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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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A B S T R A C T

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 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 μmol photons·m^{-2}·s^{-1}. The intensity of PAR was measured using a quantum-meter (LI-189, LI-COR Inc., Nebraska, USA) with a cosine collector.