nm with a 1 cm glass cuvette.

2.5. Determination of the Chlorophyll a Fluorescence in Cyanobacteria and Microalgae

The measurement of chlorophyll fluorescence was conducted using a pulse amplitude modulated (PAM) fluorometer (FMS1, Hansatech, King's Lynn, United Kingdom). After 7 days of the experiment, the fluorescence parameter F_v/F_m was analyzed. The 594 nm amber modulating beam with 4-step frequency control was used to provide illumination. The analyzed material was placed in the leaf clip on the 13 mm glass fiber filter (Whatman GF/C, Saint Louis, MO, USA). Saturating pulses of 0.7 s duration with an intensity of 4500 µmol photons m^{-2} s^{-1} were used to test airborne cyanobacterial and microalgal species. All samples were dark-adapted before the measurements.

2.6. B(a)P Analysis Using High-Performance Liquid Chromatography

To summarize, after 7 days of incubation, the cultures were filtered, and the B(a)P was isolated from the samples using solvent extraction with acetonitrile: dichloromethane (3:1 *v*/*v*) in an ultrasonic bath. The concentration of B(a)P was determined using high-performance liquid chromatography (Dionex UltiMate 3000) with a fluorescence detector (benzo(a)pyrene λex. = 296 nm, λem. = 408 nm). The chromatographic separation process was performed under gradient conditions using a mobile phase (water: acetonitrile) with a Thermo Scientific HYPERSIL GOLD C18 PAH chromatographic column (250 × 4.6 mm; 5 µm).

The solvents used for analyses were produced by Merc and were of HPLC grade. The benzo(a)pirene standard produced by Sigma-Aldrich (1000 µg/mL) was used to prepare calibration curves of the following concentrations: 0.1–10 ng·cm^{-3}. The standard solutions for calibration curves were prepared in methanol. The linearity of the method was >0.999%. However, the precision, expressed as a coefficient of variation, was less than 15%. The limit of quantification of the method (LoQ) was defined as the 10-fold signal-to-noise ratio for a sample with a very low (close to the detection limit) content of B(a)P and was 0.01 ng cm^{-3}. The recovery rate was 83% when compared to the reference material (SRM-2585). This procedure was previously used for determining the presence of B(a)P in the air. Nine strains were tested under several B(a)P concentrations (7.8, 15, 78, 312, and 624 ng L^{-1}) and several temperatures (10 °C, 15 °C, 20 °C, 25 °C, and 30 °C), as well as additional blank samples.

2.7. Statistical Analyses

To investigate the impact of B(a)P concentration and temperature on the growth, chlorophyll *a*, and fluorescence of airborne cyanobacteria and microalgae, we utilized a repeated measures ANOVA. Significant differences between the control and treatment levels were determined through a post-hoc Tukey's HSD test. Our data are reported as means ± standard deviations (SD), with statistical significance denoted by * $p < 0.05$. Prior to conducting these tests, normality and homoscedasticity were verified. Python [49] was employed to perform all statistical analyses.

3. Results

3.1. Variability in the Number of Cyanobacterial and Microalgal Cells

The experimental results indicated that the number of cyanobacterial and microalgal cells increased with the rise in ambient temperature despite the addition of benzo(a)pyrene (B(a)P, (ANOVA, $p < 0.05$) (Figure S1 in Supplement). A linear regression was performed to determine the average cell count values (based on the added concentration of B(a)P). The regression coefficient was found to be 0.95 for *Kirchneriella* sp., 0.96 for *Coccomyxa* sp., and 0.85 for *Oocystis* sp. For diatoms, the coefficient was 0.80 for *Nitzchia* sp., 0.93 for *Amphora* sp., and 0.73 for *Halamphora* sp. Meanwhile, for cyanobacteria, the coefficient was 0.64 for *Nostoc* sp., 0.96 for *Synechococcus* sp., and 0.86 for *Aphanothece* sp.

The average cell quantities for individual strains are presented in Table S1. For all tested organisms, the addition of a small amount of benzo(a)pyrene (increase in concentration from 0 to 7.8 ng mL^{-1}) led to statistically significant changes in the number of cells (ANOVA, $p < 0.05$; Table S2).

Among the three tested cyanobacteria, *Nostoc* sp. showed the highest number of cells at a temperature of 30 °C: 46.05 × 10^5 cell mL^{-1} (Figure 1). The addition of small concentrations of B(a)P (7.8 ng mL^{-1}) at the highest temperature (30 °C) led to an increase in the number of cells of *Nostoc* sp., but a further increase in B(a)P concentration did not result in a linear increase in the number of cells. The minimum number of *Nostoc* sp. cells was 1.89 × 10^5 cell mL^{-1} and was recorded at a temperature of 15 °C and a B(a)P concentration of 7.8 ng mL^{-1}. For the remaining cyanobacteria, the highest cell numbers were observed at a temperature of 30 °C. The highest cell count for *Synechococcus* sp. was 1.01 × 10^5 cell mL^{-1}, which was observed at the highest temperature with a concentration of 15 ng mL^{-1} B(a)P, whereas for *Aphanothece* sp. it was 12.21 × 10^5 cell mL^{-1} at a B(a)P concentration of 7.8 ng mL^{-1}. The lowest values of 0.23 cell mL^{-1} for *Synechococcus* sp. were recorded at a B(a)P concentration of 15 ng mL^{-1} B(a)P and a temperature of 15 °C. In the case of *Aphanothece* sp., the lowest cell counts of 1.72 × 10^5 cell mL^{-1} were observed at a B(a)P concentration of 7.8 and 15 ng mL^{-1} and a temperature of 10 °C. A linear relationship between the concentration of B(a)P and the number of cyanobacteria cells was not observed in the experiment.

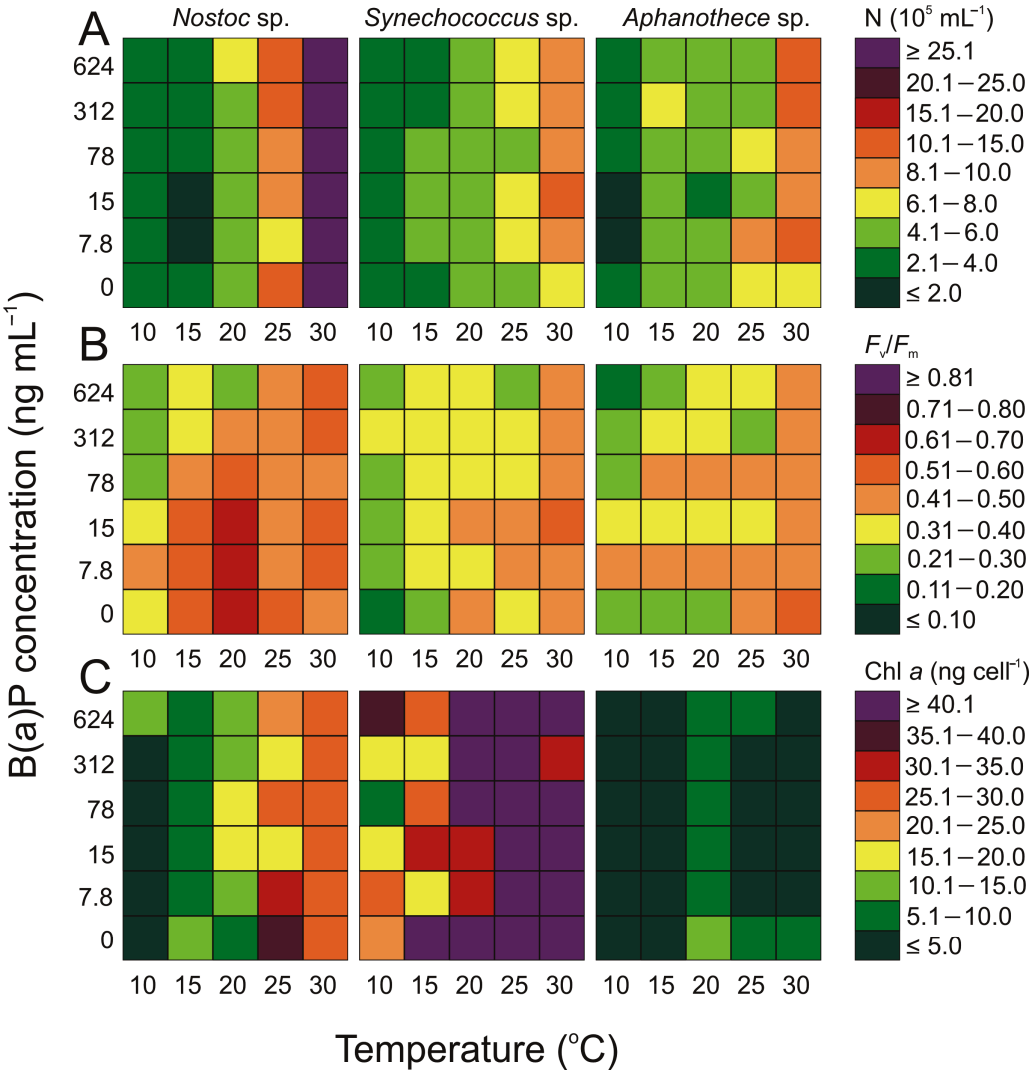

Figure 1. Changes in (**A**) the number of cells (10^5 N mL^{-1}), (**B**) maximum quantum efficiency of PSII photochemistry (F_v/F_m), and (**C**) chl *a* content (ng cell^{-1}) for airborne cyanobacteria obtained after 7 days of experiment under different B(a)P concentration (ng mL^{-1}) and temperature (°C) conditions.

In addition to the cyanobacterium of *Nostoc* sp., a high number of cells was also found for the diatoms *Amphora* sp. (37 × 10^5 cell mL^{-1}) and *Nitzschia* sp. (approximately 22 × 10^5 cell mL^{-1}), which for both of them was achieved at a temperature of 30 °C and a concentration of 15 ng mL^{-1} B(a)P. On the other hand, the highest number of cells of *Halamphora* sp., 11.37 × 10^5 cell mL^{-1}, was observed at a B(a)P concentration of 7 ng mL^{-1} and a temperature of 20 °C, whereas the lowest number of cells, 2.61 × 10^5 cell mL^{-1}, was noted at a temperature of 10 °C without the addition of benzo(a)pyrene. Under similar conditions, the lowest number of *Nitzchia* sp. and *Amphora* sp. were recorded: 1.3 × 10^5 cell mL^{-1} and 2.32 × 10^5 cell mL^{-1}, respectively. The addition of a low concentration of B(a)P (7.8 ng mL^{-1}) resulted in a slight increase in the number of cells only at temperatures below 15 °C. However, a significant decrease in the number of organisms was observed at temperatures between 15 °C and 30 °C (Tukey HSD, $p < 0.05$; Figure 2). With an increase in the concentration of B(a)P, a decrease in the number of cells of both diatom species was noted, particularly evident in the case of *Nitzschia* sp. In the case of *Amphora* sp., similarly to cyanobacteria, the addition of a low concentration of B(a)P (7.8 ng mL^{-1}) stimulated the growth of the tested organisms (ANOVA, $p < 0.05$). At the highest concentration of benzo(a)pyrene (624 ng L^{-1}), however, the number of cells ei-

ther decreased or remained at the same level as in the case of the B(a)P concentration of 312 ng L^{-1}.

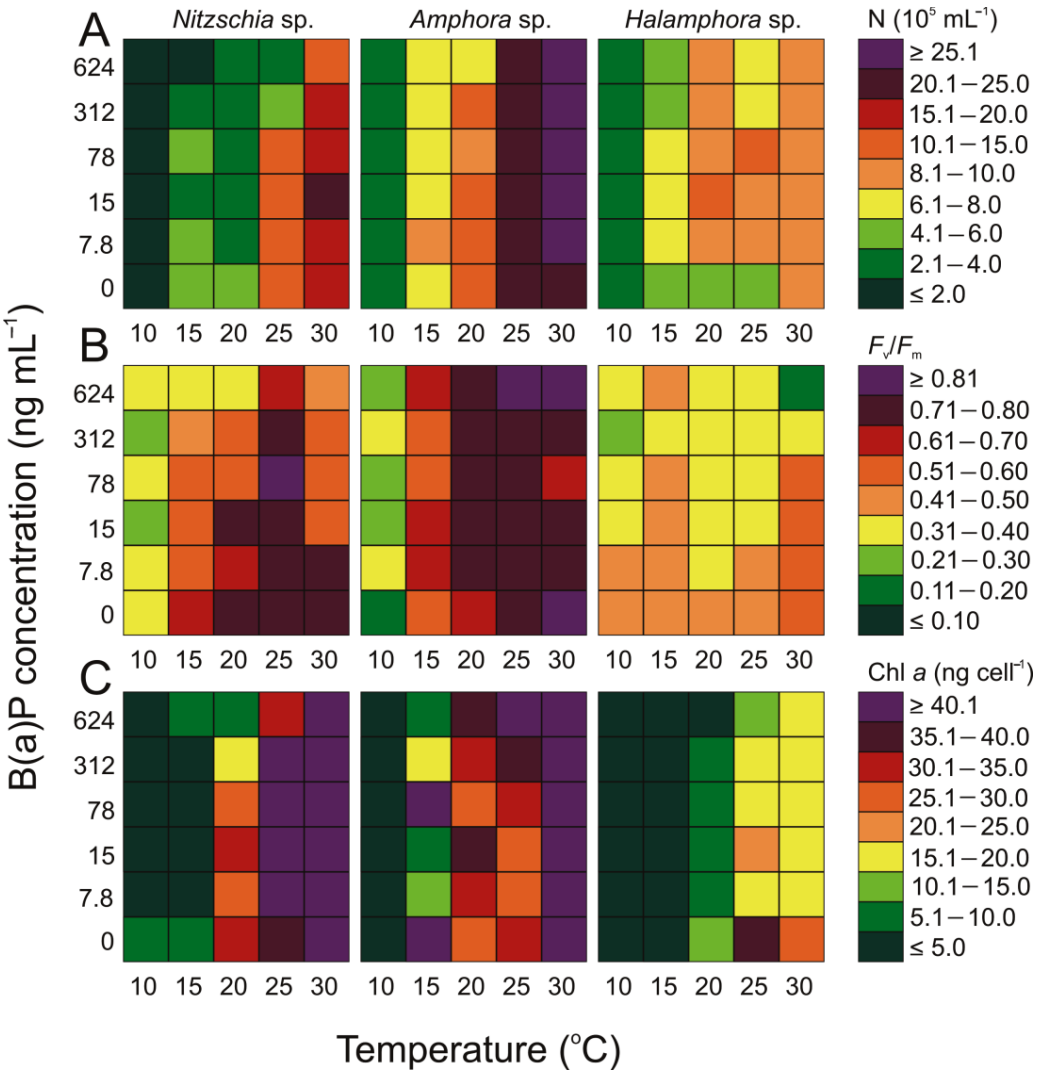

Figure 2. Changes in (**A**) the number of cells (10^5 N mL^{-1}), (**B**) maximum quantum efficiency of PSII photochemistry (F_v/F_m), and (**C**) chl *a* content (ng $cell^{-1}$) for airborne diatoms obtained after 7 days of experiment under different B(a)P concentration (ng mL^{-1}) and temperature (°C) conditions.

The green algae selected for the experiment (*Oocystis* sp., *Coccomyxa* sp., *Kirchneriella* sp.) exhibited an increase in cell numbers upon the addition of B(a)P (ANOVA, $p < 0.05$). However, temperature was again the leading factor (ANOVA, $p < 0.05$). During the present study, the highest increase in Chlorophyta cell number was recorded after the addition of B(a)P at a concentration of 78 ng mL^{-1}, which corresponds to a B(a)P concentration in the air of 5 ng m^{-3} (Figure 3). The highest number was demonstrated to be 73 × 10^5 cell mL^{-1} for *Coccomyxa* sp., 24.08 × 10^5 cell mL^{-1} for *Oocystis* sp., and 22.60 × 10^5 cell mL^{-1} for *Kirchneriella* sp. The results obtained during the present study showed that the addition of B(a)P at higher concentrations, above 78 ng mL^{-1}, resulted in a slight decrease in the cell number of green algae, especially noticeable at the highest temperature of 30 °C. On the other hand, the lowest values obtained for *Oocystis* sp. (0.4 × 10^5 cell mL^{-1}) and *Kirchneriella* sp (0.85 × 10^5 cell mL^{-1}) were achieved at a temperature of 10 °C with zero B(a)P concentration, whereas for *Coccomyxa* sp., it was at the lowest temperature but with a B(a)P concentration of 15 ng mL^{-1} (4.94 × 10^5 cell mL^{-1}).

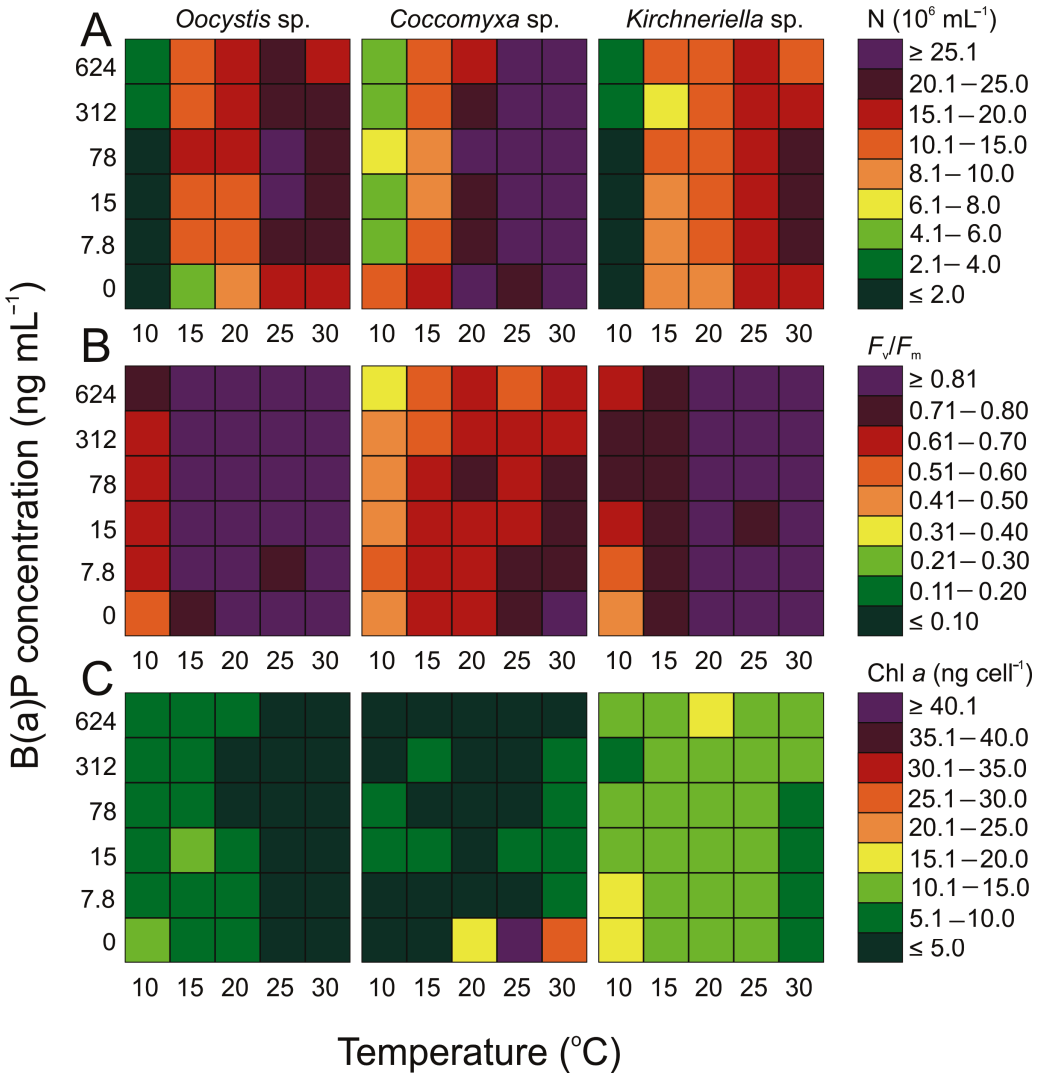

Figure 3. Changes in (**A**) the number of cells (10^5 N mL^{-1}), (**B**) maximum quantum efficiency of PSII photochemistry (F_v/F_m), and (**C**) chl *a* content (ng cell^{-1}) for airborne green algae obtained after 7 days of experiment under different B(a)P concentration (ng mL^{-1}) and temperature (°C) conditions.

3.2. Variability in Chlorophyll a Concentration

The concentration of chlorophyll *a*, recorded after a 7-day exposure to B(a)P and a control sample without B(a)P, is presented in Table S3 in the supplementary material. The significant fact is that the addition of a small amount of benzo(a)pyrene resulted in changes in the concentration of chlorophyll *a* (ANOVA, $p < 0.05$; Table S2).

Synechococcus sp. was characterized by the highest values of chlorophyll concentration among the studied cyanobacteria (with maximum 99 ng cell^{-1} at 7.8 ng mL^{-1} B(a)P and 30 °C; and minimum 5.21 ng cell^{-1} at 78 ng mL^{-1} B(a)P and 10 °C). The remaining cyanobacteria were characterized by lower concentrations of chlorophyll *a*. For *Nostoc* sp., the value of chl *a* ranged from 0.68 ng cell^{-1} (at 10 °C and no B(a)P) to 38.18 ng cell^{-1} (at 30 °C and no B(a)P), whereas for *Aphanothece* sp., the lowest value was 1.15 ng cell^{-1} (at 0 °C and 78 ng mL^{-1}) and the highest value was 13.07 ng cell^{-1} (at 20 °C and no B(a)P). Interestingly, a decrease in the content of chl *a* was observed after the addition of B(a)P for the cyanobacteria *Aphanothece* sp.

Upon analyzing the concentration of chl *a*, we found that in some diatoms (*Nitzschia* sp. and *Halamphora* sp.), the concentration of chl *a* decreased with an increase in the concentration of B(a)P (Figure 2). The maximum chl *a* concentration for *Nitzchnia* sp. was 64.01 ng cell^{-1} (at 30 °C and 7.8 ng mL^{-1} B(a)P). Similarly to the other diatoms, at the

lowest temperature, the amount of chlorophyll *a* was below the detection limit. On the other hand, for *Halamphora*, the highest value of chl *a* after adding B(a)P was 21.59 ng cell^{-1} (at 25 °C and 7.8 ng mL^{-1}), which was much lower than the blank sample for *Hamaphora* at 25 °C (37.85 ng cell^{-1}). The maximum amount of chl *a* for *Amphora* sp. was 70.47 ng cell^{-1}. It was evident that the addition of a small amount of B(a)P (from 0 ng mL^{-1} to 7.8 ng mL^{-1}) significantly reduced the chlorophyll *a* content for many diatoms, e.g., from 50.86 ng cell^{-1} to 11.02 ng cell^{-1} for *Amphora* sp. (at 15 °C).

Coccomyxa sp. had a low concentration of chl *a* and exhibited a decrease in chl *a* concentration values upon B(a)P addition, in comparison to the control samples. For example, at 25 °C the value decreased from 48.2 ng cell^{-1} to 3.95 ng cell^{-1}. Furthermore, for this microalga, the lowest temperature recorded a chlorophyll concentration below the limit of quantification, whereas the highest concentration (8.63 ng cell^{-1}) was found at the highest temperature at 78 ng mL^{-1} B(a)P. Other green algae are characterized by a higher content of chlorophyll *a* compared to *Coccomyxa* sp. after adding B(a)P. *Kirchneriella* sp. was characterized by the highest values of chl *a* concentration among the studied cyanobacteria after adding B(a)P (with maximum 16.24 ng cell^{-1} at 7.8 ng mL^{-1} B(a)P and 10 °C; and minimum 9.07 ng cell^{-1} at 15 ng mL^{-1} B(a)P and 30 °C). For *Oocystis* sp., maximum chl *a* concentration after adding B(a)P was 10.96 ng cell^{-1} at 15 °C and 15 ng mL^{-1}, which was lower than maximum chl *a* for the blank sample with *Oocystis* sp. (13.23 ng cell^{-1} at 10 °C). The minimum value was 1.84 ng cell^{-1} at 30 °C and 624 ng mL^{-1}. In the case of chl *a*, both temperature changes and also exposure to a small amount of B(a)P caused changes in the concentration parameter in the case of all strains (ANOVA, $p < 0.05$).

3.3. Variability in the Maximum Quantum Efficiency of PSII Photochemistry (F_v/F_m)

The mean values of the F_v/F_m parameter before and after the addition of a small amount of B(a)P are presented in Table S4. Both the addition of benzo(a)pyrene and temperature changes significantly affect the value of the F_v/F_m parameter (ANOVA, $p < 0.05$; Table S2).

The F_v/F_m was low for the cyanobacteria. The lowest values were obtained in samples with the highest concentration of B(a)P. The maximum F_v/F_m for *Nostoc* sp. was 0.65 at 20 °C and a B(a)P concentration of 7.8 ng mL^{-1}, which was the same as the blank sample at this temperature. The remaining cyanobacteria were characterized by a lower F_v/F_m, which for *Synechococcus* sp. at its maximum was 0.52 at 15 ng mL^{-1} B(a)P and 30 °C, and for *Aphanothece* was 0.49.

In the case of diatoms, the maximum F_v/F_m for *Nitzchia* sp. and *Amphora* sp. were similar, amounting to 0.81 at 25 °C and 78 ng mL^{-1}, and 30 °C and 624 ng mL^{-1}, respectively. For *Halamphora* sp., the maximum value was 0.58 at 30 °C and 15 ng mL^{-1}. The minimum F_v/F_m was similar to Cyanobacteria, which was 0.25, 0.22, and 0.19 for *Nitzchia* sp., *Amphora* sp., and *Halamphora* sp., respectively. The lowest value for *Nitzchia* sp. was noted at 10 °C and 312 ng mL^{-1}, for *Amphora* sp. at 10 °C and 624 ng mL^{-1}, and for *Halamphora* sp. at 30 °C and 624 ng mL^{-1}. In the case of green algae, the obtained F_v/F_m results differ significantly compared to the other studied bioaerosols.

The obtained maximum values amounted to 0.90, 0.79, and 0.89 for *Oocystis* sp., *Coccomyxa* sp., and *Kirchneriella* sp., whereas the minimum values were 0.64, 0.40, and 0.60, respectively. For the green algae, the maximum was noted at 30 °C and B(a)P 15 ng mL^{-1} for *Oocystis* sp., 7.8 ng mL^{-1} for *Coccomyxa* sp., and from 15 to 624 ng mL^{-1} for *Kirchneriella* sp. The minimum values for *Oocystis* sp. and *Kirchneriella* sp. were related at 10 °C and 7.8 ng mL^{-1}, whereas for *Coccomyxa* sp. the minimum value was found at 10 °C and 624 ng mL^{-1}. Both temperature changes and adding a small amount of B(a)P significantly affect the discussed parameter in the case of all strains (ANOVA, $p < 0.05$; Figure 3).

3.4. Variability in the Concentration of Benzo(a)pyrene after a 7-Day Exposure

The results obtained during the experiment indicate that the concentration of benzo(a)pyrene was lower after seven days of exposure for each tested alga (ANOVA,

$p < 0.05$; Figure 4, Table S5). For the purpose of this analysis, the concentrations obtained at individual temperatures were averaged. The reduction of the B(a)P concentration occurred also in the blank samples. Furthermore, the concentration of benzo(a)pyrene that remained in the samples after 7 days of exposure differed significantly between the green algae and the other groups (ANOVA, $p < 0.05$; Table S6).

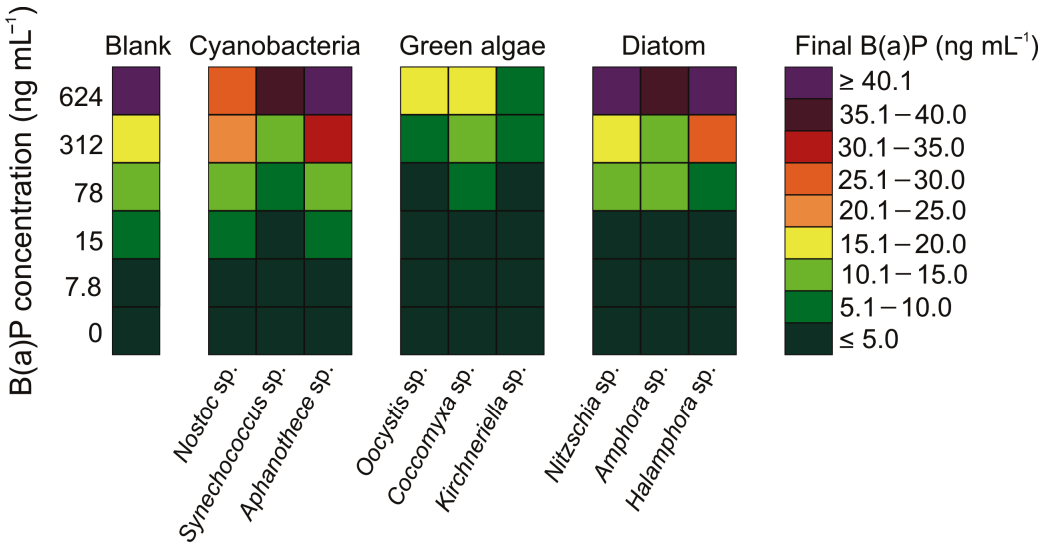

Figure 4. The final concentration of benzo(a)pyrene after 7 days of exposure for individual cyanobacteria and microalgae.

Regarding diatoms, the highest mean B(a)P concentration was recorded in *Halamphora* sp. (20.1 ng mL^{-1}), whereas the lowest mean concentration was observed in *Amphora* sp. (14.1 ng mL^{-1}). In the cyanobacteria samples, the highest B(a)P concentration was noted in *Aphanothece* sp. (23.01 ng mL^{-1}), whereas the lowest was observed in *Synechococcus* sp. (13.4 ng mL^{-1}). In contrast, the concentration of B(a)P for green algae was two to three times lower than that of the other two algae species, with values of 7.23 ng mL^{-1}, 3.3 ng mL^{-1}, and 6.2 ng mL^{-1} for *Coccomyxa* sp., *Kirchneriella* sp., and *Oocystis* sp., respectively. The averaged values divided into individual B(a)P concentrations are presented in the table (Table S5). The results obtained during our seven-day experiment allowed us to establish that the average degradation of B(a)P was the highest for *Kirchneriella* sp. (80%). Additionally, *Oocystis* sp. was found to be responsible for 63% and *Coccomyxa* sp. for 56% of the B(a)P degradation.

4. Discussion

4.1. Cyanobacteria

The experimental results indicated that the number of cells of all three tested cyanobacteria increased significantly with increasing temperature regardless of the concentration of B(a)P (Figure 1). This is mainly related to the temperature preferences of specific organisms. Therefore, when conducting an experiment, it is worth focusing particularly on the analysis of results obtained under conditions preferred by a given organism. The addition of a small concentration of B(a)P, equivalent to 0.5 ng m^{-3} in the air, significantly affects the amount of microorganism cells, but it cannot be unequivocally stated that it always leads to growth. For *Synechococcus* sp. and *Aphanothece* sp., a slight stimulation of the increase in the number of cells was observed after the addition of B(a)P, especially at higher temperatures of 25 °C and 30 °C. The temperature is not random, especially considering the cyanobacteria, with particular emphasis on those occurring in the Baltic region, which prefer high temperatures, hence the most visible effects are observed at temperatures between 25 °C and 30 °C [12,50]. However, the increase in cell number was maintained regardless of a further increase in B(a)P concentration. The results obtained suggest that in the case of these organisms,

small amounts of toxic substances can have a positive effect. On one hand, it can result in increased resistance and tolerance to stressors in certain organisms, which can be beneficial in environments with naturally occurring stressors or in areas with moderate levels of pollution. However, it can also lead to unexpected effects, such as promoting the growth of invasive species or causing the accumulation of pollutants in ecosystems.

In the case of *Nostoc* sp., the effect described above is not as pronounced as in the case of *Synechococcus* sp. and *Aphanothece* sp. The main difference between these cyanobacteria is the type of cell form. Both *Synechococcus* sp. and *Aphanothece* sp. have a microscopic size and a single-celled type, unlike filamentous *Nostoc* sp. However, the hypothesis that the cell form is the basis for differences in the occurrence of this process requires further detailed research.

Zhu and colleagues [51] have reported a similar phenomenon for *Microcystis aeruginosa*, another microscopic unicellular plankton. It appears that *M. aeruginosa* has a high tolerance to PAHs, and even low doses, this compound may have a positive effect on cell growth [51].

Moreover, in our experiment, we observed a decrease in the number of cells for each type of cyanobacteria at the lowest temperature (10 °C) after the addition of a small concentration of B(a)P (7.8 ng mL^{-1}). This could be attributed to the low number of these organisms' cells during the winter period in aerosols over the southern Baltic Sea region, when the air is known to be the most polluted with PAHs [12,43]. It also confirms the fact that the occurrence of specific cellular processes is closely related to the temperature preferences of organisms.

It is worth emphasizing that, even at concentrations as high as 624 ng mL^{-1}, there was no significant decrease in the number of cyanobacteria cells. The ability of cyanobacteria to cope with PAHs may be due to their higher growth rate at higher temperatures. As one of the earliest organisms to populate the Earth, cyanobacteria have developed various defense mechanisms to survive in unfavorable environments. Some cyanobacteria are even capable of modulating the production of toxins in response to environmental stressors [51,52].

The maximum quantum efficiency of PSII photochemistry (F_v/F_m) is a widely used parameter to describe the physiological response of cyanobacteria and microalgae. It provides information on the efficiency of the light energy absorption and conversion into chemical energy during photosynthesis. The obtained research results indicate that the addition of a small amount of benzo(a)pyrene significantly affects the change in the F_v/F_m parameter as well as the content of chlorophyll *a* in the cell, thus significantly affecting cellular stress. According to Zhang et al. [52], research on *M. aeruginosa* revealed that this organism possesses antioxidant defense enzymes such as superoxide dismutase (SOD) and catalase (CAT). The increased activity of these enzymes can provide protection against oxidative damage caused by PAHs. *M. aeruginosa* is known to produce a highly toxic compound called microcystin. However, studies have shown that this cyanobacterium can protect itself against oxidative stress caused by PAHs by stabilizing its photosynthetic apparatus and modulating protein metabolites [52–54]. As low values of F_v/F_m are often observed when photosynthetic organisms are exposed to stress, indicating photoinhibition [55], the cyanobacteria were characterized by the lowest average value of this parameter, thus representing the group most susceptible to stress.

Based on our previous research, it was found that among the selected microorganisms isolated from the air, *Synechococcus* sp. showed the highest ability to produce microcystin [12]. Future research could shed light on the concentration of toxins produced by cyanobacteria in the air, especially because an increase in temperature can lead to the growth of cyanobacterial blooms and an increase in the number of cyanobacterial cells and their toxins in aerosols. This is particularly important because even a small concentration of B(a)P in the air, such as 0.5 ng m^{-3}, could exacerbate the negative impact of airborne cyanobacteria on human health.

4.2. Ochrophyta

Among the analyzed microalgae, *Nitzschia* sp. and *Halamphora* sp. were found to be very sensitive to high concentrations of B(a)P. The growth inhibition observed in the diatom cells may suggest a lack of defense mechanisms against PAHs in these organisms at temperatures below 30 °C. Furthermore, lower concentrations of chl *a* were observed in the presence of B(a)P compared to the control sample. In the case of *Nizchia* sp. and *Halamphora* sp., the average ratio of F_v/F_m was lower in the presence of B(a)P compared to the control sample, indicating increased cellular stress. Interestingly, the diatoms found in water tend to prefer lower temperatures for growth. There are diatoms, such as *Achnanthidium* sp. and *Fragilaria* sp., that have been found to have high growth rates between 10 °C and 30 °C [52]. The diatoms used in our experiment did not exhibit a significant increase in cell numbers at low temperatures, such as 10 °C.

The literature studies suggest that PAHs may have a particularly negative effect on diatoms. For instance, in the case of *Thalassiosira pseudonana*, they can impact the metabolism of fatty acids and the formation of silica shells [56]. Othman et al. [57] have reported that the presence of PAHs can impair the formation of diatom frustules, which in turn may lead to the inhibition of cell division and a decrease in growth rates. There are also studies that confirm the negative effect of B(a)P on photosynthesis in diatoms. Exposure to B(a)P can result in the downregulation of several proteins that are involved in photosynthesis [58].

On the other hand, the stimulation of an increase in the number of *Amphora* sp. at low concentrations of B(a)P across the entire temperature range may indicate the hormetic process, which is the phenomenon of a beneficial effect of small doses of a harmful factor on organisms. In the case of *Amphora* sp., despite the decrease in chl *a* concentration in the presence of B(a)P, the average values of F_v/F_m compared to the control sample indicate that this organism slightly reduced cellular stress when in contact with B(a)P.

Diatoms are generally not commonly found in the atmosphere of the southern Baltic Sea region [3]. Their highest biomass in sea water is typically reached in the spring and winter, and their bloom typically starts at the turn of February and March [59]. At the same time, the highest concentrations of B(a)P are typically recorded in the air [40,42]. It is highly likely that the lack of defense mechanisms in diatoms against PAHs contributes to their low abundance in atmospheric aerosols. However, the reason for this can also be attributed to the larger size and weight of diatoms, resulting from their silica cell wall [3,12,59].

4.3. Chlorophyta

In general, green algae prefer to grow at temperatures between 27 °C and 32.8 °C [54]. Temperature is a factor that, on one hand, promotes organism growth. On the other hand, it may increase the toxicity of PAHs [57,60]. A study conducted by Vieira and Guilhermino [60] indicated that the toxicity of anthracene, naphthalene, and phenanthrene on *Tetraselmis chuii* increased with every 5 °C increase in temperature. In the case of green algae, regardless of the temperature, the number of cells as well as the concentration of chl *a* and F_v/F_m changed under the influence of added B(a)P. Green algae differ from the microorganisms described above in many respects. Above all, they are the only microorganisms discussed here in which cellular stress decreased in the presence of B(a)P compared to the control sample.

Green algae appear to possess highly efficient defense mechanisms, as variations have been documented even among individual strains. In the case of *Kirchneriella* sp. and *Oocystis* sp., it can be observed that the concentration of B(a)P had a positive effect on the increase in the number of cells, but there was a limiting concentration beyond which the number of cells decreased. These organisms are characterized by a very high F_v/F_m, indicating an absence of cellular stress in the presence of B(a)P. The F_v/F_m for *Kirchneriella* sp. and *Oocystis* sp. remained at a similar, constant value at temperatures above 10 °C, which suggests that these organisms were not stressed by the presence of B(a)P and were better able to tolerate PAHs even at higher concentrations (Figure 3). The difference in chl *a* concentration in the cells of these two green algae (*Kirchneriella* sp. and *Oocystis* sp.) and

Cocomyxa sp. may be a key factor in the differences in their interaction with B(a)P. However, this hypothesis requires further investigation.

The results obtained indicate that Chlorophyta are the least sensitive of the analyzed algae to PAHs. Perhaps this is the reason why they are detected in the air of the coastal zone of the southern Baltic Sea throughout the year, even in winter when the concentration of B(a)P in the air is at its highest [12]. These organisms have a higher likelihood of occurring in the air during periods of high B(a)P concentrations compared to other cyanobacteria and microalgae, as observed in the southern Baltic Sea region [12]. However, our experiment indicated that all the green algae used in the experiment showed a reduction in the number of cells under the highest concentration of B(a)P (624 ng mL^{-1}). At this point, it would be worth asking if there is a concentration limit of B(a)P that leads to this. It is worth considering whether this phenomenon is dependent on the air temperature or the amount of green algae bloom in seawater, or if there is a concentration limit of B(a)P that leads to this. Further research on this subject is advisable.

4.4. The Role of Microalgae and Cyanobacterial Cell in the Degradation of B(a)P

Reducing the amount of B(a)P in both control and other samples indicates that light, as a constant parameter in all samples, has an influence on the decomposition of the PAH itself. However, the presence of certain types of microorganisms may enhance this effect. According to Alegbeleye et al. [28], marine algae have been shown to transform B(a)P into diols and quinones, a process that can take between 5 and 6 days. However, Warshawsky et al. [29] pointed out that not all algae are able to exclude B(a)P and its phototoxic products through a physical barrier such as the cell wall, and that only certain algae have the appropriate dioxygenase enzymes to metabolize B(a)P into dihydrodiols and phototoxic products.

Scientists have indicated that green algae are capable of absorbing B(a)P and metabolizing it into dihydrodiols, whereas diatoms and cyanobacteria were unable to metabolize this PAH. Therefore, the obtained results indicate a statistically significant difference in the B(a)P content after 7 days of exposure between cyanobacteria and diatoms, as well as green algae. Figure 4 shows that the variability of B(a)P concentrations was very similar between diatoms and cyanobacteria. Thus, it was clear that green algae had the highest role in comparison to other bioaerosols. The differences between the amount of benzo(a)pyrene after a 7-day exposure between green algae and other types algae amounted to over 60%. However, the precise understanding processes that benzo(a)pyrene undergoes in the presence of cyanobacteria and diatoms require further intensive research to unequivocally confirm that these organisms do not degrade B(a)P.

The results obtained for the green algae indicate that *Kirchneriella* sp. has the highest ability to degrade benzo(a)pyrene. *Kirchneriella* sp. had the highest concentration of chlorophyll *a* and a high resistance to stress, which differed from other green algae. It should be acknowledged, however, that other green algae also have a high ability to degrade B(a)P. Green algae are abundant in chl *a*, which can absorb light energy for photosynthesis and is a major active substance that generates a high level of singlet oxygen. This singlet oxygen can catalyze the photo-transformation of B(a)P to quinones [35]. It may explain why *Kirchneriella* sp. had the highest efficiency in the removal of benzo(a)pyrene. The high degradability of B(a)P by green algae may favor their presence in the atmosphere throughout the year, including in winter when PAH emissions are at their highest. These organisms are recorded in Poland in the atmosphere also in the cold months when B(a)P concentrations in the air are exceeded. Thus, the above-described properties may favor their presence in the atmosphere. On the other hand, these studies indicate the potential use of green algae in air purification processes to remove this pollutant. A significant increase in the number of green algae cells in aerosols compared to other microorganisms may be responsible for the observed difference in B(a)P removal efficiency. Furthermore, scientific research confirms that even dead cells of green algae can effectively remove more than 90% of B(a)P [35]. In the presented studies, we did not assume the determination

of the number of dead cells. However, in difficult weather conditions, a higher mortality of microorganisms than in water could favor the removal of pollutants. However, this hypothesis requires further research. It should be emphasized, however, that in our research, we did not observe the complete degradation of B(a)P by microalgae or cyanobacteria. Such a process is possible, but it is extremely rare [61].

5. Conclusions

Due to climate change, research on cyanobacteria and microalgae present in both sea and air has become increasingly important, as they play a significant role in the environment. Cyanobacteria and microalgae isolated from the air were subjected to B(a)P concentrations at varying temperature values. The addition of this compound, which is dangerous for humans, did not result in the complete death of any of the strains. On the contrary, many cyanobacteria and microalgae showed an increase in the number of cells after the addition of even small concentrations of B(a)P. The addition of benzo(a)pyrene caused significant changes in the cell number, concentration of chlorophyll *a*, and the maximum PSII quantum efficiency. The stimulation of growth of cyanobacteria and microalgae in the presence of low concentrations of benzo(a)pyrene can be significant in the context of biotechnology development and environmental protection.

Whether the given parameter decreased or increased depended individually on the strain, not the phylum. Both an increase in cell number and low cellular stress promote high B(a)P degradation. On the other hand, the concentration of chlorophyll *a* may be a key factor responsible for the differences in degradation between strains of green algae. Green algae proved to be a group with the greatest potential to degrade B(a)P, which may be considered as a promising bioremediation path. Of particular note is the green algae *Kirchneriella* sp., which stands out for its high B(a)P degradation among the studied bioaerosols. Low cellular stress and high degradability of pollutants may favor microorganisms inhabiting the atmosphere, especially during colder periods when B(a)P concentrations are at their highest.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cells12071073/s1. Figure S1: variability of cyanobacteria and microalgae quantity with respect to sample incubation temperature in the presence of B(a)P (a) and without B(a)P (b); Table S1: the average, minimum, and maximum cell quantities for individual strains after 7 days of B(a)P addition and without B(a)P addition. Table S2: three-way factorial ANOVA of cell concentration, fluorescence, and pigment content measured in tested strains growing at different temperatures (°C) and B(a)P concentration (ng mL^{-1}) in the range of 0 to 7.8 ng mL^{-1}, df—degrees of freedom; F—Fisher's F-test statistic; Mss—mean sum of squares and; Ss—sum of squares. Levels of significance were * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Table S3: the average, minimum, and maximum chlorophyll *a* for individual strains after 7 days of B(a)P addition and without B(a)P addition. Table S4: the average, minimum, and maximum F_v/F_m for individual strains after 7 days of B(a)P addition and without B(a)P addition. Table S5: the average content of B(a)P [ng mL^{-1}] after 7 days of exposure. Table S6: Two-way factorial ANOVA of B(a)P concentration after 7 days of exposure for tested taxa (divided as group of cyanobacteria, green algae, and diatoms), df—degrees of freedom; F—Fisher's F-test statistic; Mss—mean sum of squares; and Ss—sum of squares. Levels of significance were * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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AUTHORS CONTRIBUTION STATEMENT

We hereby confirm that the specific contribution to the publication:

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were as follows:

Wiśniewska Kinga Areta – 50%:
Preparation of the research concept, development of methods, acquisition of funds, sample collection, gathering and analysis of literature data, conducting the experiment and laboratory research, sampling preparation for benzo(a)pyrene concentration measurements, analysis of results, graphical and statistical data processing, preparation of manuscript.

Lewandowska Anita – 20%:
Preparing the concept of work, developing methods, supervising the work progress, preparation of manuscript, revision the manuscript, proofreading the manuscript.

Śliwińska-Wilczewska Sylwia – 20%:
Preparing the concept of work, developing methods, supervising the work progress, graphical data processing, conducting laboratory research, statistical data analysis, revision the manuscript.

Staniszewska Marta – 5%:
Developing methods, measurement of benzo(a)pyrene concentration

Budzałek Gracjana – 5%:
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1 SUMMARY OF THE OBTAINED RESULTS

10 PODSUMOWANIE UZYSKANYCH REZULTATÓW BADAŃ

1.1 VERIFICATION OF THE FIRST HYPOTHESIS

10.1 WERYFIKCJA PIERWSZEJ HIPOTEZY

H1. Cyanobacteria and microalgae are present in the atmosphere of the coastal zone of the Gulf of Gdansk throughout the year, probably due to increase in air temperature in recent decades.

The increasing air temperature, both on a global and regional scale, particularly in the Baltic Sea region, is a scientifically documented fact (Neumann et al., 2012; Allison et al., 2015; Kahru et al., 2016; Marosz et al., 2023). Marosz and co-authors (2023) estimated that the air temperature in Poland has increased on average by 0.28℃ per decade over the last 71 years. It gives a total change in this period exceeding 2℃. The largest temperature variation has been stated for winter (0.36℃ per decade) (Marosz et al., 2023). Since 1871 a significant air temperature increase has been also noted in the Baltic Sea region (Ahola et al., 2021). The annual mean temperature trends showed an increase between 0.08°C (south of 60°N) and 0.11°C (north of 60°N) per decade and was higher than the trend of the global mean temperature (about 0.05°C per decade) for the period 1861 to 2000) (HELCOM, 2013). The increasing air temperature already brought numerous consequences for the environment. The Baltic Sea surface temperature has increased by 0.59℃ per decade (for 1990-2018) and it is expected to warm (HELCOM, 2013; Rutgersson et al., 2014; Kniebush et al., 2019; Siegel and Gerth, 2019).

The increase in air temperature and milder winters also lead to a reduction in the duration of the ice cover period and a decrease in its maximum extent. In the Baltic Sea region, this trend is estimated to have started around 1800, with a record low extent observed in 2019-2020 (EEA, 2022). The frequency of mild ice winters (having the maximum ice cover of less than 130,000 km^2) has increased from 7 years in a 30-year period (1950-1979) to 16 years during the period 1993-2022. On the other hand, the frequency of severe winters with high ice cover (at least 270,000 km^2 of ice) has decreased from six to one year during the same periods. Forecasts indicate a further decrease in sea ice extent over the Baltic Sea, and according to some scenarios, largely ice-free winters can be expected by the end of this century (Luomarantaet al., 2014; EEA, 2022). The described phenomenon can facilitate the year-round occurrence of phytoplankton in the sea. Furthermore, increased human activity over the past century has caused significant eutrophication of the Baltic Sea. Consequently, this resulted in an increase of the phytoplankton biomass in the sea and a higher frequency of cyanobacterial blooms (Łysiak-Pastuszak et al., 2004; Neumann et al., 2012, Ahola et al., 2021). An illustration of this can be observed in the Baltic Proper, where the growing season duration has doubled from approximately 110 days in 1998 to 220 days in 2013, as reported by Kahru et al. (2016).

Based on the above observations, it was assumed that the lengthening of the phytoplankton growing season in the sea may lead the presence of cyanobacteria and microalgae in the air of the Gulf of Gdansk coastal zone all year round, including the winter period. This hypothesis was positively verified (**Publication II**). During the sampling period from January to December 2020, cyanobacteria and microalgae belonging to eight phyla were detected. In general, in taken samples the presence of 29 taxa was determined. The number

of cyanobacteria and microalgae ranged from zero to 1685 cells m^{-3}. The most abundant phylum was cyanobacteria, which constituted 63% of all detected phyla, while picocyanobacteria *Synechococcus* sp. dominated. It was not surprising, like cyanobacteria are the most recorded organisms among this group of bioaerosols worldwide (**Publication I**). The research let to conclude, that the highest number and greatest taxonomic diversity of cyanobacteria and microalgae in the air occurs in the warm months of the year, from April to September, during phytoplankton blooms in the Baltic Sea. It was mainly related to an increase in temperature (in average 19.6°C) and a decrease in wind speed (in average 2.3 m s^{-1}). The highest number of taxa (15 taxa) was recorded in July. During this month also the highest number of cyanobacteria and microalgae in the air was noted, in average equal to 479 cells m^{-3} (with the maximum of 1685 cells m^{-3}) (**Publication II**). The amount of cyanobacteria and microalgae detected in the air corresponded with the findings of Reisser (2001), who observed the presence of 300-500 cells m^{-3} of microalgae and cyanobacteria on sunny summer days. The daily changes in both the quantity and taxonomic composition of analysed organisms are not as spectacular as the seasonal changes (**Publication II**). The differences in the amount of cyanobacteria and microalgae in the air during the day and night were recorded in July and August. Surprisingly, a much greater number of cyanobacteria and microalgae occurred at night in July (48% on average), conversely the opposite occurred in August (13% on average). However, the daily variability of cyanobacteria and microalgae still requires further scientific research. The direct proportional relationship between the occurrence of cyanobacteria and microalgae in the air and biochemical processes in the sea was confirmed also during the summer phytoplankton bloom season of July–September 2019 (**Publication III**). Again, the increase in the biomass of cyanobacteria and microalgae in seawater could have been related to the highest air temperature (up to 31.2°C) and the relatively low wind speeds (mean of 1.3 m s^{-1}). In addition, it was found, that the amount of cyanobacteria and microalgae in precipitation was directly proportional to the concentration of NO_3^- in the seawater.

Research conducted in the coastal zone of the Gulf of Gdansk has indicated that marine-origin bioaerosols can be present in the air even during the winter season. However, only cyanobacteria and green algae were detected, and no significant dominance of any particular group could be identified. The number of microorganisms was relatively small, particularly when compared to the period of intense phytoplankton blooms in the sea. For example, in January only 2 taxa were recorded. The results obtained in winter pointed out a decrease of airborne microalgae with increasing wind speed (mean of 5.8 m s^{-1}). It cannot be ruled out that with a further increase in air temperature and winter warming, the presence of cyanobacteria and microalgae in the air may increase in the coming decades (**Publication II**). Regional scenarios project an annual mean near surface temperature increase over the Baltic Sea of 1.4°C - 3.9°C by the end this century, compared to 1976-2005 (Gröger et al., 2021). Looking ahead, climate change predictions for the Baltic Sea indicate a trend towards warmer sea temperatures and a decrease in sea ice coverage in the future (Ahalo et al., 2021). These changes are expected to have significant impacts on increase in phytoplankton concentration in sea water. Continued eutrophication together with a longer phytoplankton growth season and higher sea surface temperature, will intensify bacterially mediated transformation of organic matter, CO_2 production, and oxygen consumption in the Baltic Sea (Wohler et al., 2009; Semenza, 2017). On the other hand, the increase in winter temperatures may lead to an increase in the attractiveness of the region for tourism, even during the winter season. Like ongoing temperature increase favour more frequent cyanobacterial blooms, human exposure to these organisms and toxins they produce may not be limited to the summer months.

1.2 VERIFICATION OF THE SECOND HYPOTHESIS

10.2 WERYFIKACJA DRUGIEJ HIPOTEZY

H2. Among the meteorological factors determining the presence of cyanobacteria and microalgae in the atmosphere of the Gulf of Gdansk coastal zone, rainfall is the most significant.

The number and taxonomic diversity of cyanobacteria and microalgae in atmospheric aerosols within the coastal zone of the Gulf of Gdansk exhibit seasonal variations and are influenced by various meteorological parameters (**Publication II**, **Publication III**). The main of them are air temperature and humidity, wind speed and direction, air masses advection, rainfall, and the number of sunshine hours (photoperiod). Depending on the prevailing weather conditions cyanobacteria and microalgae can be emitted from water reservoirs or reemitted from other surfaces to the atmosphere (Rosas et al., 1989; Sharma et al., 2006; Singh et al., 2018). The process is most effective during a period of high primary productivity (**Publication II, Publication III**). Some of meteorological factors can promote transport of these microorganisms over the land and other their removal from the air (Sharma and Singh, 2010, Lewandowska et al., 2017; **Publication I - IV**).

The results obtained in this study indicate that the presence of cyanobacteria and microalgae in the atmosphere can be stimulated by an increase in air temperature, similar to how it can lead to an increase in phytoplankton biomass in the sea (**Publication I**). A direct proportional positive relationship (Spearman rank correlation r=0.755) between the amount of airborne cyanobacteria and microalgae and air temperature has been noted in the coastal zone of the Gulf of Gdansk (**Publication II**). The relationship seems to be so strong that even influence of benzo(a)pyrene, being indicator of air pollution with polycyclic aromatic hydrocarbons (PAHs), does not disturb this process (**Publication IV**).

Wind speed is another significant meteorological factor that influences the abundance and taxonomic diversity of cyanobacteria and microalgae in the atmosphere both globally and in the region of the Gulf of Gdansk (**Publications I - IV**). Wind is necessary for drying, fragmentation, and airborne transportation of algae. In general, the role of wind should be the same for both bioaerosols and other particulate matter present in the air. Higher wind speeds affect the production of bioaerosols and facilitate their transport over longer distances (Sharma et al., 2006b; Lewandowska et al., 2017). When a waterbody is rough, it produces three types of droplets that contribute to the emission of bioaerosols: spume drops, film drops, and jet drops. It is suggested that spume drops are effectively torn off the waves when wind speeds exceed 7.0 – 11.0 m s^{-1} (Löndahl, 2014). Although, the studies conducted in the coastal zone of the Gulf of Gdansk indicated that an increase in the amount of cyanobacteria and microalgae in the atmosphere occurs rather with a decrease in wind speed (**Publication II, Publication III**). The results showed that the wind speed that is conducive to high levels of cyanobacteria and microalgae in the air is around 2.3 – 2.7 m s^{-1}. On a yearly scale, in the region of the research, such a wind speed was noted in the period of phytoplankton bloom in the Baltic Sea. During the winter period, the wind speed was higher (an average of 5.8 m s^{-1}). In the sea water a rapid increase of phytoplankton concentration during the vegetative season was noted under low wind speed (in average equal to 1.3 m s^{-1}) and after several days with high air temperature (above 30°C) (**Publication III**). Probably the lack of high waves and almost windless conditions, together with higher air temperatures favoured phytoplankton blooms in the surface sea water and promoted higher amount of cyanobacteria and microalgae in the air. It can be concluded that wind speed and air temperature are closely

related factors that play a significant role in the effective emission of microorganisms. The interplay between these two factors is crucial for the emission of microorganisms into the atmosphere.

Airborne microorganisms can subsequently incorporate into clouds before undergoing wet and/or dry deposition (Marshall and Chalmers 1997, Tesson et al., 2016; Lewandowska et al., 2017; **Publication I**). According to Marshall and Chalmers (1997), air humidity is an important meteorological parameter influencing the emission of cyanobacteria and microalgae into the atmosphere. Authors found that desiccation could increase the possibility of algae becoming airborne. Our results demonstrated that relative humidity itself did not have as significant of an impact on the presence of cyanobacteria and microalgae in the air compared to other meteorological factors **(Publication II, Publication III)**. The only relation that was found is that rainfall was less effective in washing out microalgae and cyanobacteria during periods when relative humidity was increasing during the day (**Publication III**).

Results obtained in this study indicated that among all meteorological parameters, rainfall seems to be the most important factor affecting the amount of cyanobacteria and microalgae in the air over the coastal zone of the Gulf of Gdansk (**Publication III**). The occurrence of rainfall affects the presence of those microorganisms in two ways. Firstly, rainfall can be responsible for effectively washing out cyanobacteria and microalgae from the atmosphere (**Publication III**). The findings indicated that following each rainfall event, the number of cyanobacteria and microalgae cells in aerosols was reduced by 21-87% compared to their levels before the rainfall. This amount appears to be significant, since for bacteria present in the atmosphere washout process causes only about 40% reduction (Ouyang et al., 2020). On the other hand, rainfall can promote an increase in the taxonomic diversity of algae on the sea and land surfaces. Along with rainfall, cyanobacteria and microalgae present in clouds or/and deposited on surfaces such as e.g., tree leaves may be washed out (Schlichting 1969; Dillon et al., 2020). Rainfall can also facilitate the re-emission of previously deposited cyanobacteria and microalgae back into the air (Joung et al., 2017; **Publication III**). Measurements conducted in the coastal zone of the Gulf of Gdansk indicated that the taxonomic composition of cyanobacteria and microalgae in the air can differ after rainfall compared to before rainfall. However, there were no cases of a specific taxon being completely removed from the air by short lasting rainfall event. An exception to this pattern was observed with *Synechococcus* sp., which was completely washed out during rainfall events lasting more than 24 hours. Additionally, it was found, that there may be other taxa present in the rainfall which was not present in aerosols before the rain. Such an example can be *Nodularia* cf. *harveyana* (**Paper III**). This could have been related to the almost daily change in the direction of the air mass trajectory, whereby other taxa of microorganisms may have been supplied from slightly different source regions. Other studies have confirmed that the presence of new microalgae in a sample can be associated with a change in the air mass flowing over the measurement station (Lewandowska et al., 2017; Wiśniewska et al., 2020; **Publication III**). The direction of air masses advection, in addition to wind speed, is an important meteorological factor influencing the number and taxonomic diversity of cyanobacteria and microalgae in the air over the coastal zone. This factor is responsible for transporting microorganisms over long distances (Lewandowska et al., 2017; Wiśniewska et al., 2020).

The latest scientific reports based on the example of the Baltic Sea indicate that the amount of rainfall per year is increasing. However, it should be noted that this mainly applies to the winter periods (Ahalo et al., 2021). This is related to the continuing increase in air temperature, which leads to increased evaporation of water into the atmosphere, followed by intense rainfall. The forecasts suggest that in the northern parts of the Baltic Sea region, the majority of simulations indicate a rise in summer precipitation, whereas the outlook for the intermediate and southern parts is unclear (Ahalo et al., 2021). Although the process of washing out cyanobacteria and microalgae from the atmosphere, especially after a bloom in the sea and their subsequent emission, appears to be beneficial, it is worth considering that this may contribute to increased biodiversity of cyanobacteria and microalgae in various environments.

1.3 VERIFICATION OF THE THIRD HYPOTHESIS

10.3 WERYFIKACJA TRZECIEJ HIPOTEZY

H3. Cyanobacteria and microalgae suspended in the air can pose a potential threat to human health as a source of toxins and through the transfer of benzo(a)pyrene, which is an indicator of air pollution by polycyclic aromatic hydrocarbons.

Air quality is currently recognized as one of the most significant environmental threats, with implications for human health and well-being. The presence of chemical substances in the atmosphere has been a prominent subject of study for scientists for many years. Extensive research has been devoted to understanding this topic, as reported by Manisalidis et al. (2020). The issue of air pollution caused by particulate matter of various sizes (PMx) and polycyclic aromatic hydrocarbons (PAHs) present in it, has been thoroughly described for the southern Baltic Sea region (Staniszewska et al., 2013; Gaffke et al., 2015; Witkowska et al., 2016 a and b; Lewandowska et al., 2018; Skalska et al., 2019; Wiśniewska et al., 2019b; Buch et al., 2021, i.e.). In addition to chemical pollutants, bioaerosols, which include bacteria, viruses, fungi, pollen, cyanobacteria, and microalgae, can also have adverse effects on human health (Fröhlich-Nowoisky et al., 2016; Jang et al., 2018; Habibi-Yangje et al., 2020; **Publication I**). The state of knowledge about cyanobacteria and microalgae in the Baltic Sea, as well as toxin they produce has been the subject of research for many scientists (Wasmund and Uhlig, 2003; Mazur-Marzec et al., 2008; Stoń-Egiert and Ostrowska, 2022). On the other hand, there has been limited information available regarding the presence of cyanobacteria and microalgae in the atmosphere (**Publication I**).

For reasons explained above, one of the questions addressed in this doctoral dissertation aimed to determine whether airborne cyanobacteria, microalgae, and the products of their microbiological degradation could pose a health threat to individuals residing in the coastal zone of the Gulf of Gdansk (**Publication II).** Based on numerous scientific studies Genitsaris et al. (2011) compiled a list of cyanobacteria and microalgae present in the atmosphere, which can pose a health risk to humans if inhaled. During the research on airborne cyanobacteria and microalgae conducted in the coastal zone of Gulf of Gdansk in 2020, 29 taxa were recorded (**Publication II**). Among them, several taxa were noted that can have a negative impact on human health when inhaled. These include *Amphora* sp. *Bracteacoccus* sp., *Chlorococcum* sp., *Chlorosarcinopsis* sp., *Oocystis* sp., *Stichococcus* sp., *Nodularia* sp., *Nostoc* sp., *Synechocystis* sp., *Chrysochromulina* sp., and *Gymnodinium* sp. (**Publication II**). These organisms, once they enter the human respiratory system, can cause respiratory problems, skin irritation and rashes, eye irritation and redness, headaches, nausea, dizziness, allergic reactions, asthma exacerbations and neurotoxic effects (Genitsaris et al., 2011; Hofbauer 2021; Juay et al., 2023). They can also produce toxins, what have been confirmed in studies conducted in the coastal zone of Gulf of Gdansk (**Publication II**). There is currently no scientifically confirmed data on the amount of toxins that must be inhaled by a human to cause a negative health effect. However, some researchers have confirmed that microcystins have toxic effects on organisms when inhaled even at lower doses (Sahu and Tangutur, 2014). For this reason microcystin-LR (MC-LR) was selected as the toxin serving as an indicator. Belonging to hepatotoxins, it is the best-known toxin produced by cyanobacteria. Microcystins, in addition to impairing liver function, can also promote the development of liver tumours and induce apoptosis and necrosis of hepatocytes (Rzymski, 2009). Concentrations of MC-LR obtained in described study ranged from below the detection limit to 420 fg cell^{-1} (**Publication II**). The presence of MC-LR has been found

in *Nostoc* sp., *Nostoc edaphicum*, *Pseudanabaena galeata*, *Pseudanabaena catenata*, *Leptolyngbya* sp., *Synechococcus* sp., *Gloeocapsa* sp., and *Rivularia* sp. from the CCAA collection. The highest concentration of this toxin (420 fg cell^{-1}) was noted for picocyanobacterium *Synechococcus* sp. CCAA 46 (**Publication II**). Interestingly, *Synechococcus* sp. is one of the most common photoautotrophic microorganisms on the Earth (Whitton and Potts, 2000). However, it is worth emphasizing that individual strains or different species of the same genus may produce varying amounts of toxins (**Publication II**). Usually, the risk of inhaling harmful organisms and their toxins increase during algae blooms (**Publication I**, **Publication II**). The present study indicated that the highest concentrations of microcystin LR were recorded in May 2020 (**Publication II**). On the other hand, it was found that in August 2020, during the intense bloom of cyanobacteria in the coastal zone of Gulf of Gdansk, the presence of cyanobacteria *Nodularia* sp. was noted in aerosols. It is toxin known for its harm to human health. In the region of measurements cyanobacteria toxic blooms and nodularin production usually occur in the summer season (Lehtimaki et al., 1997; Paldaviciene et al., 2009). Even the amount of microcystin LR was lower in August than in May, the presence of *Synechococcus* sp. and *Chroococcus* sp., as well as *Nodularia* sp., *Phormidium* sp., and *Pseudanabaena* sp. in the atmosphere was noted. Thus, it is recommended that people avoid spending extended periods in the coastal zone of the Baltic Sea during the intense algae bloom.

Another fundamental issue described in the thesis was the connection between the size of bioaerosols and their deposition in the human respiratory system. As with particulate matter (PMx), it can be expected that smaller bioaerosols will penetrate deeper into the human respiratory tract and will settle in the bronchial and acinar airways, leading to many illnesses (Fröhlich-Nowoisky et al., 2016; Lewandowska et al., 2017; Facciponte et al., 2018). To quantitatively analyze the airborne algae and cyanobacteria, a six-cascade impactor was used as a substitute for the respiratory tract (**Publication I**). It could collect particles of appropriate diameter in six size ranges: >7 μm, 4.7–7 μm, 3.3–4.7 μm, 2.1–3.3 μm, 1.1–2.1 μm, and ≤1.1 μm. Research conducted in the coastal zone of the Gulf of Gdansk indicated that the total number of cyanobacteria and microalgae cells was the highest among the coarse particles (>2.1 μm) and constituted 61% of all cells detected in bioaerosols (6901 cells m^{-3}). Particles of this size can be deposited in the upper respiratory tract and do not penetrate deeper than the secondary bronchi. While the lowest number was detected in the finest particles, with a diameter <1.1 μm (1100 cells m^{-3}) (**Publication II**). Also, Facciponte et al. (2018) conducted research involving volunteers, confirmed that cyanobacteria were detected at a high frequency in the upper respiratory tract (92.2%) of the participants.

In addition to the described above, an attempt was made to identify taxonomic groups potentially hazardous to human health (Genitsaris et al., 2011) in different particle diameter ranges. During the study, it was observed that among the harmful organisms present in aerosols, including *Amphora* sp. *Bracteacoccus* sp., *Chlorococcum* sp., *Chlorosarcinopsis* sp., *Oocystis* sp., *Stichococcus* sp., *Nodularia* sp., *Nostoc*sp., *Synechocystis* sp., *Chrysochromulina* sp., and *Gymnodinium* sp. approximately 30.0% were recorded in particles small enough to reach secondary bronchi (<2.1 μm). In the context of human health, it is good news, that the majority of harmful microorganisms were detected in coarser aerosols, which do not reach the deeper parts of the human respiratory system. However no statistically significant differences were found in the quantity of cyanobacteria and microalgae depending on the bioaerosol size distribution (KW test, $p > 0.05$). This implies that it is not possible to attribute individual organisms to only one size fraction. The reason for this can be that the coccoid algae vary in diameter from a few to several dozen μm, which determines the size of organisms that can pass through the impactor nozzles of a given diameter. The situation is more complex in the case of filamentous organisms, whose length and width range from a few to several μm. Therefore, an organism arranged in a shorter plane can pass through nozzles with a smaller diameter and be deposited deeper in the human respiratory tract. Therefore, in the case of significant emissions of toxic

cyanobacteria and microalgae, such as during toxic cyanobacterial blooms, it should be considered that these organisms can also get into human alveoli (**Publication II**).

Since reports on avian flu, measles, and SARS (including COVID-19), have demonstrated that viruses and bacteria pose a more significant threat to human health when present in polluted air (Cu et al., 2003; Su et al., 2019; Frontera et al., 2020; Peng et al., 2020; Yao et al., 2020, Annesi-Maesano et al., 2021; Pansini et al., 2021), it was deemed important to investigate the potential role of cyanobacteria and microalgae in such scenarios **(Publication IV**). In many regions of the world, including southern Baltic Sea, one commonly reported air pollutant is benzo(a)pyrene. It is one of the most harmful compounds with toxic, mutagenic, and carcinogenic properties (Tobiszewski and Namieśnik, 2012). The International Agency for Research on Cancer (IARC) has classified benzo(a)pyrene as a class 1 carcinogen, which means it is highly carcinogenic to humans. The acceptable average annual concentration for this compound in PM10 in EU countries is 1 ng m^{-3} (Directive 2004/107/WE). Although the level of benzo(a)pyrene pollution in the Gdynia region is one of the lowest in Poland, its daily concentration, even in aerosols of the smallest diameter, exceeds the established annual norm several times throughout the year (e.g., Staniszewska et al., 2013, Wiśniewska et al. 2019b). **Publication IV** concentrated on the interaction between cyanobacteria and microalgae with benzo(a)pyrene in the air, in the context of human health. For this purpose, laboratory experiment was conducted. Selected strains of cyanobacteria and microalgae isolated from the atmosphere were subjected to different concentrations of benzo(a)pyrene, ranging from relatively low (standard solution of 7.8 ng L^{-1} corresponding to 0.5 ng m^{-3} in the air) to very high levels (standard solution of 624 ng L^{-1} corresponding to 40 ng m^{-3} in the air). Interestingly, the addition B(a)P, which is so dangerous for humans, did not result in the complete death of any of the strains (**Publication IV**). Moreover, many cyanobacteria and microalgae after the addition of even small concentrations of B(a)P showed an increase in the number of cells as well as changes in the content of assimilatory pigments and the capacity to carry out photosynthesis. Therefore, even slight air pollution with benzo(a)pyrene is likely to facilitate the growth of airborne cyanobacteria and microalgae.

The key objective of this study was to determine whether the present benzo(a)pyrene in the air can be degraded by cyanobacteria and microalgae. It was noted that at the end of the experiment significant difference in the concentration of benzo(a)pyrene in the presence of green algae in the comparison to cyanobacteria and diatoms occurred (**Publication IV**). Whether cyanobacteria and diatoms are capable of degrading benzo(a)pyrene requires further investigation. The obtained results allowed to conclude that green algae can degrade benzo(a)pyrene. It is consistent with other scientific reports (Warshawsky et al., 1995; Alegbeleye et al., 2017). In this situation, the question arises whether the concentration of benzo(a)pyrene in the air in coastal regions such as Gdynia, is significantly lower than in other regions of Poland, due to the presence of green algae in the air? Would the lack of green algae in the air result in higher concentration of this chemical compound dangerous to human health? Certainly, further steps that require additional research include determining which components benzo(a)pyrene is decomposed into by green algae. Although the process of removing benzo(a)pyrene from the environment may seem like a positive phenomenon, there is a possibility that in the case of decomposition into peroxides, quinones, sulphur, and nitric derivatives, the obtained compounds may still be harmful to living organisms (Papageorgopoulou et al., 1999; Chetwittayachan et al., 2002). Nevertheless, airborne green algae have the potential as a promising bioremediation path (**Publication IV**).

2 CONCLUSIONS

11 WNIOSKI

The presented thesis provides extensive knowledge on the biodiversity of cyanobacteria and microalgae in the atmosphere over the coastal zone of the Gulf of Gdansk (**Publications I-IV**). It indicated the quality and quantity variations that these organisms undergo depending on synoptic situations. It also provides globally unique knowledge regarding the potential threat of airborne cyanobacteria and microalgae to human health.

The most important conclusions drawn from this work are as follows:

I. Cyanobacteria and microalgae are present in the atmosphere of the Gulf of Gdansk all year round. The increase in their number and taxonomic diversity in the air occurs during periods of intense phytoplankton blooms in the Baltic Sea. Forecasts related to upcoming temperature increase let to conclude that the significance of this bioaerosols will also increase.

II. Rain seems to be the most important meteorological factor responsible for shaping the amount and taxonomic composition of cyanobacteria and microalgae in the atmosphere. It can be responsible for the removal of over 80% of these microorganisms from the atmosphere. Although it does not selectively wash out specific microorganism taxa.

III. In the atmosphere over the coastal zone of the Gulf of Gdansk microorganisms classified as dangerous to human health as well as those which can produce toxins have been recorded. Due to the small size of particles, in which they occur (<2.1 μm of diameter), it may be possible their transport into the deepest parts of the human respiratory system. However, they constitute a definite minority (30%) in relation to cyanobacteria and microalgae occurring in coarser bioaerosols (>2.1 μm in diameter).

IV. Between analysed taxa, the green algae species demonstrated the highest potential for B(a)P degradation, thereby suggesting a promising avenue for bioremediation. At low levels of benzo(a)pyrene concentrations cyanobacteria and microalgae can have considerable implications for the advancement of biotechnology.

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5 APPENDIX

14 ZAŁĄCZNIKI

5.1 APPENDIX PUBLICATION I

Appendix 1 The list of airborne algae and cyanobacteria found in the bioaerosols studies.

Airborne algae and cyanobacteria	Location	Literature
Cyanobacteria		
Anabaena sp.	Nederland	van Overeem, 1936
	United States	Brown, 1964
	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
	India	Sharma et al., 2006
	Greece	Genitsaris et al., 2011
	Malaysia	Chu et al., 2013
	India	Sahu and Tangutur, 2014
	Poland	Lewandowska et al., 2017
Anacystis sp.	United States	Brown, 1964
	India	Sahu and Tangutur, 2014
Aphanocapsa sp.	Nederland	van Overeem, 1936
	United States	Schlichting, 1969
	India	Sharma et al., 2006;
	Poland	Lewandowska et al., 2017
Aphanothece sp.	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Poland	Lewandowska et al., 2017
Arthrospira sp.	United States	Brown, 1964;
	India	Sharma et al., 2006
Brasilonema sp.	Hawaii United States	Singh et al., 2018
Calothrix sp.	Taiwan	Chang, 1967
	Hawaii United States	Brown, 1971
	Hawaii United States	Carson and Brown, 1971
	India	Sharma et al., 2006
	Egypt	El Gamal, 2008
	Hawaii United States	Singh et al., 2018
Chamaesiphon sp.	Poland	Lewandowska et al., 2017
Chlorogloea sp.	Mexico	Rosas et al., 1989
Chroococcidiopsis sp.	Hawaii United States	Singh et al., 2018
Chroococcus sp.	Nederland	van Overeem, 1936
	United States	Brown, 1964
	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
	India	Sharma et al., 2006
	Egypt	El Gamal, 2008
	Greece	Genitsaris et al., 2011
Cyanodictyon sp.	Poland	Lewandowska et al., 2017
Cylindrospermum sp.	Hawaii United States	Brown, 1971;
	India	Sharma et al., 2006

Entophysalis sp.	Hawaii United States	Carson and Brown, 1976
Fremyella sp.	United States	Brown, 1964
Geitlerinema sp.	Greece	Genitsaris et al., 2011
Gloeocapsa sp.	Nederland	van Overeem, 1936
	United States	Brown, 1964
	Taiwan	Chang, 1967
	Hawaii United States	Brown, 1971
	India	Sharma et al., 2006
	Egypt	El Gamal, 2008
	India	Sahu and Tangutur, 2014
Gloeothece sp.	Taiwan	Chang, 1967
	India	Sharma et al., 2006;
	Poland	Lewandowska et al., 2017
Haplosiphon sp.	Hawaii United States	Brown, 1971
	India	Sharma et al., 2006
Homoeothrix sp.	Greece	Genitsaris et al., 2011
Hydrocoleum sp.	Egypt	El Gamal, 2008
Jaaginema sp.	Greece	Genitsaris et al., 2011
Leptolyngbya sp.	Malaysia	Ng et al., 2011
	Poland	Lewandowska et al., 2017
	Hawaii United States	Singh et al., 2018
Limnothrix sp.	Greece	Genitsaris et al., 2011
Lyngbya sp.	United States	Brown, 1964
	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Hawaii United States	Carson and Brown, 1976
	Hawaii United States	Brown, 1971
	United States	Lee and Eggleston, 1989
	Mexico	Rosas et al., 1989
	India	Sharma et al., 2006
	Egypt	El Gamal, 2008
	India	Sahu and Tangutur, 2014
Merismopedia sp.	United States	Brown, 1964
	India	Sharma et al., 2006
	India	Sahu and Tangutur, 2014
	Poland	Lewandowska et al., 2017
Microchaete sp.	India	Sharma et al., 2006
Microcoleus sp.	United States	Brown, 1964
	United States	Luty and Hoshaw, 1963
	Hawaii United States	Brown, 1971
	Egypt	El Gamal, 2008
Microcystis sp.	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
	India	Sharma et al., 2006
	Poland	Lewandowska et al., 2017
Mixosarcina sp.	United States	Brown, 1964
	Mexico	Rosas et al., 1989
	Egypt	El Gamal, 2008
Nodularia sp.	Egypt	El Gamal, 2008
Nostoc sp.		Brown, 1964
	United States	Luty and Hoshaw, 1963
	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
	Mexico	Rosas et al., 1989
	India	Sharma et al., 2006
	Egypt	El Gamal, 2008
	Malaysia	Ng et al., 2011

	Hawaii United States	Singh et al., 2018
Oscillatoria sp.	United States	Brown, 1964
	United States	Luty and Hoshaw, 1963
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
	Hawaii United States	Carson and Brown, 1976
	India	Sharma et al., 2006
	Greece	Genitsaris et al., 2011
	Malaysia	Chu et al., 2013
	Hawaii United States	Singh et al., 2018
Pelogloea sp.	United States	Schlichting, 1969
Phormidium sp.	Nederland	van Overeem, 1936
	United States	Brown, 1964
	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
	Mexico	Rosas et al., 1989
	India	Sharma et al., 2006
	Egypt	El Gamal, 2008
	Greece	Genitsaris et al., 2011
	Malaysia	Ng et al., 2011
	Malaysia	Chu et al., 2013
	India	Sahu and Tangutur, 2014
Planktolyngbya sp.	Greece	Genitsaris et al., 2011
Plectonema sp.	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	United States	Brown, 1971
	Mexico	Rosas et al., 1989
	India	Sharma et al., 2006
	Egypt	El Gamal, 2008
Pleurocapsa sp.	United States	Luty and Hoshaw, 1963
	Taiwan	Chang, 1967
	India	Sahu and Tangutur, 2014
Pseudanabaena sp.	Egypt	El Gamal, 2008;
	Greece	Genitsaris et al., 2011
	Malaysia	Chu et al., 2013
	Poland	Lewandowska et al., 2017
Rhabdoderma sp.	Poland	Lewandowska et al., 2017
Schizothrix sp.	United States	Brown, 1964
	United States	Lee and Eggleston, 1989
	Egypt	El Gamal, 2008
Scytonema sp.	United States	Brown, 1964
	United States	Luty and Hoshaw, 1963
	Taiwan	Chang, 1967
	United States	Brown, 1971
	India	Sharma et al., 2006
	India	Sahu and Tangutur, 2014
	Hawaii United States	Singh et al., 2018
Symploca sp.	Hawaii United States	Carson and Brown, 1976
Synechococcus sp.	United States	Brown, 1964
	India	Sharma et al., 2006
	Poland	Lewandowska et al., 2017
Synechocystis sp.	Hawaii United States	Carson and Brown, 1976
	India	Sharma et al., 2006
	Poland	Lewandowska et al., 2017
Tolupothrix sp.	United States	Brown, 1964
	Taiwan	Chang, 1967
	Hawaii United States	Brown, 1971
	India	Sharma et al., 2006

Trichodesmus sp.	Egypt	El Gamal, 2008
Woronichinia sp.	Poland	Lewandowska et al., 2017
Xenococcus sp.	Egypt	El Gamal, 2008
Chlorophyta		
Actinastrum sp.	Nederland	van Overeem, 1937
Ankistrodesmus sp.	United States	Schlichting, 1969
Asterococcus sp.	Taiwan	Chang, 1967
	United States	Schlichting, 1969
Borodinella sp.	United States	Brown, 1964
Botryokorine sp.	Mexico	Rosas et al., 1989
Bracteacoccus sp.	United States	Luty and Hoshaw, 1963
	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
Characium sp.	Hawaii United States	Carson and Brown, 1976
Chlamydomonas sp.	United States	Luty and Hoshaw, 1963
	United States	Brown, 1964
	Hawaii United States	Brown, 1971
	United States	Schlichting, 1969
Chlorasphaeropsis sp.	Hawaii United States	Brown, 1971
Chlorella sp.	Nederland	van Overeem, 1937
	United States	Luty and Hoshaw, 1963
	United States	Brown, 1964
	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
	Hawaii United States	Carson and Brown, 1976
	United States	Lee and Eggleston, 1989
	Mexico	Rosas et al., 1989
	India	Sharma et al., 2006
	Greece	Genitsaris et al., 2011
	Malaysia	Chu et al., 2013
	Poland	Lewandowska et al., 2017
Chlorococcum sp.	United States	Luty and Hoshaw, 1963
	United States	Brown, 1964
	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
	Hawaii United States	Carson and Brown, 1976
	United States	Lee and Eggleston, 1989
	Mexico	Rosas et al., 1989
	India	Sharma et al., 2006
	Malaysia	Chu et al., 2013
	India	Sahu and Tangutur, 2014
	Poland	Lewandowska et al., 2017
Chlorosarcina sp.	United States	Brown, 1964
	Hawaii United States	Brown, 1971
Chlorosarcinopsis sp.	United States	Luty and Hoshaw, 1963
	United States	Brown, 1964
	United States	Lee and Eggleston, 1989
Coccomyxa sp.	United States	Chang, 1967
Coelastrella sp.	Poland	Lewandowska et al., 2017
	Hawaii United States	Singh et al., 2018
Coelastrum sp.	United States	Brown, 1964
Coleochaete sp.	Taiwan	Chang, 1967
Cylindrocystis sp.	United States	Brown, 1964
	Hawaii United States	Brown, 1971
Dictyochloris sp.	United States	Brown, 1964

Didymocystis sp.	Greece	Genitsaris et al., 2011
Eudorina sp.	United States	Schlichting, 1969
Friedmannia sp.	United States	Brown, 1964
Gloeococcus sp.	Taiwan	Chang, 1967
Gloeocystis sp.	Taiwan	Chang, 1967
	United States	Schlichting, 1969
Hormidium sp.	Nederland	van Overeem, 1937
	United States	Luty and Hoshaw, 1963
	United States	Brown, 1964
	Taiwan	Chang, 1967
	Hawaii United States	Brown, 1971
	Hawaii United States	Carson and Brown, 1976
	Mexico	Rosas et al., 1989
	India	Sharma et al., 2006
Hormotilopsis sp.	United States	Brown, 1964
Mesotaenium sp.	Mexico	Rosas et al., 1989
Microspora sp.	United States	Schlichting, 1969
Monoraphidium sp.	Greece	Genitsaris et al., 2011
	Poland	Lewandowska et al., 2017
Mougeotia sp.	India	Sahu and Tangutur, 2014
Myrmecia sp.	Taiwan	Chang, 1967
Nannochloris sp.	United States	Brown, 1964
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
	Hawaii United States	Singh et al., 2018
Neochloris sp.	United States	Brown, 1964
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
Oedogonium sp.	United States	Schlichting, 1969
	India	Sharma et al., 2006
Oocystis sp.	United States	Luty and Hoshaw, 1963
	United States	Brown, 1964
	Taiwan	Chang, 1967
	Hawaii United States	Carson and Brown, 1976
	United States	Lee and Eggleston, 1989
Ourococcus sp.	United States	Brown, 1964
Palmella sp.	United States	Brown, 1964
Palmellococcus sp.	United States	Brown, 1964
	Taiwan	Chang, 1967
	United States	Schlichting, 1969
Pediastrum sp.	Greece	Genitsaris et al., 2011
Phacotus sp.	Hawaii United States	Carson and Brown, 1976
Planktosphaeria sp.	United States	Luty and Hoshaw, 1963
	Taiwan	Chang, 1967
Pleurastrum sp.	United States	Brown, 1964
Prasiola sp.	Nederland	van Overeem, 1936
Protococcus sp.	United States	Brown, 1964
	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Hawaii United States	Carson and Brown, 1976
Protosiphon sp.	United States	Brown, 1964
	United States	Schlichting, 1969
Pseudochlorella sp.	Greece	Genitsaris et al., 2011
Pseudococcomyxa sp.	Poland	Lewandowska et al., 2017
Pseudoulvella sp.	United States	Brown, 1964
Radiococcus sp.	United States	Brown, 1964
	Greece	Genitsaris et al., 2011
Radiosphaera sp.	United States	Brown, 1964
Rhizoclonium sp.	United States	Schlichting, 1969

Roya sp.	United States	Brown, 1964
	Taiwan	Chang, 1967
Scenedesmus sp.	United States	Brown, 1964
	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	Hawaii United States	Brown, 1971
	Mexico	Rosas et al., 1989
	India	Sharma et al., 2006
	India	Sahu and Tangutur, 2014
Selenastrum sp.	India	Sharma et al., 2006
Sphaerocystis sp.	United States	Schlichting, 1969
Spongiochloris sp.	Hawaii United States	Brown, 1964
	United States	Luty and Hoshaw, 1963
	United States	Schlichting, 1969
Stichococcus sp.	Nederland	Van Overeem, 1936
	United States	Luty and Hoshaw, 1963
	United States	Brown, 1964
	Hawaii United States	Brown, 1971
	United States	Lee and Eggleston, 1989
	India	Sharma et al., 2006;
	Poland	Lewandowska et al., 2017
Tetraedron sp.	United States	Schlichting, 1969
Tetraspora sp.	United States	Brown, 1964
Trebouxia sp.	United States	Brown, 1964
	Taiwan	Chang, 1967
	Hawaii United States	Singh et al., 2018
Trentepohlia sp.	Taiwan	Chang et al., 1965
Ulothrix sp.	United States	Brown, 1964
	United States	Schlichting, 1969
	Mexico	Rosas et al., 1989
Watanabea sp.	Hawaii United States	Singh et al., 2018
Westella sp.	United States	Brown, 1964
	United States	Luty and Hoshaw, 1963
Zygnema sp.	Hawaii United States	Brown, 1971
Bacillariophyta		
Amphora sp.	Greece	Genitsaris et al., 2011
Cocconeis sp.	United States	Lee Eggleston, 1989
Coscinodiscus sp.	United States	Schlichting, 1969
Cyclotella sp.	Greece	Genitsaris et al., 2011
Diatonema sp.	Greece	Genitsaris et al., 2011
Eunotia sp.	Greece	Genitsaris et al., 2011
Fragilaria sp.	Greece	Genitsaris et al., 2011
Gomphonema sp.	Taiwan	Chang, 1967
Grammatophora sp.	Greece	Genitsaris et al., 2011
Hantzschia sp.	United States	Brown, 1964; 1963
	United States	Luty and Hoshaw, 1963
	Taiwan	Chang, 1967
	Greece	Genitsaris et al., 2011
Leptocylindrus sp.	Greece	Genitsaris et al., 2011
Licmophora sp.	Greece	Genitsaris et al., 2011
Melosira sp.	United States	Brown, 1964
Navicula sp.	Nederland	Van Overeem, 1936
	United States	Brown, 1964
	United States	Schlichting, 1969
	United States	Lee Eggleston, 1989
	India	Sharma et al., 2006
	Greece	Genitsaris et al., 2011
	Poland	Lewandowska et al., 2017

Nitzchia sp.	Taiwan	Chang, 1967
	United States	Schlichting, 1969
	United States	Lee Eggleston, 1989
	India	Sharma et al., 2006
	Greece	Genitsaris et al., 2011
Phaeodactylum sp.	Poland	Lewandowska et al., 2017
Pinnularia sp.	India	Sharma et al., 2006
Pleurosigma sp.	Greece	Genitsaris et al., 2011
Surirella sp.	Greece	Genitsaris et al., 2011
Synedra sp.	Greece	Genitsaris et al., 2011
Tabellaria sp.	Greece	Genitsaris et al., 2011
Ochrophyta		
Botrydiopsis sp.	United States	Brown, 1964
	India	Sharma et al., 2006
Chrysocapsa sp.	United States	Schlichting, 1969
Dictyocha sp.	Greece	Genitsaris et al., 2011
Dinobryon sp.	Greece	Genitsaris et al., 2011
Heterococcus sp.	United States	Brown, 1964
	India	Sharma et al., 2006
Monallantus sp.	Hawaii United States	Carson and Brown, 1976
Monocilia sp.	United States	Brown, 1964
	Hawaii United States	Carson and Brown, 1976
Nannochloropsis sp.	Poland	Lewandowska et al., 2017
Tribonema sp.	United States	Brown, 1964
Vaucheria sp.	United States	Schlichting, 1969
Charophyta		
Closterium sp.	Greece	Genitsaris et al., 2011
	India	Sharma et al., 2006
Cosmarium sp.	Greece	Genitsaris et al., 2011
	United States	Brown, 1964
Euglenozoa		
Euglena sp.	Greece	Genitsaris et al., 2011
Miozoa		
Ceratium sp.	Greece	Genitsaris et al., 2011
Ceratium sp.	United States	Lee and Eggleston, 1989
Peridinium sp.	Greece	Genitsaris et al., 2011
Prorocentrum sp.	Greece	Genitsaris et al., 2011

Appendix 2.

Figures 2, 3, 4, 5 were made based on data provide by van Overeem, 1937; Brown et al., 1964; Cheng, 1967; Luty and Hoshaw, 1967; Schlichting et al., 1969; Brown et al., 1971; Carson and Brown, 1976; Lee and Eggleston, 1989; Rosas et al., 1989; Broady, 1996; Sharma et al., 2006; El Gamal 2008; Genitsaris et al., 2011; Ng et al., 2011; Chu et al., 2013; Sahu and Tangutur, 2014; Lewandowska et al., 2017; Singh et al., 2018. Present articles was selected because they provide all taxa that had been collected. To obtained consistent database, authors determine taxa from algal and cyanobacterial species. Based on the above data using the ARcMap 10.6.1 software by ESRI, authors determined the locations (points) where the research was carried out, and in the attribute table assigned taxa, the group to which they belong and whether they pose a threat to human health (Genitsaris et al., 2011). Maps showing points and pie charts were created in the ArcMap 10.6.1 software.

5.2 APPENDIX PUBLICATION II

SUPPLEMENTARY INFORMATION:

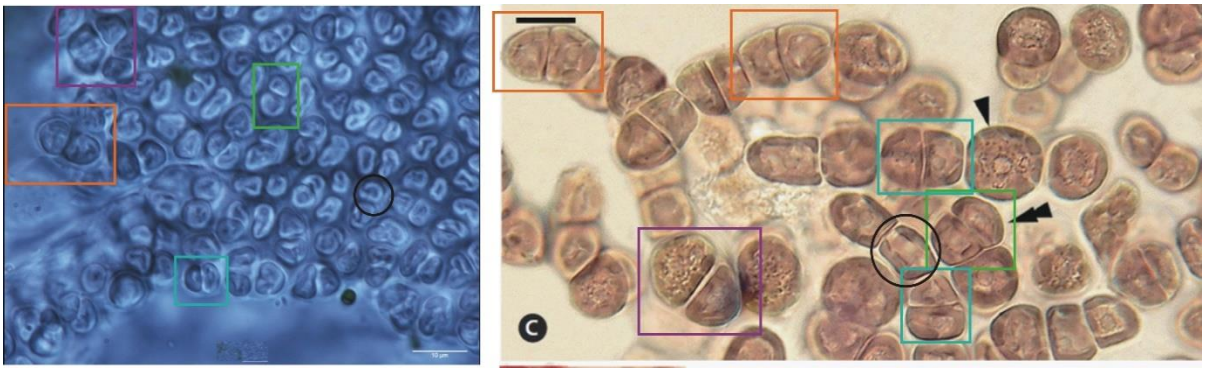

Fig. S1. A photo of an unidentified Rhodophyta species (left) along with the *Tsunamia transpacifica* J.A.West, G.I.Hansen, Zuccarello & T.Hanyuda 2016 (right) which it resembles (https://www.e-algae.org/journal/Figure.php?xn=algae-2016-31-4-289.xml&id=)

Table S1. A list of species of airborne algae and cyanobacteria from the southern Baltic Sea region isolated in 2018-2020 and included into CCAA collection

Number	Species	Phylum	Date of isolation
CCAA 23	*Chlorella* sp.	Chlorophyta	22.05.18
CCAA 24	*Chlorella minutissima*	Chlorophyta	24.05.18
CCAA 47	*Halamphora* sp.	Bacillariophyta	05.06.18
CCAA 49	*Rivularia* sp.	Cyanobacteria	22.06.18
CCAA 10	*Chlorella minutissima*	Chlorophyta	24.06.18
CCAA 38	*Kirchneriella* sp.	Chlorophyta	24.06.18
CCAA 39	*Nostoc edaphicum*	Cyanobacteria	24.06.18
CCAA 40	*Nostoc edaphicum*	Cyanobacteria	24.06.18
CCAA 26	*Pseudanabaena catenata*	Cyanobacteria	24.06.18
CCAA 25	*Scenedesmus* sp.	Chlorophyta	24.06.18
CCAA 44	*Synechococcus* sp.	Cyanobacteria	24.06.18
CCAA 43	*Bracteacoccus* sp.	Chlorophyta	26.06.18
CCAA 27	*Chlorella* sp.	Chlorophyta	26.06.18
CCAA 41	*Navicula* sp.	Bacillariophyta	26.06.18
CCAA 42	*Nostoc edaphicum*	Cyanobacteria	26.06.18
CCAA 13	*Pseudanabaena catenata*	Cyanobacteria	26.06.18
CCAA 30	*Nostoc* sp.	Cyanobacteria	29.06.18
CCAA 31	*Nostoc* sp.	Cyanobacteria	29.06.18
CCAA 32	*Nostoc* sp.	Cyanobacteria	29.06.18
CCAA 33	*Nostoc* sp.	Cyanobacteria	29.06.18
CCAA 21	*Cocomyxa* sp.	Chlorophyta	06.07.18
CCAA 20	*Oocystis* sp.	Chlorophyta	06.07.18
CCAA 9	*Oocystis* sp.	Chlorophyta	06.07.18
CCAA 14	*Synechococcus* sp.	Cyanobacteria	06.07.18
CCAA 18	*Gloeocapsa* sp.	Cyanobacteria	08.07.18
CCAA 11	*Planktolyngbya contorta*	Cyanobacteria	08.07.18
CCAA 12	*Pseudanabaena catenata*	Cyanobacteria	08.07.18
CCAA 7	*Oocystis* sp.	Chlorophyta	22.07.18
CCAA 8	*Pseudanabaena galeata*	Cyanobacteria	22.07.18
CCAA 28	*Chlorella* sp.	Chlorophyta	26.07.18
CCAA 29	*Nostoc* sp.	Cyanobacteria	26.07.18
CCAA 6	*Kirchneriella* sp.	Chlorophyta	08.08.18
CCAA 15	*Leptolyngbya foveolarum*	Cyanobacteria	08.08.18
CCAA 35	*Nostoc edaphicum*	Cyanobacteria	08.08.18
CCAA 36	*Nostoc edaphicum*	Cyanobacteria	08.08.18

CCAA 37	*Nostoc edaphicum*	Cyanobacteria	08.08.18
CCAA 1	*Nostoc* sp.	Cyanobacteria	08.08.18
CCAA 2	*Nostoc* sp.	Cyanobacteria	08.08.18
CCAA 3	*Nostoc* sp.	Cyanobacteria	08.08.18
CCAA 5	*Nostoc* sp.	Cyanobacteria	08.08.18
CCAA 19	*Pseudanabaena catenata*	Cyanobacteria	08.08.18
CCAA 46	*Synechococcus* sp.	Cyanobacteria	08.08.18
CCAA 4	*Nostoc* sp.	Cyanobacteria	12.08.18
CCAA 45	*Synechococcus* sp.	Cyanobacteria	12.08.18
CCAA 34	*Amphora* sp.	Bacillariophyta	03.09.18
CCAA 48	*Aphanothece* sp.	Cyanobacteria	15.09.18
CCAA 17	*Nitzschia* sp.	Bacillariophyta	15.09.18
CCAA 16	*Nostoc* sp.	Cyanobacteria	15.09.18
CCAA 22	*Nostoc* sp.	Cyanobacteria	15.09.18
CCAA 50	*Bracteacoccus* sp.	Chlorophyta	11.07.19
CCAA 52	*Chlorella minutissima*	Chlorophyta	15.07.19
CCAA 51	*Chlorococcum* sp.	Chlorophyta	15.07.19
CCAA 53	*Chlorella minutissima*	Chlorophyta	30.07.19
CCAA 54	*Oocystis* sp.	Chlorophyta	20.08.19
CCAA 55	*Oocystis* sp.	Chlorophyta	20.08.19
CCAA 56	*Stichococcus* sp.	Chlorophyta	22.08.19
CCAA 57	*Microthamnion* sp.	Chlorophyta	29.08.19
CCAA 59	*Chlorococcum* sp.	Chlorophyta	11.09.19
CCAA 60	*Klebsormidium* sp.	Charophyta	26.09.19
CCAA 61	*Vaucheria* sp.	Ochrophyta	15.11.19
CCAA 58	*Pseudococcomyxa* sp.	Chlorophyta	04.08.20

Table S2. The average of the meteorological parameters – air temperature, relative humidity, wind speed, pressure, and precipitation during the sampling months

Months	**Temp [°C]**	**Rh [%]**	**Ws [m s^{-1}]**	**P [hPa]**	**Rain [mm]**
Jan	4.0	76.7	3.3	1009.8	0.1
Feb	4.7	67.6	3.5	1003.8	0.1
Mar	4.7	62.3	3.1	1015.0	0.1
Apr	7.9	56.3	2.8	1015.0	0.1
May	11.1	69.1	2.6	1015.5	0.1
Jun	16.6	74.9	2.2	1010.9	0.1
Jul	17.7	66.9	2.7	1011.6	0.1
Aug	19.6	68.8	2.3	1012.1	0.1
Sep	16.5	68.4	2.7	1013.6	0.1
Oct	11.9	73.1	3.4	1009.2	0.2
Nov	7.8	73.4	3.1	1018.9	0.1
Dec	2.7	76.9	5.8	1010.0	0.1

Table S3. The nutrient composition in the Gulf of Gdansk (Baltic Sea), blue green (B-G) algae biomass, phytoplankton biomass (Phytopl.), the primary production (PP) and sea water temperature (http://model.ocean.univ.gda.pl)

Month	NO_3^{2-} [mg m^{-3}]	PO_4^{3-} [mg m^{-3}]	B-G Algae [mg m^{-3}]	Phytopl. [mg m^{-3}]	PP [mg m^{-2} d^{-1}]	Temp [°C]
Jan	192.2	37.9	0	12.2	7.8	4.5
Feb	161.5	36.1	0	1.7	0.7	4.5
Mar	168.0	36.6	0	7.8	4.2	4.5
Apr	60.2	16.7	0	166.3	110.1	6.8
May	29.6	16.4	0	82.2	40.6	9.3
Jun	32.9	22.4	0.2	69.1	27.3	14.2
Jul	48.5	29.8	2.5	71.2	26.8	16.9
Aug	187.1	46.4	10.7	27.3	7.2	18.3
Sep	141.3	40.1	9.8	42.4	8.7	16.9
Oct	85.1	30.9	0.4	94.7	33.9	16.1
Nov	116.6	34.6	0	22.9	2.5	10.1
Dec	155.5	37.1	0	4.7	28.9	7.5

Table S4. The detected microalgae and cyanobacteria of occurrence in the air

Species	Class	Phylum	Occurrence (%)
Amphora sp.	Bacillariophyceae	Bacillariophyta	0.177
Cyclotella sp.	Bacillariophyceae	Bacillariophyta	0.177
Fragilariopsis sp.	Bacillariophyceae	Bacillariophyta	0.177
Halamphora sp.	Bacillariophyceae	Bacillariophyta	0.177
Hyalotheca sp.	Zygnematophyceae	Charophyta	0.354
Streptosarcina sp.	Klebsormidiophyceae	Charophyta	0.354
Ankistrodesmus sp.	Chlorophyceae	Chlorophyta	0.177
Bracteacoccus sp.	Chlorophyceae	Chlorophyta	1.416
Chlorella sp.	Trebouxiophyceae	Chlorophyta	10.442
Chlorococcum sp.	Chlorophyceae	Chlorophyta	1.947
Chlorosarcinopsis sp.	Chlorophyceae	Chlorophyta	0.531
Coccomyxa sp.	Trebouxiophyceae	Chlorophyta	1.239
Kirchneriella sp.	Chlorophyceae	Chlorophyta	0.708
Oocystis sp.	Trebouxiophyceae	Chlorophyta	4.956
Stichococcus sp.	Trebouxiophyceae	Chlorophyta	12.212
Aphanocapsa sp.	Cyanophyceae	Cyanobacteria	0.177
Aphanothece sp.	Cyanophyceae	Cyanobacteria	0.354
Chroococcus sp.	Cyanophyceae	Cyanobacteria	16.991
Nodularia sp.	Cyanophyceae	Cyanobacteria	0.177
Nostoc sp.	Cyanophyceae	Cyanobacteria	1.593
Phormidium sp.	Cyanophyceae	Cyanobacteria	0.531
Pseudanabaena sp.	Cyanophyceae	Cyanobacteria	1.416
Synechococcus sp.	Cyanophyceae	Cyanobacteria	39.292
Synechocystis sp.	Cyanophyceae	Cyanobacteria	1.593
Woronichinia sp.	Cyanophyceae	Cyanobacteria	0.177
Chrysochromulina sp.	Coccolithophyceae	Haptophyta	0.708
Gymnodimium sp.	Dinophyceae	Miozoa	0.354
Xanthonema sp.	Xanthophyceae	Ochrophyta	1.416
Unidentified	Stylonematophyceae	Rhodophyta	0.177

Table S5. MC-LR content for individual strains of airborne cyanobacteria and microalgae. Values show arithmetic means (n = 3) and are followed by standard deviations in brackets

CCAA index	Target Cyanobacteria	MC-LR (μg L^{-1})	MC-LR (fg $cell^{-1}$)
CCAA 01	*Nostoc sp.*	ND	ND
CCAA 02	*Nostoc sp.*	ND	ND
CCAA 03	*Nostoc sp.*	0.078 (0.014)	3.843 (0.890)
CCAA 04	*Nostoc sp.*	ND	ND
CCAA 05	*Nostoc sp.*	0.031 (0.004)	3.705 (2.370)
CCAA 08	*Pseudanabaena galeata*	0.035 (0.006)	0.291 (0.050)
CCAA 11	*Planktolyngbya contorta*	ND	ND
CCAA 12	*Pseudanabaena catenata*	0.354 (0.009)	15.880 (0.152)
CCAA 13	*Pseudanabaena catenata*	ND	ND
CCAA 14	*Synechococcus* sp.	ND	ND
CCAA 15	*Leptolyngbya foveolarum*	0.602 (0.005)	16.076 (0.327)
CCAA 16	*Nostoc* sp.	ND	ND
CCAA 18	*Gloeocapsa* sp.	0.633 (0.03)	80.671 (2.314)
CCAA 19	*Pseudanabaena catenata*	0.320 (0.009)	13.151 (0.491)
CCAA 22	*Nostoc* sp.	0.051 (0.007)	21.161 (2.783)
CCAA 26	*Pseudanabaena catenata*	ND	ND
CCAA 29	*Nostoc* sp.	ND	ND
CCAA 30	*Nostoc* sp.	ND	ND
CCAA 31	*Nostoc* sp.	0.334 (0.001)	7.529 (0.084)
CCAA 32	*Nostoc* sp.	0.303 (0.008)	7.750 (0.208)
CCAA 33	*Nostoc* sp.	0.112 (0.010)	2.995 (0.206)
CCAA 35	*Nostoc edaphicum*	0.275 (0.033)	19.046 (2.001)
CCAA 36	*Nostoc edaphicum*	0.034 (0.013)	1.166 (0.451)
CCAA 37	*Nostoc edaphicum*	ND	ND
CCAA 39	*Nostoc edaphicum*	ND	ND
CCAA 40	*Nostoc edaphicum*	0.226 (0.001)	12.162 (0.554)
CCAA 42	*Nostoc edaphicum*	ND	ND
CCAA 44	*Synechococcus* sp.	0.097 (0.007)	195.574 (13.647)
CCAA 45	*Synechococcus* sp.	0.078 (0.010)	85.903 (10.515)
CCAA 46	*Synechococcus* sp.	0.238 (0.006)	419.952 (8.093)
CCAA 48	*Aphanothece* sp.	ND	ND
CCAA 49	*Rivularia* sp.	0.033 (0.007)	11.365 (2.531)

ND—not detected.

5.3 APPENDIX PUBLICATION III

Supplementary material

Sample ID	Date of sampling	NO_3^{2-} [mg L^{-1}]	PO_4^{3-} [mg L^{-1}]
R0119	*05/07/19*	-	-
R0219	*06/07/19*	-	-
R0319	*07/07/19*	0.5	0.01
R0419	*08/07/19*	2.4	0.05
R0519	*09/07/19*	3.4	0.02
R0619	*16/07/19*	0.8	1.23
R0719	*20/07/19*	3.2	5.64
R0819	*23/07/19*	0.8	0.90
R0919	*28/07/19*	0.6	0.03
R1019	*04/08/19*	-	0.82
R1119	*05/08/19*	0.8	0.05
R1219	*06/08/19*	1.2	0.09
R1319	*09/08/19*	0.9	0.08
R1419	*01/09/19*	1.6	1.65
R1519	*09/09/19*	0.7	0.32
R0120	*25/08/20*	-	-
R0220	*25/08/20*	-	-
R0320	*25/08/20*	0.8	0.14
R0420	*26/08/20*	-	-
R0520	*27/08/20*	0.6	0.36
R0620	*28/08/20*	0.9	1.18
R0720	*31/08/20*	1.1	0.54
R0820	*01/09/20*	0.5	0.05

Table S1. Nutrients measured in the rain sample (http://model.ocean.univ.gda.pl).

Date of sampling	NO_3^{2-} [mg m^{-3}]	PO_4^{3-} [mg m^{-3}]	B-G Algae [mg m^{-3}]	PP [mg m^{-2} d^{-1}]
05/07/19	11.6	14.3	3	15.7
06/07/19	11.2	14.2	3	12.1
07/07/19	12.4	14.3	3	7.76
08/07/19	13.6	14.5	3.2	5.96
09/07/19	16.1	14.4	3.1	2.85
16/07/19	24.6	14.1	8.3	4.38
20/07/19	45.6	17.6	14.1	5.72
23/07/19	81.4	25.2	9.7	2.35
28/07/19	287	58.7	6.3	4.96
04/08/19	185	41.7	22	5.84
05/08/19	196	42.4	26.1	8.23
07/08/19	191	42.3	26.4	6.37
29/08/19	208	40.8	57.1	17.2
01/09/19	181	39.8	59.2	23.1
09/09/19	159	36.4	28	19.5
25/08/20	163	48.3	6.7	6.15
26/08/20	147	48.3	6.6	8.69
27/08/20	153	45.9	7.5	8.22
28/08/20	152	44.5	10.5	6.7

29/08/20	160	45.3	6.3	5
30/08/20	161	46.7	7	7.81
31/08/20	164	44.7	8.7	5.27
01/09/20	163	45	11.5	7.39
02/09/20	179	45.7	10.2	4.44

Table S2. The nutrient composition in the Gulf of Gdansk (Baltic Sea), phytoplankton biomass – blue green algae biomass and the primary production (http://model.ocean.univ.gda.pl).

	Rainfall	T_{mean}	Rh	hPa	Ws	NO_3^{2-}	PO_4^{3-}	BG biomass	PP
Microalgae and cyanobacteria in rain	-0.098	0.604	-0.105	0.78	-0.302	0.588	0.549	0.890	0.165
p value	>0.05	*<0.05	>0.05	***<0.001	>0.05	*<0.05	>0.05	***<0.001	>0.05

Table S3. Spearman rank correlation coefficients between number of microalgae and cyanobacteria in the rain (cells L^{-1}) and: daily records for rainfall [mm], mean temperature [°C], relative humidity [%], atmospheric pressure [hPa], wind speed [m s^{-1}], NO_3^{2-} [mg m^{-3}] and PO_4^{3-} [mg m^{-3}] concentration in sea water, blue green algae biomass [mg m^{-3}] and primary production [mg m^{-2} d^{-1}] in the Baltic Sea.

Environment	Type of bioaerosol	Type of research	References
Aerosols	Cyanobacteria	Quality	El-Gamal [44]
Aerosols	Cyanobacteria, microalgae	Quantity and quality	Genitsaris et al. [2]
Aerosols	Cyanobacteria, microalgae	Quality	Lee and Eggleston [43]
Aerosols	Cyanobacteria, microalgae	Quality	Lewandowska et al. [6]
Aerosols	Cyanobacteria, microalgae	Quantity and quality	Marshall et al. [15]
Aerosols	Cyanobacteria	Quantity	Murby and Haney [10]
Aerosols	Cyanobacteria, microalgae	Quality	Ng et al. [46]
Aerosols	Bacteria, cyanobacteria	Quality and quantity	Jang et al. [57]
Aerosols	Cyanobacteria, microalgae	Quantity and quality	Rosas et al. [12]
Aerosols	Cyanobacteria, microalgae	Quality and quantity	Sharma and Singh [45]
Aerosols	Cyanobacteria, microalgae	Quantity and quality	Sharma et al. [13]
Aerosols	Cyanobacteria, microalgae	Quantity and quality	Singh et al. [14]
Aerosols	Cyanobacteria, microalgae	Quality and quantity	Tormo et al. [54]
Aerosols	Cyanobacteria, microalgae	Quality	Wiśniewska et al. [31]
Aerosols, rainfall	Cyanobacteria, microalgae	Quantity and quality	Ouyang et al. [55]
Aerosols, soil	Cyanobacteria, microalgae	Quantity and quality	Carson et al. [16]

Aerosols, soil, buildings	Cyanobacteria, microalgae	Quality and quantity	Chu et al. [50]
Aerosols, soil, water	Cyanobacteria, microalgae	Quality and quantity	Sharma et al. [49]
Cloud water	Bacteria, Cyanobacteria	Quantity and quality	Kourtev et al. [19]
Cloud water	Bacteria, Cyanobacteria	Quantity and quality	Xu et al. [48]
Rainfall	Bacteria	Quantity and quality	Joung et al. [56]
Rainfall, clouds	Cyanobacteria, microalgae	Quantity and quality	Dillon et al. [47]
Aerosols, rainfall	Cyanobacteria, microalgae	Quantity and quality	Current study

Table S4. Comparing the information on the type of bioaerosols, the location of airborne cyanobacteria and microalgae as well as the type of research performed.

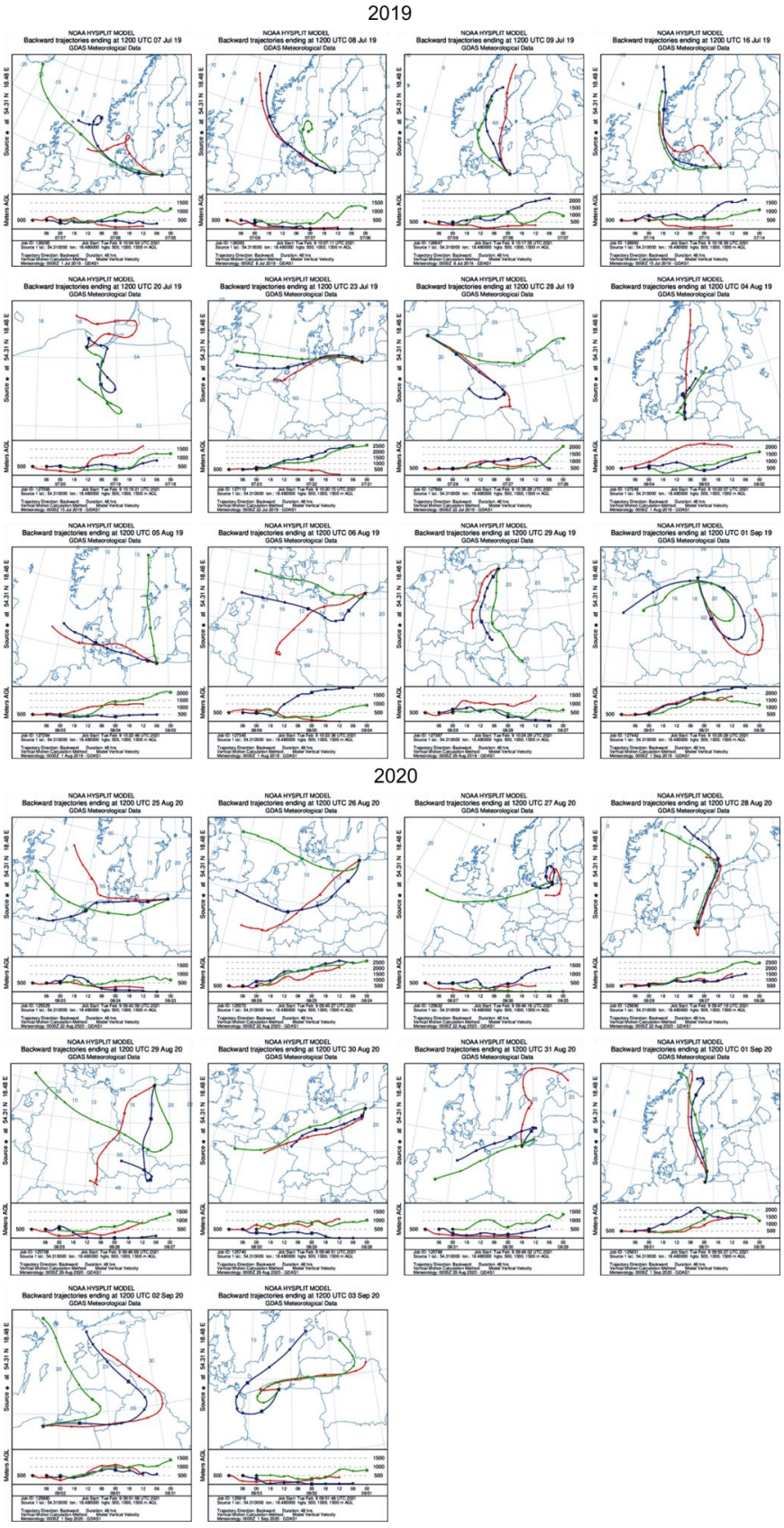

Figure S1. Representative 48 h backward trajectories of air masses during sampling period (HYSPLIT https://www.ready.noaa.gov).

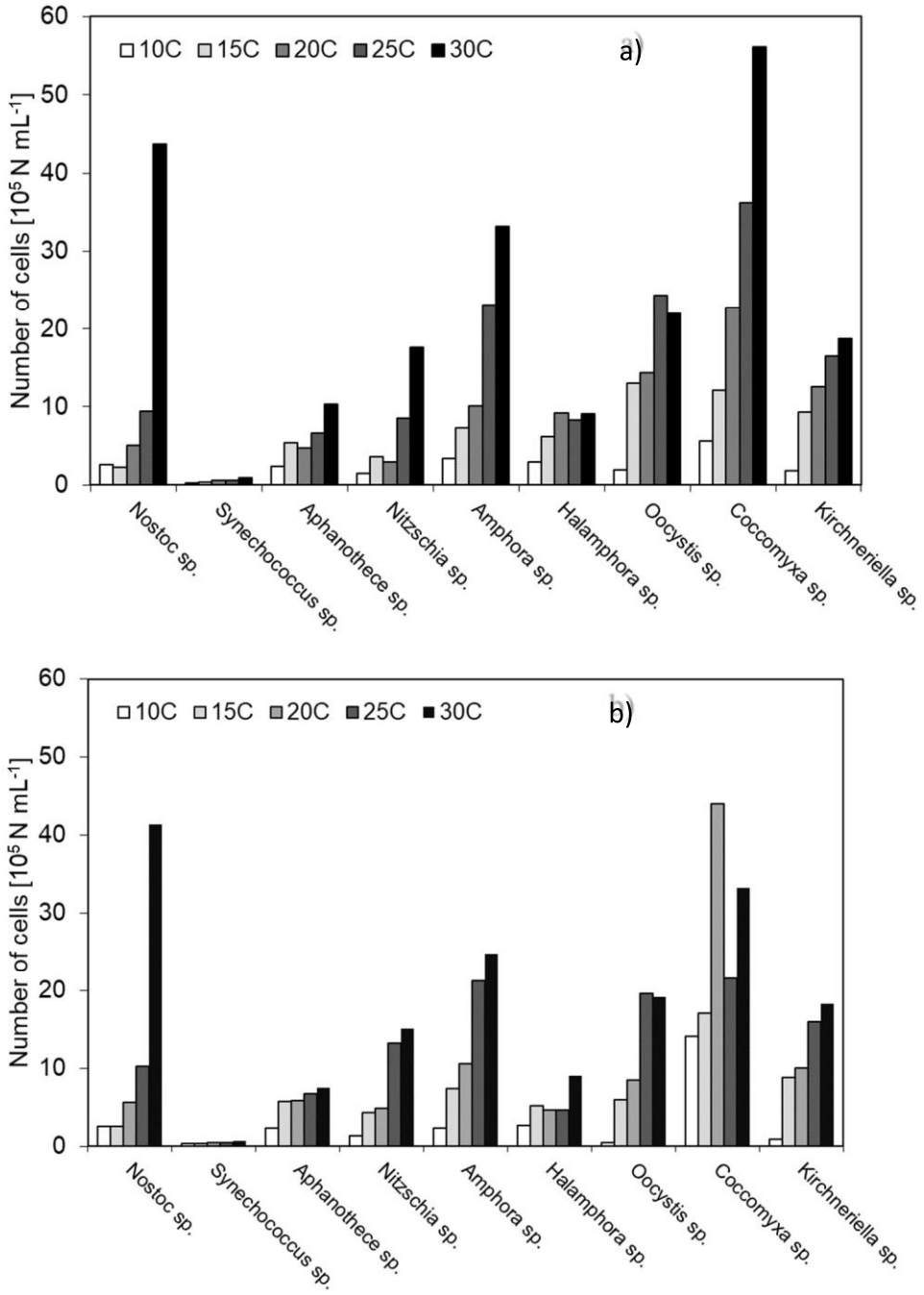

Fig. S1. Variability of cyanobacteria and microalgae quantity with respect to sample incubation temperature a) in the presence of B(a)P b) without B(a)P.

Table S1. The average, minimum and maximum cell quantities for individual strains after 7 days of B(a)P exposition and without B(a)P exposition.

	Nostoc sp.	Synechococcus sp.	*Aphanothece sp.*	*Nitzschia sp.*	*Amphora sp.*	*Halamphora sp.*	*Oocystis sp.*	*Coccomyxa sp.*	*Kirchneriella sp.*
Number of cells after adding B(a)P [·105 N x mL^{-1}]									
Av.	12.6	0.5	5.9	6.8	15.4	7.2	15.2	26.5	11.8
Min.	1.9	0.2	1.7	1.4	3.2	2.7	1.0	5.0	1.5
Max	46.1	1.0	12.2	22.0	37.4	10.4	27.2	73.0	22.6
Number of cells without B(a)P [·105 N x mL^{-1}]									
Av.	12.5	0.4	5.6	7.8	13.2	5.2	10.7	26.0	10.78
Min.	2.6	0.3	2.3	1.3	2.3	2.6	0.4	14.1	0.9
Max	41.4	0.7	7.5	15.1	24.7	9.1	19.6	44.0	18.2

Table S2. Three-way factorial ANOVA of cells concentration, fluorescence and pigment content measured in tested strains growing at different temperatures (0°C) and B(a)P concentration (ng mL^{-1}) in the range of 0 to 7.8 ng mL^{-1}: df – degrees of freedom; F – Fisher's F-test statistic; Mss – mean sum of squares; Ss – sum of squares. Levels of significance were: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

	SS	Degr. of Freedom	MS	F	p
	Number of cells (cel mL^{-1})				
Intercept	8994.494	1.000	8994.494	20307.157	0.000***
Strain	5909.914	8.000	738.739	1667.875	0.000***
Temperature	4084.866	4.000	1021.216	2305.633	0.000***
B(a)P	20.138	1.000	20.138	45.467	0.000***
Strain*Temperature	7113.780	32.000	222.306	501.907	0.000***
Strain*B(a)P	45.220	8.000	5.653	12.762	0.000***
Temperature*B(a)P	26.366	4.000	6.591	14.882	0.000***
Strain*Temperature*B(a)P	130.340	32.000	4.073	9.196	0.000***
Error	79.726	180.000	0.443		
	Fluorescence (Fv/Fm)				
Intercept	93.778	1.000	93.778	35179.892	0.000***
Strain	5.818	8.000	0.727	272.799	0.000***
Temperature	2.352	4.000	0.588	220.598	0.000***
B(a)P	0.011	1.000	0.011	4.163	0.043*
Strain*Temperature	0.920	32.000	0.029	10.790	0.000***
Strain*B(a)P	0.072	8.000	0.009	3.360	0.001**
Temperature*B(a)P	0.070	4.000	0.017	6.546	0.000***
Strain*Temperature*B(a)P	0.170	32.000	0.005	1.990	0.003**
Error	0.480	180.000	0.003		
	Chl *a* (ng cel^{-1})				
Intercept	1444.490	1.000	1444.490	5981.596	0.000***
Strain	4721.586	8.000	590.198	2443.996	0.000***
Temperature	69.140	4.000	17.285	71.576	0.000***
B(a)P	0.768	1.000	0.768	3.181	0.076

Strain*Temperature	435.580	32.000	13.612	56.366	0.000***
Strain*B(a)P	1.163	8.000	0.145	0.602	0.775
Temperature*B(a)P	5.298	4.000	1.325	5.485	0.000***
Strain*Temperature*B(A)P	38.964	32.000	1.218	5.042	0.000***
Error	43.468	180	0.241		

Table S3. The average, minimum and maximum chlorophyll *a* for individual strains after 7 days of B(a)P exposition and without B(a)P exposition

	Nostoc sp.	Synechococcus sp.	*Aphanothece sp.*	*Nitzschia sp.*	*Amphora sp.*	*Halamphora sp.*	*Oocystis sp.*	*Coccomyxa sp.*	*Kirchneriella sp.*
Chl *a* after adding B(a)P [ng cell^{-1}]									
Av.	15.2	47.1	3.8	24.2	29.8	9.0	5.7	4.3	12.2
Min.	1.4	5.2	1.2	0	0	0	1.8	0	9.1
Max	38.2	98.8	13.1	64.0	70.5	37.9	11.0	8.23	16.2
Chl *a* cells without B(a)P [ng cell^{-1}]									
Av.	18.6	55.9	7.1	24.7	33.7	16.3	6.7	18.4	12.8
Min.	0.7	20.1	2.0	6.4	2.6	0	2.1	3.0	7.7
Max	38.2	94.0	19.1	41.7	55.0	37.9	13.2	48.2	16.5

Table S4. The average, minimum and maximum Fv/Fm for individual strains after 7 days of B(a)P exposition and without B(a)P exposition

	Nostoc sp.	Synechococcus sp.	*Aphanothece sp.*	*Nitzschia sp.*	*Amphora sp.*	*Halamphora sp.*	*Oocystis sp.*	*Coccomyxa sp.*	*Kirchneriella sp.*
Fv/Fm after adding B(a)P									
Av.	0.5	0.4	0.4	0.5	0.6	0.4	0.8	0.6	0.8
Min.	0.2	0.2	0.2	0.3	0.2	0.2	0.6	0.4	0.6
Max	0.6	0.5	0.5	0.8	0.8	0.6	0.9	0.8	0.9
Fv/Fm cells without B(a)P									
Av.	0.5	0.4	0.4	0.7	0.6	0.5	0.8	0.6	0.8
Min.	0.4	0.2	0.3	0.4	0.2	0.5	0.5	0.5	0.5
Max	0.6	0.5	0.5	0.8	0.8	0.5	0.9	0.7	0.9

Table S5. The average content of B(a)P [ng mL^{-1}] after 7 days of exposure

Added B(a)P [ng mL^{-1}]	*Nostoc* sp.	*Synechococcus* sp.	*Aphanothece* sp.	*Nitzschia* sp.	*Amphora* sp.	*Halamphora* sp.	*Oocystis* sp.	*Coccomyxa* sp.	*Kirchneriella* sp.	Blank sample
	B(a)P concentration after 7 days of exposition [ng mL^{-1}]									
7.8	3.6	3.0	1.4	4.1	2.1	1.3	2.2	1.6	1.5	2.1
15	9.5	3.2	5.1	2.3	2.3	1.9	0.9	0.9	2.0	6.5
78	10.8	9.5	10.5	10.6	13.8	7.9	3.2	5.6	1.1	10.0
312	22.3	14.2	30.1	15.8	14.9	29.2	6.9	10.6	6.2	19.0
624	25.9	37.1	74.8	43.8	37.5	70.1	17.8	17.6	5.6	48.2

Table S6. Two-way factorial ANOVA of B(a)P concentration after 7 days of exposure for tested taxa (divided as group of cyanobacteria, green algae, and diatoms): df – degrees of freedom; F – Fisher's F-test statistic; Mss – mean sum of squares; Ss – sum of squares. Levels of significance were: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

	SS	Degr. of Freedom	MS	F	p
	B(a)P concentration after 7 days of exposure (ng mL^{-1})				
Intercept	8076.261	1.000	8076.261	102.298	0.000***
B(A)P	7278.184	4.000	1819.546	23.047	0.000***
Strain	1368.927	2.000	684.463	8.670	0.001**
B(A)P*Strain	1528.676	8.000	191.085	2.420	0.038*
Error	2368.448	30.000	78.948		