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Identification of cyanobacteria and microalgae in aerosols of various sizes in the air over the Southern Baltic Sea☆

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ABSTRACT

Bioaerosols were collected between April and November 2015 on land (Gdynia) and at sea (Southwestern Baltic), using six-step microbiological pollutant sampler. It was determined that picoplanktonic cyanobacteria of the genus *Synechococcus, Synechocystis, Aphanocapsa, Aphanothece, Microcystis, Merismopedia, Woronichinia* and *Cyanodictyon* were the most commonly found in aerosols both over land and at sea. Chlorophyta were also numerous (*Chlorella vulgaris*, *Stichococcus bacillaris*), as were Bacillariophyta and Ochrophyta (*Phaeodactylum* sp., *Navicula* cf. *perminuta* and *Nannochloropsis* cf. *gaditana*). As primary production and phytoplankton concentration in sea water grew, so did the diversity of the microorganisms identified in bioaerosols. Over the sea cyanobacteria and microalgae occurred more often in large aerosols (> 3.3 µm). Over land they were mainly the components of smaller particles. In respirable particles species both capable of producing harmful secondary metabolites and potentially toxic ones were identified. We assume that bioaerosols pose the actual threat to human health in Baltic Sea region.

1. Introduction

The advance of civilisation, which occurred in the late-18th/early-19th centuries, resulted in the transformation by human activity of most areas of land. As industry developed, toxic and harmful substances that had not previously been components of the air, or that occurred in small quantities with no negative effect on the natural environment, began to be released into the atmosphere. Later on, with the intensification of scientific development and air quality research, that could not have remained insignificant. However, components from natural sources also play a significant role in the atmosphere and among them, bioaerosols are commonly present. These are formed of a diverse complex of particles which include viruses, protozoa, bacteria, mycelium fragments, fungus spores, and algae (Genitsaris et al., 2011). Products of microbiological metabolism such as endotoxins, enterotoxins, enzymes and mycotoxins are also present (Law et al., 2001; Agranovski et al., 2002). Bioaerosols have a significant influence on the formation of clouds and precipitation, and thus on the hydrological cycle, and the Earth's climate (Pöschl et al., 2010). They can also penetrate into the human body via the respiratory system and affect human health negatively.

“The golden age of aerobiology” was in the years 1861–1882 (Comtois and Isard, 1999) and it was at that time that Ehrenberg discovered the presence of cyanobacteria and microalgae in aerosols collected by Darwin on the Atlantic Ocean (Ehrenberg, 1844). The cyanobacteria are morphologically diverse as a phylum (e.g. Whitton and Potts, 2012). Cyanobacteria belong taxonomically to bacteria, are also defined as photosynthetic protists (Després et al., 2012). They tolerate a wide range of environmental factors, and for this reason they can be found in almost all ecological niches. However, a broad majority of them inhabit water basins. Cyanobacteria perform an important ecological function in oceans, contributing to global changes in carbon and nitrogen cycles (Stewart and Falconer, 2008). Microalgae and cyanobacteria are organisms that have different size ranges. The smallest being cyanobacteria and picoplankton (0.2–2 µm) and the largest being unicellular organisms of about 500 µm (Finlay, 2002; Tesson et al., 2016). They are omnipresent and are characterised by a fast growth rate. These organisms drift in the water column or are attached to the surface, and are introduced into the atmosphere when, for example, they are lifted by the wind from the land or water surface. The process of algae lifting and introduction to the atmosphere, along with sea salt, organic matter and bacteria, has been previously described by e.g. Gregory (1973), Blanchard (1989) and Marshall and Chalmers (1997). The air, however, is not a favourable environment for the growth and

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reproduction of cyanobacteria or microalgae and for this reason they only spend short periods of time there. In the atmosphere, such organisms are limited by lack of sufficient nutrients, periodic water deficits or solar radiation. On the other hand, the atmosphere can allow microorganisms present in aerosols to travel with air masses. The small sizes of aerosol of which they are constituent can represent a helpful factor in the distribution of cyanobacteria or microalgae over land. This is due to the fact that coastal sites display specific meteorological patterns, like onshore/offshore breeze phenomena, and play an important role in the dispersion, transformation, removal or accumulation of particles (Gariazzo et al., 2007). So that bioerosols which originate at sea could be transformed in the coastal area as a result of fraction and enrichment with terigenic and anthropogenic particles (Lewandowska and Falkowska, 2013).

While cyanobacteria and algae living in freshwater and sea water are well recognised, those that are the components of aerosols are rarely the focus of scientific research, especially in the Baltic Sea region. In recent decades, scientists have focused mainly on fungi and bacteria, and their presence in the air has been linked to ailments including infectious diseases, poisonings, allergies, asthma and even cancer (Peccia et al., 2011). Their influence on the respiratory system is particularly well-documented (Verhoeff and Burge, 1997; Douwes et al., 2003; Lee et al., 2005). On the other hand, the knowledge of microalgae and cyanobacteria in terms of their influence on health is scant. Studies conducted in the 1940s showed a correlation between the presence of algae in the air and the incidence of hay fever, asthma or allergies, while patients who had been exposed to cyanobacterial extracts were observed to have severe skin reactions (Heise, 1949). The consequence of algae being present in various environments is their ability to produce and discharge a range of secondary metabolites (Rastogi and Sinha, 2009). Among them are compounds bearing neurotoxic and hepatotoxic properties, and compounds with allelopathic properties. Despite the fact that secondary metabolites of cyanobacteria can be used in medicine and the pharmaceutical industry as compounds of antibacterial, antiviral and antifungal properties (Berry et al., 2008; Garima et al., 2013), most literature reports indicate their negative effect on the growth and functioning of live organisms (Hamilton et al., 2014; Rzymski et al., 2014; Poniedziałek et al., 2015). However phytoplancton toxins concentrated at the sea surface become aerosolized after lysis or caught up in bubblemediated transport and can impact human health through the respiratory route (Landsberg, 2002; Ferrante et al., 2013). The symptoms commonly recorded during inhalation are weakness and malaise, respiratory distress, abnormalities incardiac function and impairment of the neuromuscular apparatus. Inflammatory reactions typical of inhalational contact are also observed (Deeds and Schwartz, 2009; Tubaro et al., 2011; Ferrante et al., 2013). As yet, little is known about the role that microalgae and cyanobacteria play in the respiratory transportation of radionuclides, heavy metals, pesticides, herbicides and carcinogenic and mutagenic agents into the human organism.

Scientific studies into bioaerosols tend to focus on bacteria and fungi, both in Poland and worldwide (Szadkowska-Stańczyk et al., 2010; Li et al., 2011; Urbano et al., 2011). For this reason the principal aim of the present studies, which were carried out on aerosols of various sizes during the vegetation season in the coastal zone and over the open-waters of the Baltic Sea, was rather the identification of microalgae and cyanobacteria. Investigation of this topic fits in with the widespread need to create regional and global bioaerosol maps (atmospheric biogeography) (Després et al., 2012). Furthermore, as biological components of aerosols can penetrate into the human organism via the respiratory system just as chemical compounds do, another key aim of these studies was to determine whether among the identified microalgae and cyanobacteria there were any capable of posing a potential threat to human health.

2. Materials and methods

2.1. Preparing microbiological culture media

Prior to the collection of samples a standard sterile mineral f/2 culture medium was prepared (Guillard, 1975) on the basis of sea water with a salinity of 8 PSU. After delivery to the laboratory, the water was filtered through GF/C Whatman glass filters. Next the culture medium was poured into a 250 ml Erlenmeyer flask, to which 1% of agar was added, and the flask was sterilised for 20 min in an autoclave at 120 °C temperature and 1.2 atm pressure. 27 ml of the liquid culture medium was then applied to the Petri dishes used for the collection of cyanobacteria and microalgae, and exposed to UV radiation for 20 min. All Petri dishes (prepared with agar) were kept refrigerated until required for sample collection.

2.2. Collecting microbiological samples

Aerosol studies were conducted with varied frequency, in cycles ranging from 30 min (June, sea stations) to 1.5 h (all samples at land station), from April to November 2015 on land (Gdynia) and at sea (Gulf of Gdansk, Southwestern Baltic) (Fig. 1, Table 1). In Gdynia, measurements were performed on the roof of the Institute of Oceanography, where the atmospheric chemistry coastal station together with the meteorological station have existed for several years. The height of the building (20 m) enables taking measurements above tree canopies and neighbouring buildings. The Institute is located a few hundred meters from the sea coast (Gulf of Gdansk). Sea stations were located along the Polish sea coast. Station 1 was located the closest to the sea shore, in the internal water of the Puck Bay. Samples obtained on sea stations No. 2–4 were located in the external water, a few kilometers from the coast. Stations 2 and 3 were located the furthest from the coast near Bornholm Island. Samples from the sea station were collected on ship and altitude of sampling was always 20 m.

All samples were collected using a six-step microbiological pollutant particle sampler by Tisch Environmental, Inc. The air was aspirated by a vacuum pump characterised by constant air flow of $28.3\,\mathrm{l\,min^{-1}}$. Each of the impactor nozzles directed the exhaust air onto a Petri dish covered with agar, collecting particles of various sizes 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 and 6. < 1.1 μm in diameter).

During measurements meteorological data, i.e. wind speed, air temperature and humidity was obtained using an automatic Milos 500 station by Vaisala located on the roof of the Institute of Oceanography building as well as on the ship deck (Table 1). The sea water temperature was calculated using the PM3D hydrodynamic model updated daily on the basis of current remote-sensing data during data assimilation (http://www.satbaltyk.pl). In addition 48-h air-mass backward trajectories at the height of 20 m above ground level for each sampling period were constructed using the atmospheric model HYSPLIT (Draxler and Rolph, 2003; Rolph, 2003) and the data from the meteorological database at the National Oceanic and Atmospheric Administration (NOAA), accessible on the Internet (http://www.arl.noaa.gov/ready.html). A detailed description of the trajectories has been presented in previous papers (Lewandowska et al., 2010; Lewandowska and Falkowska, 2013).

2.3. Sample preservation until analysis

Analysed samples were grown for 30 days under constant conditions of 20 °C, on a 16:8 h light:dark cycle at 10 $\mu\mathrm{mol\ photons{\cdot}m^{-2}{\cdot}s^{-1}}$. The intensity of PAR was measured using a quantum-meter (LI-189, LI-COR Inc., Nebraska, USA) with a cosine collector.

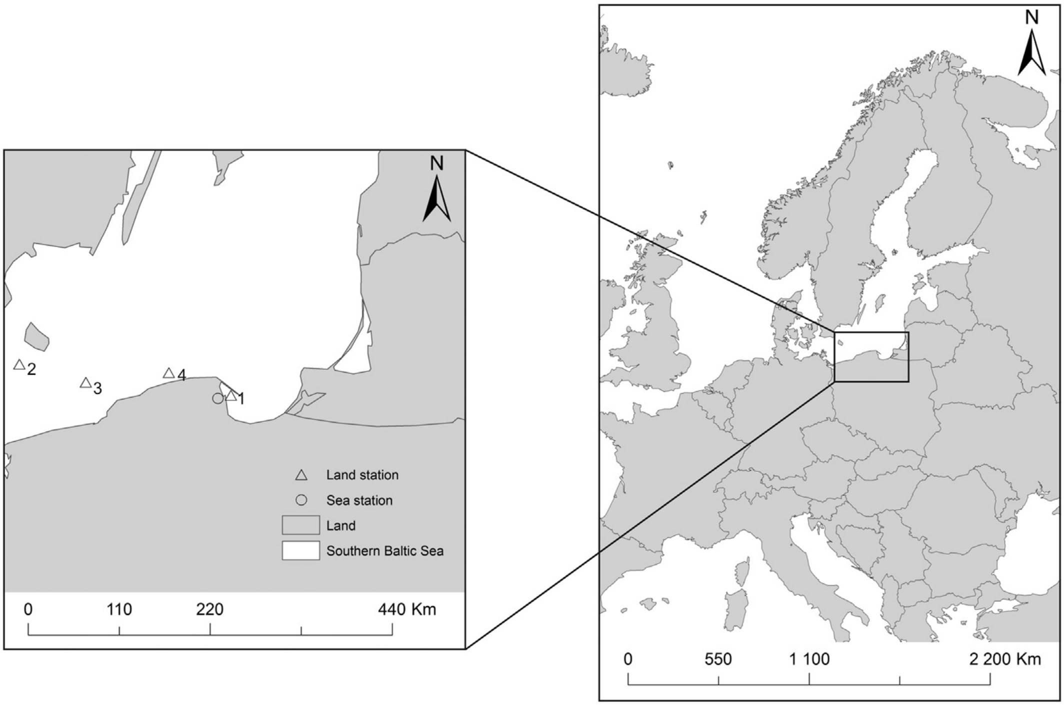

Fig. 1. Sample collection sites (marine stations Δ; land stations ○).

2.4. Identification of the collected material

The taxonomic composition and number of identified taxa were analysed under a Nikon eclipse 80i microscope at a magnification of 1000 × (objective = 100 ×). In addition, in order to verify the studied material, an epifluorescence microscope (Nikon Eclipse 80i) with green excitation/block filter (EX 510-560, DM 575, BA 590, 6-2A) was used. The latter proves chlorophyll *a* content in identified taxa and in turn the ability to photosynthesis process conduction. The analysed material was collected from Petri dishes and checked under a light and epifluorescence microscope. Phytoplankton organisms were identified to the species level or, if this was impossible, assigned only to a genus. Taxa were identified using keys and world literature (Huber-Pestalozzi, 1950; Lind and Brook, 1980; Komarek and Fott, 1983; Popovski and Pfiester, 1990; Cox, 1996; Komarek and Anagnostidis, 1999; Hindák, 2001).

3. Results and discussion

3.1. Identified species of cyanobacteria and microalgae in aerosols

In 120 bioaerosol samples (42 from marine stations and 78 from the land station), 41 taxa of cyanobacteria and microalgae were identified. They were the components of both small aerosols (< 3.3 μm in diameter) and larger particles (> 3.3 μm in diameter) (Table 2, Appendices 1 and 2).

The studies made it possible to determine that both over land and over the sea the most commonly found were picocyanobacteria

Table 1
The locations of measurement stations, times of sample collection and environmental conditions during the sampling period.

Station	Location and symbol	Date	T_{sea} [°C]	T_{air} [°C]	Rh [%]	V [$m\cdot s^{-1}$]	Air mass advection
Land station	(○) 54°30′ N 18°32′ E	10.04.15	4.4	13.1	44.3	1.6	NW
		21.04.2015	5.3	9.2	53.3	2.9	N
		22.04.2015	5.4	9.5	43.7	1.7	N
		23.04.2015	5.9	16.4	39.0	6.5	NW
		24.04.2015	5.7	12.1	56.6	3.4	NW
		07.08.2015	5.3	29.0	46.2	1.9	SW
		8.08.2015	18.9	28.2	42.5	1.8	SE
		14.08.2015	19.3	19.9	59.6	2.0	NE
		18.08.2015	19	21.0	50.2	2.5	NE
		12.10.2015	18.9	7.8	49.7	2.5	SE
		16.11.2015	19.0	6.7	76.6	1.6	NW
		23.11.2015	12.2	2.9	77.1	2.4	NE
		30.11.2015	12.2	5.8	70.9	4.4	NW
Sea station	(Δ1) 54°42′ N 18°35′ E	10.06.2015	11.1	19.1	63.9	0.3	NE
		12.06.2015	11.8	20.2	58.7	3.7	SW
	(Δ2) 54°55′ N 14°02′ E	28.06.2015	12.2	15.1	68.9	6.	NW
	(Δ3) 54°48′ N 15°58 E	29.06.2015	12.2	15.6	69.6	5.7	SW
	(Δ4) 54°54′ N 17°57 E	30.06.2015	15.2	16.3	71.3	1.7	SE

Note: T_{sea} - sea water temperature [°C], T_{air} - air temperature [°C], Rh - relative humidity [%], V - wind speed [$m\cdot s^{-1}$].

Table 2

Taxa list of airborne cyanobacteria and microalgae found in aerobiological studies in 6 fractions sampled. (^{l}) depicts taxa found with microscopic observation in the air over land, (^{s}) depicts taxa found in the air over sea and (^{har}) depicts potentially harmful taxa.

Group	Species	Fractions
Cyanobacteria (Cyanophyceae)	*Chamaesiphon* cf. *polymorphus*^{s}	> 7 µm
	Chroococcidiopsis cf. *cubana*^{s}	4.7–7 µm
	Merismopedia cf. *tenuissima*^{l,s,har}	2.1–3.3 µm
	Microcystis cf. *viridis*^{l,s,har}	2.1–3.3 µm
	Chamaesiphon cf. *polonicus*^{s}	> 7 µm
	Synechococcus cf. *elongatus*^{l,s,har}	2.1–3.3 µm
	Aphanocapsa cf. *rivularis*^{l,s,har}	1.1–2.1 µm
	Leptolyngbya cf. *foveolarum*^{s}	> 7 µm
	Rhabdoderma cf. *lineare*^{s}	3.3–4.7 µm
	Gloeothece sp.^{s}	4.7–7 µm
	Aphanocapsa cf. *planctonica*^{l,s,har}	1.1–2.1 µm
	Synechocystis sp.^{l,s,har}	2.1–3.3 µm
	Aphanocapsa cf. *delicatissima*^{l,s,har}	1.1–2.1 µm
	Cyanodictyon cf. *imperfectum*^{l,s}	2.1–3.3 µm
	Synechococcus sp.^{l,s,har}	1.1–2.1 µm
	Synechococcus cf. *leopoliensis*^{l,s,har}	1.1–2.1 µm
	Aphanothece cf. *saxicola*^{l,s}	2.1–3.3 µm
	Nostoc sp.^{s,har}	> 7 µm
	Phormidium cf. *autumnale*^{s,har}	> 7 µm
	Pseudanabaena cf. *limnetica*^{s}	> 7 µm
	Leptolyngbya cf. *tenuis*^{s}	> 7 µm
	Anabaena cf. *variabilis*^{s,har}	> 7 µm
	Woronichinia sp.^{l,s}	2.1–3.3 µm
	Synechococcus elongatus^{l,s,har}	2.1–3.3 µm
	Synechocystis cf. *salina*^{l,s,har}	2.1–3.3 µm
	Cyanodictyon cf. *planctonicum*^{l,s}	2.1–3.3 µm
	Aphanothece cf. *stagnina*^{l,s}	1.1–2.1 µm
	Chroococcus cf. *dispersus*^{s}	4.7–7 µm
	Aphanothece cf. *paralleliformis*^{l,s}	1.1–2.1 µm
	Aphanothece cf. *minutissima*^{l,s}	1.1–2.1 µm
	Synechocystis cf. *aquatilis*^{l,s,har}	2.1–3.3 µm
	Leptolyngbya cf. *granulifera*^{s}	> 7 µm
	Aphanocapsa cf. *delicatissima*^{l,s,har}	2.1–3.3 µm
	Rhabdoderma cf. *ineare*^{s}	3.3–4.7 µm
	Pseudanabaena cf. *catenata*^{s}	> 7 µm
	Cyanodictyon cf. *planctonicum*^{l,s}	2.1–3.3 µm
Chlorophyta (Trebouxiophyceae)	*Stichococcus bacillaris*^{s}	3.3–4.7 µm
	Chlorella vulgaris^{l,s,har}	4.7–7 µm
	Coccomyxa cf. *subellipsoidea*^{s}	3.3–4.7 µm
Chlorophyta (Chlorophyceae)	*Monoraphidium* cf. *litorale*^{s}	4.7–7 µm
	Chlorococcum cf. *hypnosporum*^{s}	4.7–7 µm
	Coelastrella cf. *oocystiformis*^{s}	4.7–7 µm
Charophyta (Conjugatophyceae)	*Spirotaenia* cf. *condensata*^{s}	4.7–7 µm
Bacillariophyta (Bacillariophyceae)	*Navicula* cf. *perminuta*^{l}	3.3–4.7 µm
	Phaeodactylum sp.^{l}	2.1–3.3 µm
Ochrophyta (Eustigmatophyceae)	*Nannochloropsis* cf. *gaditana*^{l}	3.3–4.7 µm

(Cyanobacteria, Cyanophyceae) of the following genus: *Synechococcus, Synechocystis, Aphanocapsa, Aphanothece, Microcystis, Merismopedia, Woronichinia* and *Cyanodictyon* (Fig. 2). Moreover, in the samples the authors identified species of filamentous cyanobacteria *Nostoc* sp., *Phormidium* sp., *Anabaena* cf. *variabilis*, *Leptolyngbya* cf. *foveolarum*, *Pseudanabaena* cf. *limnetica* and *Leptolyngbya* cf. *tenuis*. Chlorophyta were also present in large number of taxa (e.g. *Chlorella vulgaris* and *Stichococcus bacillaris*). Bacillariophyta (Bacillariophyceae) found to be present in aerosols were representative of only two taxa (*Phaeodactylum* sp., and *Navicula* cf. *perminuta*) and Ochrophyta (Eustigmatophyceae) of one taxa (*Nannochloropsis* cf. *gaditana*) which constituted 3% of all the identified organisms. The presence of cyanobacteria in areosols in such large numbers (49%) may result from the fact that they exhibit great tolerance to a wide range of environmental factors. They can be found practically everywhere in the world, from oceans, freshwater and brackish water basins, to land environment (Baracaldo et al., 2005; Thajuddin and Subramanian, 2005). Cyanobacteria occur even in desert and polar areas (Whitton and Potts, 2012). However, a large percentage share of the identified cyanobacteria (48%) are typical of the moderate climate (Schlichting, 1961).

In their studies conducted in Tesaloniki (Greece) between August 2007 and November 2008, Genitsaris et al. (2011) arrived at similar proportions of particular taxa to those determined in the Southern Baltic Sea. They identified 353 algae species, among which the highest proportions were reached by Cyanobacteria (37.4%) and Chlorophyta (35.4%) (Genitsaris et al., 2011). Other numerous taxonomic groups included: Bacillariophyceae (15.3%) and a newly formulated phylum - Streptophyta (4%). The rest consisted of other phyla such as Pyrrophyta, Euglenophyta and Cryptophyta. The presence of cyanobacteria in aerosols was also found in Varanasi (India), where similarly to the Baltic Sea coast, they were more numerous than Chlorophyta and Bacillariophyta. Such species as *Phormidium* sp. and *Nostoc* sp. were observed by the authors throughout the two-year sampling period (2003–2004) (Sharma et al., 2008). Both these species were also identified within the present studies. On the other hand in 2008 year in Cairo (Egypt), regardless of the season, the following species were found to be present in aerosols: *Chroococcus limenticus*, *Lyngbya lagerheimii* and *Schizothrix purpurascens* (El-Gamal, 2008). Out of the species listed above, only the first one was identified in the atmosphere of the Southern Baltic Sea.

Cyanobacteria and Chlorophyta meanwhile were present in the aerosols of the Southern Baltic Sea coastal zone throughout the sample collection period (Fig. 3). The number of identified taxa (cyanophyta) was at its highest in April 2015 over land station ($n = 25$), stayed high in June over open sea stations ($n = 18$) and dropped successively over the next measurement months over the land (Fig. 3). In November only one species was identified in aerosols (*Aphanocapsa* cf. *planctonica*), probably due to the gradual disappearance of cyanobacteria and microalgae in the Baltic basin as the vegetation period came to an end and winter began (Mazur-Marzec et al., 2013). In the Baltic Sea, the algae growth season starts in spring as the amount of light increases (Putnam and Duke, 1974). At that time cyanobacteria occur in the sea water in the form of individual cells or small colonies (Mazur-Marzec et al., 2013), which increases the likelihood of them being lifted up into the atmosphere. In addition to this, a large number of species identified in aerosols were helped by the wind speed in springtime, which on average amounted to $3.2\ m\cdot s^{-1}$ (Lewandowska and Falkowska, 2013). Some cyanophyta and Chlorophyta present in aerosol collected on land could come from land, e.g. lakes or human-related habitation, but the air mass originated from over the sea (Table 1) pointing rather to their marine origin. In summer, when in the discussed study area they are the dominant taxa in sea water, cyanobacteria occur in marine plankton in large quantities, forming larger colonies and aggregates (Putnam and Duke, 1974; Mazur-Marzec et al., 2013). This is conducive to their quicker lifting towards the surface. The average wind speed over land station in the discussed period was lower than in spring and amounted to $2.0\ m\cdot s^{-1}$. This indicates more stable conditions and a lack of large waves. In such conditons the number of cyanobacterial cells grows quickly close to the water surface (Stal et al., 2003) and this can result in the presence of this organisms in aerosols over sea (June) and over land (August). However, their lifting into the atmosphere is not as effective as in spring. The higher directly proportional correlation between the number of identified cyanobacteria and wind speed was weak, but statistically significant ($r^2 = 0.29$, $p < 0.05$). It follows from HELCOM data for 2015 that, owing to low temperatures in June, the surface cyanobacterial bloom in the Baltic started late- on the last day of July, and continued for four weeks until 25th August (www.helcom.fi). In the first half of August the bloom was observed mainly in the Baltic Proper, and in the second half of the month it was

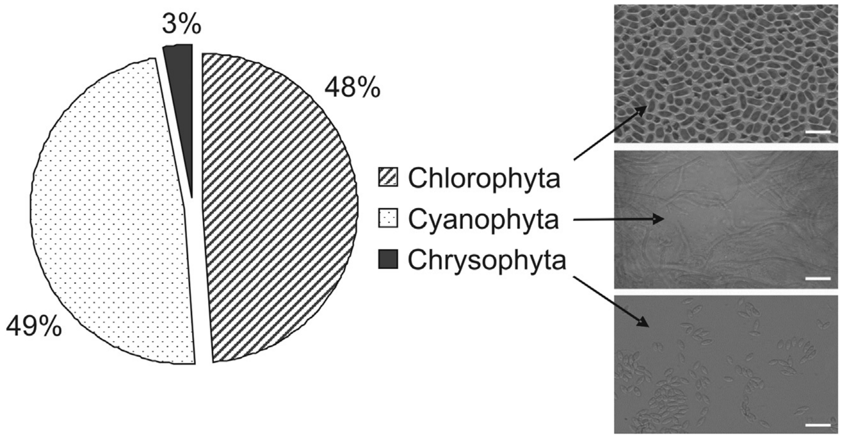

Fig. 2. The percentage share of the phyla identified in aerosols in the Southern Baltic region. Scale bars = 10 μm.

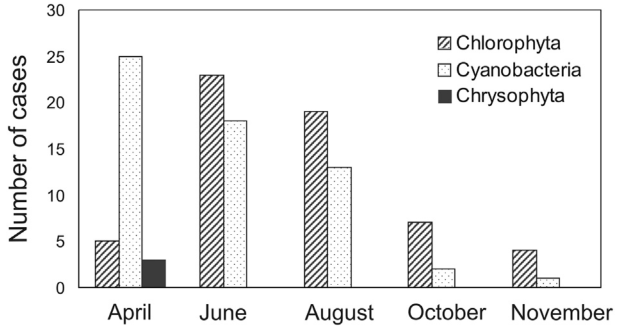

Fig. 3. Variability of the numbers of identified microorganisms in aerosols collected in 2015 in the Southern Baltic Sea zone in terms of particular phyta. In June all taxas were collected on the sea stations while in the remaining months at the land station in Gdynia.

concentrated in the southern part of the Sea of Bothnia. Its last signs in the Baltic Proper were observed on 28th August (www.helcom.fi). Cyanophyta were nevertheless present in water until November 2015 (http://model.ocean.univ.gda.pl), although their concentration was three orders of magnitude lower than in summer (32.3 $\mathrm{mg \cdot m^{-3}}$ and 0.03 $\mathrm{mg \cdot m^{-3}}$, respectively).

Apart from water temperature and the availability of light, another factor regulating the intensity of cyanobacterial bloom in the Baltic Sea is the amount of phosphorus available in surface water. At times when bioaerosol samples were collected, in the period from April to November, phosphates were continually present in sea water (http://model.ocean.univ.gda.pl). Their concentration was at its lowest in June in the open water of the Baltic Sea (12.7 $\mathrm{mg \cdot m^{-3}}$), and peaked in August in the Gulf of Gdansk (39.0 $\mathrm{mg \cdot m^{-3}}$). Moreover, a weak, reversely proportional correlation was found between the number of identified cyanobacteria and phosphate concentration in the sea water ($r^2 = 0.31$, $p < 0.05$). Nitrogen is a limiting compound, yet cyanobacteria are capable of binding this element from the atmosphere and overcome this limitation, forming widespread blooms in July and August and delivering the bound nitrogen to other elements of the ecosystem. It is the end of phosphorus supplies that results in the end of the bloom (Thamm et al., 2004). The variability of nitrate concentration in sea water was of similar nature to that of phosphates (http://model.ocean.univ.gda.pl). The lowest values were observed in June in the open water of the Baltic (2.1 $\mathrm{mg \cdot m^{-3}}$), and reached a peak in August in the Gulf of Gdansk (167.0 $\mathrm{mg \cdot m^{-3}}$). Such high concentrations of both the compounds in the summer period could have been the reasons for the large number of identified cyanobacteria in aerosols collected over sea (June) and over land (August).

In the case of Chlorophyta, the number of identified species was nearly five-fold lower in April over land than in June over sea station (5 and 23 species, respectively) and then decreased successively over land until November 2015, when only 4 species were identified in aerosols. Chlorophyta are a large and very diverse group of eucaryotic algae. They include single-cell organisms, as well as string and multicellular organisms. Among the single-cell organisms the prevalent kind are plankton species that drift in the water column, sometimes very close to the surface, and this may explain their high presence in aerosols (48%). In the Baltic Sea, cyanobacteria occur mainly in summer, while their quantities in spring and autumn are very small (Wasmund and Uhlig, 2003). That was also confirmed by the present studies, in which it was found that as water temperature grows (Table 1), there is a directly proportional increase in the number of cyanobacteria species identified in aerosols ($r^2 = 0.85$, $p < 0.05$). On the other hand, a drop in the concentration of nitrates in sea water translated to a smaller number of these organisms in aerosols ($r^2 = 0.98$, $p < 0.05$). Generally we noted that the dynamic of species in the air was a reflection of their dynamics in the sea water.

All three Bacillariophyta and Ochrophyta taxa (*Phaeodactylum* sp., *Navicula* cf. *perminuta* and *Nannochloropsis* cf. *gaditana*) were present only in aerosols collected over the land in April 2015. In the Baltic Sea the spring microalgae bloom, consisting mainly of Bacillariophyceae, begins when water temperature reaches 2 °C to 4 °C, and the growth pace of these species exceeds one cell division per day (Schultz-Zehden and Matczak, 2012). Seeing as at the starting point of the measurements (10th April 2015) the water temperature was 4.4 °C (Table 1), the development of microalgae was not limited. The Bacillariophyta phylum includes mainly eucaryotic single-cell algae, which are found both in freshwater and seawater. Some Bacillariophyceae have the ability to form mass blooms, sometimes even neustonic ones (Wasmund et al., 1998), and this could be the reason for their presence in bioaerosols. In the Baltic Sea, a mass occurrence of these organisms is observed in spring and in autumn. A lack of Bacillariophyceae in autumn samples, as well as the lack of species belonging to Pyrrophyta, may be related to the occurrence of larger or string organisms, such as *Chaetoceros* sp. or *Skeletonema* sp. at that time, which cannot be actively transported in aerosols (Wasmund et al., 1998).

3.2. *The influence of advection direction on the presence of cyanobacteria and microalgae in aerosols*

Air masses arriving from over the sea can transport nutrients and microorganisms over land, constituting their effective transfer between those two ecosystems (Weathers et al., 2000). Our observation let us to determine that in the Southern Baltic atmosphere the number and type of identified species was affected by the measurement station location and the dominant advection direction. The determination of air mass trajectories (http://ready.arl.noaa.gov/HYSPLIT.php) made it possible to establish that the air over both the land and the sea station during the studies came mainly from over the sea (Fig. 4). In relation to the above,

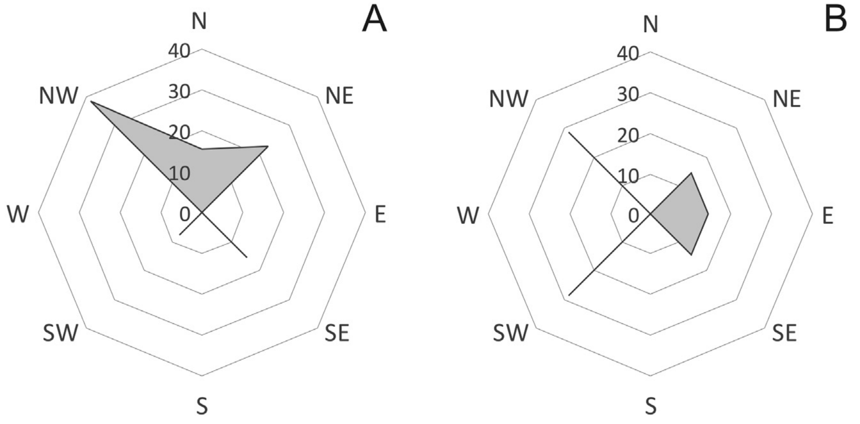

Fig. 4. The dominant advection direction, determined on the basis of trajectories of air mass movement (http://ready.arl.noaa.gov/HYSPLIT.php) over the land station (A) and the marine station (B) in the study period.

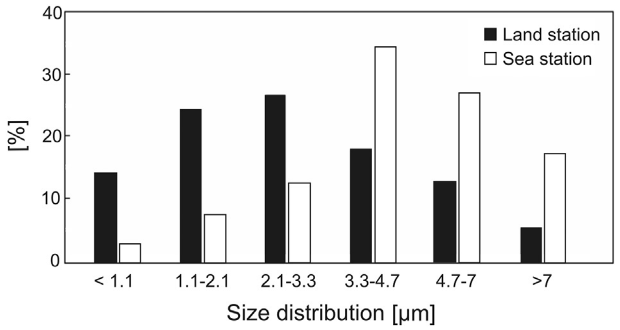

Fig. 5. The percentage share of identified species in the distribution function of aerosol sizes and locations of measurement sites.

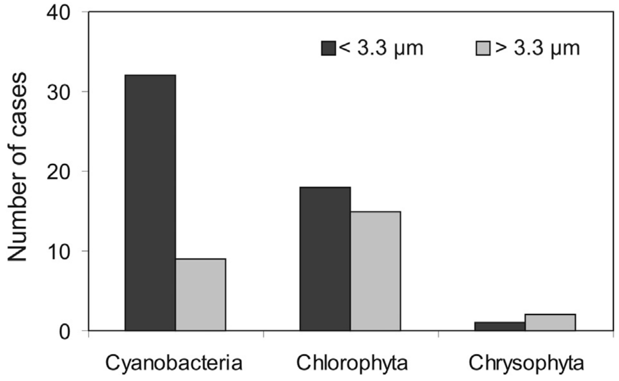

Fig. 6. The classification of the studied microorganisms into phyta with respect to particle size at the land station.

the reasons for the differing distributions of bioaerosol sizes in the atmosphere over the land and over the sea need to be looked for in the duration of particle transportation from the emission source to destination. Over the sea, bioaerosols generally were identified in particles of > 3.3 µm in diameter (78%) (Fig. 5). Over land there was a reverse tendency and as much as 65% of cyanobacteria and microalgae occurred more frequently in aerosols not exceeding 3.3 µm in diameter. Atmospheric movements can affect the spread of water microorganisms over long distances from their emission source, regardless of whether they originate from pelagic or bottom habitats. In situations when algal and cyanobacterial blooms occur, with advection from the sea, the organisms are transported over land areas (Lacey and West, 2006; Després et al., 2012). Small size particles are particularly likely to be transported (Marshall and Chalmers, 1997; Finlay, 2002; Lacey and West, 2006). Depending on several factors, the average residence time of various biological particles in the atmosphere can range from less than a day to a few weeks (Després et al., 2012). The time spent by bioaerosols in the atmosphere is a result of the forces of attraction (mainly gravitational force connected to the mass of the organism itself) and repulsion, which keeps the organism in the atmosphere (mainly resistance related to the size, density and shape of the organism), as well as such meteorological factors as wind speed or direction, and atmospheric precipitation (Rosas et al., 1989; Figueiras et al., 2006; Tesson et al., 2016). During the measurement period the mean wind speed was higher over the sea, where it amounted to 4.1 m·s^{-1} (0.3–6.7 m·s^{-1}), than in Gdynia, where it was on the level of 2.7 m·s^{-1} (1.6–6.5 m·s^{-1}). Wind speeds at such levels can affect the generation of marine aerosols and their transport on both a local and regional scale (Lewandowska and Falkowska, 2013). Over the sea most cyanobacteria and microalgae could have therefore been related to their emission with aerosols from the sea surface and smaller aerosol particles reached the air over land. In addition, we have determined that over land the potentially toxic cyanobacteria and microalgae were also most often present in small aerosols, not exceeding 3.3 µm in diameter (Fig. 6). All of the above-mentioned taxa are characteristic of the Baltic Sea and as part of smallest particles they were able to remain in the atmosphere for longer and be carried over long distances from the source (Marshall and Chalmers, 1997; Finlay, 2002; Lacey and West, 2006). Similar results obtained Marshall and Chalmers (1997) in their long-term aerobiological studies conducted in the Antarctic. They have found Chlorophyta endospores, as well as Cyanobacteria strings, which confirmed their ability to be carried over long distances with air masses. An interesting example of algae from remote areas being transported over the land station in Gdynia can be found in *Gloeothece* sp., which is untypical for the Baltic Sea but was identified in aerosols on 23th April 2015 in the atmosphere over Gdynia. According to literature, this species could originate from the Arkona basin as it is very rarely found in the Southern Baltic Sea (Hällfors, 2004). Its presence could have resulted from the transportation of organic matter with northwestern air mass advection at a mean wind speed equal to 6.5 m·s^{-1} (Table 1).

Presence of potentially toxic cyanobacteria and microalgae in small aerosols, not exceeding 3.3 µm in diameter in the atmosphere over land can indicate a higher threat to human health. These are aerosols which, owing to their small dimensions, penetrate deeper into the human body, can settle in the bronchi and air sacs, and consequently lead to a number of illnesses such as cardiovascular problems, respiratory illnesses or even cancers (Franck et al., 2003; Zhang et al., 2014). Daily an adult inhales about 12.3 m^3 (i.e. 16 kg) (Mahajan, 2006), therefore every component of the inhaled mixture has an effect on the functioning of the human organism, even those occurring in small concentrations.

3.3. *Toxic species in aerosols over land*

The lifting of matter from surface water results in its transfer to the atmosphere. This can lead to e.g. emission of pathogenic bacteria or toxic microalgae, which negatively affect human health (Dueker et al., 2012). Among the identified cyanobacteria and algae, over 15% trigger allergies or produce toxins (Genitsaris et al., 2011). In the current study six Cyanobacteria and Chlorophyta taxa that might produce harmful secondary metabolites and negatively affect human health were identified. These include Cyanobacteria of the genus *Microcystis*, *Synechococcus, Synechocystis, Aphanocapsa* and *Merismopedia*, as well as one Chlorophyta species, *C. vulgaris*.

Toxins are the best identified out of all the compounds produced by cyanobacteria. This is due to their influence on human health and economic loss resulting from the disruption in the functioning of the natural environment. The most common cyanobacterial toxins are hepatoxins: nodularin and microcystin, the former of which is produced by *Nodularia spumigena* - an inhibitor of protein phosphatase of serine/threonine type 1 and 2A (Rastogi and Sinha, 2009; Jakubowska and Szeląg-Wasilewska, 2015; Mazur-Marzec et al., 2015). In terms of chemical structure, nodularin is a cyclic peptide composed of five aminoacid parts. This compound is well-soluble in seawater. Owing to the polar nature of the particle, nodularin transportation through biological membranes of animal, plant and bacteria cells is restricted. The only exception is the liver, where a presence of active nodularin transport has been discovered in hepatocytes. Nodularin is one of the most potent natural toxins ($LD_{50} = 50\ \mu g\cdot kg^{-1}$ body mass) (Jakubowska and Szeląg-Wasilewska, 2015). In the Southern Baltic area, though, during studies conducted in 2015 into bioaerosols collected both over land and over sea, *N. spumigena* was not identified. However, there were four types of planktonic cyanobacteria: *Synechococcus*, *Synechocystis, Aphanocapsa* and *Microcystis,* which are able to produce microcystins (Jakubowska and Szeląg-Wasilewska, 2015). Meanwhile, Furtado et al. (2009) observed that other picoplanktonic cyanobacteria of the *Merismopedia* type are also capable of producing microcystins. ELISA assays detected a concentration of microcystins in these *Merismopedia* sp. cultures of $2.2\ \mu g\cdot l^{-1}$. Owing to the polarity of microcystins, similarly to nodularins, their transportation through cell membranes is possible only via carriers enabling the transport of bile acids. An effect of microcystin activity is liver deficiency, internal liver haemorrhage, and in cases of severe poisoning, even death. Moreover, microcystins trigger apoptosis and necrosis of liver cells, and are promotors of cancer tumours (Rastogi and Sinha, 2009; Jakubowska and Szeląg-Wasilewska, 2015).

The latest literature reports indicate that, apart from hepatoxins, picocyanobacteria are capable of producing and discharging many other secondary metabolites. Picocyanobacteria, including a few strains of *Synechococcus, Synechocystis* and *Aphanocapsa*, synthesize 2-methylisoborneol and geosmin (1,2,7,7-tetramethyl-2-norborneol), secondary metabolites (Jakubowska and Szeląg-Wasilewska, 2015). These organic compounds can cause serious problems as the human taste and odour detection thresholds for these compounds are between 5 and $10\ ng\cdot l^{-1}$ (Graham et al., 2008). However, Journey et al. (2011) noted concentrations of picocyanobacteria reaching as high as $100\ ng\cdot l^{-1}$, as observed in Lake Bowen (South Carolina, USA). Moreover, other studies conducted on *Synechococcus* sp. BP-1 have found that they have the ability to synthesize a unique compound - thionsulfolipid. It has been shown that the compound has toxic properties on HL 60 human lymphoma cells (Kunimitsu et al., 1993). Additionally, Śliwińska-Wilczewska et al. (2016) showed that Baltic picocyanobacterium of the genus *Synechococcus* are able to produce and release unidentified allelopathic compounds that have a negative effect on the surrounding ecosystem.

Beside the abovementioned Cyanobacterial species, Chlorophyta were also present in the aerosols of the coastal zone of the sea. Some of them (e.g. *C. vulgaris*) have the ability to excrete active secondary metabolites. One of them is chlorellina, which was isolated at the beginning of the 20th century by Pratt et al. (1944). Moreover, *C. vulgaris* is capable, during growth, of producing acetic acid, glycolic acid, lactic acid, pyruvic acid, tx-ketaglutaric acid, and acetoacetic acid (Dakshini, 1994).

4. Conclusions

In the period from April to November 2015, studies into bioaerosols were conducted in Gdynia (coastal zone of the Southern Baltic) and in the open Baltic Sea (the Gulf of Gdansk, South-Western Baltic). It was found that cyanobacteria and microalgae, having been identified both in the atmosphere over the sea and over land during the entire study period, are omnipresent microbiological air pollutants. Some of them had been transported from remote areas, such as *Gloeothece* sp. - a species not typical for the Southern Baltic Sea and could originate from the Arkona Basin.

The higher the primary production in sea water and the concentration of phytoplankton in it, the greater the diversity in terms of the microorganisms observed in the collected bioaerosol samples. Other important factors were: water temperature, accessibility of light and the amount of available phosphorus and nitrogen in the surface water.

In the atmosphere over land, microorganisms dominated in aerosol particles of smaller dimensions ($< 3.3\ \mu m$ in diameter). Over the sea, there was a reverse situation. That resulted from the fact that smaller aerosols could be better distributed over long distances. Among the identified microorganisms were species which pose a threat to human health and life: *Synechocystis* sp., *Synechococcus* sp., *Microcystis* sp. and *C. vulgaris*. Seeing as in the surrounding air, part of the everyday environment of the human habitat, those species were incorporated into small, respirable particles, it is necessary to conduct further research. Only in this way will it be possible to gauge the risk related to bioaerosols in terms of their influence on human health. Future characterisations should focus on the role of microalgae and cyanobacteria present in the air in the transportation of radionuclides, heavy metals, pesticides and herbicides, as well as carcinogenic and mutagenic substances, into living organisms including humans.

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