Phototrophic microflora colonizing substrates of man-made origin in Billefjorden Region, Central Svalbard

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Abstract

Substrates created by human have a significant impact on Arctic terrestrial environment. These substrates are new potential niche for microbial biota, which may have several essential chemical agents supporting microbial growth. Wood, concrete, brick, ceramic and other different building materials, which have been introduced by human in this isolated environment, are colonized with terrestrial and aero-terrestrial microorganisms living in the natural niches near the substrates like soil, rocks, *etc.*, but these materials newly-introduced to Svalbard terrestrial ecosystems can also work as vectors for invasion of new species into the environment. We have collected different types of artificial substrates mainly in the region of Petuniabukta bay and studied the species composition of microbial phototrophs living there. A total of 25 taxa of cyanobacteria and algae were documented on different types of substrates like brick walls, concrete, glass, iron, wood and plastic. A commonality in species diversity was observed with similar substrates in temperate climatic regions. *Fottea stichococcoides*, *Sphaerococcomyxa olivacea*, *Polysphaera composita* and *Diplosphaera chodatii* were first time recorded from Svalbard Archipelago.

Key words: cyanobacteria, algae, anthropogenic substrates, Arctic

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Introduction

The study on the cyanobacterial and algal flora of Svalbard is represented by a few principal works only (*e.g.* Oleksowicz et Luścinska 1992, Skulberg 1996, Leya et al. 2000, Kaštovská et al. 2005, Stibal et al. 2006, Matuła et al. 2007, Kim et al. 2008, Richter et al. 2009, Kim et al. 2011, Kvíderová 2012, Raabová et Kováčik 2013). Arctic environments are considered to be relatively pristine and stable because of the absence of intensive local human activities. Ecosystems in Arctic are, however, highly sensitive to human impact (Wojtuń et al. 2013). Most of the artificial substrates are introduced in these ecosystems by human only. These substrates such as glass, brick, concrete, asphalt and metal provide a suitable habitat for colonization of phototrophic microorganisms. The major sources of cyanobacteria and algae, which colonize on such "new habitats" are soil and aquatic environments around (Brown et al. 1964, Broady 1996, Sharma et al. 2007, Sahu et Tangutur 2014). Terrestrial cyanobacteria and algae are pioneer organisms, which colonize habitats potentially unavailable for living organisms and transform them, allowing other groups of organisms to colonize there (Kováčik 2000, Graham et al. 2009, Nowicka-Krawczyk et al. 2014). The colonization process starts typically with the less complex organisms (*e.g.* bacteria, cyanobacteria, algae and fungi) and tends to more complex ones like mosses and plants (Coutinho et al. 2015). Microorganisms growing on such habitats are therefore frequently subjected to extremely high UV irradiance and extreme dehydration (Karsten et al. 2005, Rindi 2007). Cyanobacteria and algae are also capable of forming specialized structures like *e.g.* heterocyst in filamentous cyanobacteria fixing nitrogen and together with other survival strategies, such as the production of protective sheaths, which allow them to survive in hostile environments by creating biofilms. The biofilms allow the retention of large amounts of water into its structure protecting the cells from rapid desiccation (Coutinho et al. 2015). The development of biofilms depends on the values of certain environmental parameters, such as high humidity and presence of light (Mandrioli et al. 2003). Once established, algal biofilms attack heterotrophic bacteria, fungi and protozoa thereby forming complex microbial biocenoses (Karsten et al. 2007). Green algae are the most ubiquitous members of microbial flora colonizing the aero-terrestrial substrates (Karsten et al. 2010). The species composition on the anthropogenic substrates depends on the microclimatic conditions and the sources of biota near the substrates, thus showed a wide variation in different ecoclimatic zones around the globe (Mandrioli et al. 2003, Coutinho et al. 2015). Cyanobacteria dominate the aero-algal flora of tropical regions, while chlorophytes dominate the temperate regions (Brown et al. 1964, Sharma et al. 2007, Adhikary et Kováčik 2010). Identification of most of the terrestrial cyanobacteria and microalgae based on morphological features is difficult and requires extensive observation both natural samples as well as their different growth phases in laboratory culture (Broady 1996, Rindi 2007). Thus in most of the published records the identified taxa were up to genus level only (Rindi 2007).

In polar regions, the aero-terrestrial habitats like artificial substrates were poorly studied. The newly introduced man-made substrates are colonized by cyanobacteria and green algae, which principally grow in epiphytic and epilithic conditions (Karsten et al. 2007, Raabová et Kováčik 2013). The study of these substrates showed an abundance of cyanobacteria and chlorophytes (Broady 1996, Olech 1996, Marshall et Chalmers 1997). It depends on the microbial composition on nearby natural habitats, from where the microbes migrate to the artificial substrates (Mandrioli et al.

2003, Sharma et al. 2007). The dominant species within these communities are mainly filamentous forms of cyanobacteria and chlorophytes along with some unicellular chlorophytes (Ortega-Calvo et al. 1995, Kováčik 2000). These substrates may house several novel organisms as well as their process of colonization is interesting to study.

The aim of this study was to characterize the biodiversity of photosynthetic microorganism communities on these anthropogenic substrates specifically in Arctic region.

Material and Methods

Samples were collected from central part of Svalbard Archipelago in Billefjorden region during August 2014 by specialists studying different man-made origin substrates like wood, stone wall, iron part of meteo-station, glass window, *etc.* (Table 1, Fig. 1). The substrates were categorized into four groups: (1) brick, concrete, masonry, ceramic tile, (2) metal, (3) wood, (4) glass and plastic parts of instruments and buildings. Samples were collected in Eppendorf tubes using a staminode. The tubes were transported to the laboratory and isolated to unialgal cultures following standard laboratory techniques. The cultures were maintained in Petri dishes and test tubes of 2% agar with Z medium (Zehnder in Staub 1961) or agar solid medium with 1.5% agar containing the mineral nutrient medium BG11 (Rippka et al. 1979). Cultures were maintained in a growth chamber with 12:12 hr light:dark photoperiod of cool-white fluorescent illumination (4400 lx, PAR 12 $W.m^{-2}$, 48.6 μmol photon m^{-2} s^{-1}) at 15 – 18°C. Detailed morphology of isolated phototrophs was analyzed using the light microscope Leica DM 2500 with Nomarski DIC optics equipped with Leica DFC 290 HD digital camera. Images were worked up with Leica Application Suite Version 3.5.0 – Build: 710 software. Several monographs (Ettl et Gärtner 1995, Komárek et Anagnostidis 2005) and research papers (Broady 1979, Kostikov et al. 2002, Matuła et al. 2007) were followed for identification of the algal strains. Quantification occurrence of individual species cultured in Petri dishes were broadly categorized into three types (1) *rare species*, represented only by one or two colonies on Petri dish, (2) *more frequent species*, represented up to 25% of the colonies on Petri dish, and (3) *dominant species*, represented by more than 25% of the colonies on Petri dish. All the strains were deposited at the Working Culture Collection of Algae at Department of Botany, Comenius University in Bratislava.

No.	Sampling site	Habitat	Geographic coordinate
1	Longyearbyen, airport runway	small concrete pillar	N 78.2468/E 15.4914
2	Longyearbyen, airport	iron road sign	N 78.2468/E 15.4915
3	Mathiesondalen, lighthouse	iron part	N 78.5739/E 16.5589
4	Pyramiden, Swedish Houses	outside masonry	N 78.6578/E 16.3214
5	Pyramiden, Swedish Houses	inside masonry, near ground	N 78.6578/E 16.3214
6	Pyramiden, walk near Post Office	concrete sidewalk	N 78.6552/E 16.3081
7	Pyramiden, building near Post Office	outside brick wall	N 78.6552/E 16.3081
8	Pyramiden, wall of kindergarten house	ceramic tile	N 78.6537/E 16.3262
9	Petuniabukta, Czech Station hut	glass window	N 78.6804/E 16.4546
10	Petuniabukta, Czech Station hut	wood wall	N 78.6804/E 16.4546
11	Petuniabukta, Czech Station hut	plastic part of equipment	N 78.6804/E 16.4546
12	Petuniabukta, Czech Station hut	wood board	N 78.6804/E 16.4546
13	Petuniabukta, meteo AWS	iron construction	N 78.7013/E 16.4645

Table 1. General characteristics of sampling sites (*No. - locality number*)

Taxon/ Sample site	1	2	3	4	5	6	7	8	9	10	11	12	13
CYANOBACTERIA													
Nostoc commune		-	-	-	-	-	-	2	-	-	-	-	-
Nodosilinea epilithica	1	-	-	1	-	-	-	-	-	-	-	-	-
Leptolyngbya sp.	-	-	-	1	-	-	-	-	-	-	-	-	-
OCHROPHYTA													
Tribonema sp.	-	-	-	-	1	-	-	-	-	1	-	-	-
Xanthonema exile	-	-	-	-	-	-	-	1	-	-	-	-	-
CHLOROPHYTA													
Diplosphaera cf. *chodatii*	-	1	-	-	-	-	-	2	-	-	-	-	-
Myrmecia sp.	-	-	-	-	-	-	-	-	-	2	1	-	-
Myrmecia bisecta	-	-	-	-	-	-	-	1	-	2	-	1	-
Muriella sp.	2	-	-	-	-	-	-	-	3	-	-	-	-
Muriella decolor	-	-	-	-	-	2	-	2	-	-	-	-	-
Leptosira sp.	-	-	-	-	-	-	-	-	-	1	-	-	-
Stichococcus minor	-	-	1	-	-	-	-	3		3	-	-	-
S. bacillaris	3	3	3	-	3	3	-	-	-	-	-	3	3
S. chloreloides	-	2	-	3	-	-	-	-	-	-	-	-	-
S. minutissimus	-	-	-	2	-	-	-	-	-	-	-	-	-
S. undulatus	-	-	-	-	2	-	-	-	-	2	-	-	-
Prasiola cf. *crispa*	-	-	-	-	-	-	1	-	-	-	-	-	-
Bracteacoccus minutus	-	-	-	-	-	-	-	2	-	-	-	-	-
B. minor	-	-	-	-	-	-	3	-	-	2	3	-	-
Coleochlamys oleifera	-	-	-	2	-	-	-	-	-	-	-	-	-
Sphaerococcomyxa olivacea	-	-	-	-	-	-	-	1	-	1	-	-	-
Polysphaera composita	-	-	-	-	-	-	-	-	-	2	-	-	-
Fottea stichococcoides	-	-	-	-	-	-	-	-	-	1	-	-	-
STREPTOPHYTA													
Klebsormidium sp.	-	-	-	-	-	1	-	1	-	-	-	-	-

Table 2. List of determined taxa from man-made origin substrates with numerical values for the number of colonies. (1 – *rare species*, represented only by one or two colonies on Petri dish, 2 – *more frequent species*, represented up to 25% of the colonies on Petri dish and 3 – *dominant species*, represented by more than 25% of the colonies on Petri dish).

Results

A total of 25 cyanobacterial and algal taxa were identified on the man-made origin substrates of investigated area (Table 2, Fig. 2). However, in most morphospecies, the exact identification into traditional morphologically defined species was not possible. Consequently, most taxa were designated by names indicating their taxonomic affinity according to their morphological characteristics. Their exact taxonomic evaluation will be made through polyphasic approach in future. Chlorophyta were the most abundant on this type of substrate. Cyanobacteria, Ochrophyta and Streptophyta were also presented, but not in adequate amount. The most abundant genus was *Stichococcus*, present abundantly in almost each sample. Poor diversity was observed on the groups 2 and 4 substrates, with only 1 – 3 taxa per site. The most abundant genus on these types of substrates was *Stichococcus*, along with less abundant *Myrmecia, Muriella* and *Diplosphaera*. Groups 1 and 3 were rich on algae and cyanobacteria as well, which is may be due to the high porosity and better chemical composition of these substrates. The species were most abundant on localities 8 and 10, *i.e.* ceramic tile on wall of kindergarten house in Pyramiden and wooden wall on Czech Station hut in Petuniabukta. The most abundant genera on these substrates were *Stichococcuss*, *Muriella* and *Bracteacoccus*. Green algal taxa, *Fottea stichococcoides*, *Sphaerococcommyxa olivacea, Polysphaera composita* and *Diplosphaera chodatii* were the new distributional records from the Svalbard Archipelago.

***Sphaerococcomyxa olivacea* (Boye-Petersen) Kostikov, Darienko, Lukešová et Hoffmann 2002 (Fig. 2B)**
Syn.: *Coccomyxa olivacea* (Boye-Petersen 1915)
Cells spherical or ellipsoid with rounded ends. Chloroplast single, parietal, without pyrenoid. Cells 5 – 8(10) µm long and 2.5 – 3(5) wide. Reproduction generally by 2 autospores liberated after extension and partial gelatinisation of the sporangial cell wall with formation of Gloeocapsa-like clusters inside colonies.

***Fottea stichococcoides* Hindák 1981 (Fig. 2C)**
Solitary or individual cells irregularly grouped in colonies, embedded in homogeneous mucilaginous layer of varying shape. Cells cylindrical, straight or sometimes slightly curved, with broad rounded ends. Chloroplast single, parietal and massive without pyrenoid. Cells (5)10 – 15 µm long and (2.5)3 – 5 µm wide. Reproduction by aplanospores.

***Polysphaera composita* Reisigl 1964 (Fig. 2D)**
Cells sphaerical or ellipsoid with firm cell wall. Each cell with 4 or more chloroplasts without pyrenoid. Cells form compact irregular colonies, sometimes with foliaceous-like structures. Cells 2.5 – 5 µm long and 1 – 3(5) µm wide. Reproduction by aplanospores.

***Diplosphaera chodatii* (Bialosuknia 1909) em. Visher 1960 (Fig. 2E)**
Syn.: *Stichococcus diplosphaera* (Chodat 1913), *S. chodatii* (Bialosuknia) Heering 1919, *Protococcus chodatii* (Bialosuknia) R. et F. Chodat 1924
Single cells sphaerical or elipsoides, (5)6 – 10 µm long and (2.5)3 – 5 µm wide. Chloroplast (chromatosphore?) parietal, pale green with one small indistinct pyrenoid. Cells appear usually in clusters, pairs and single cells are also visible. Reproductions by autospores only.

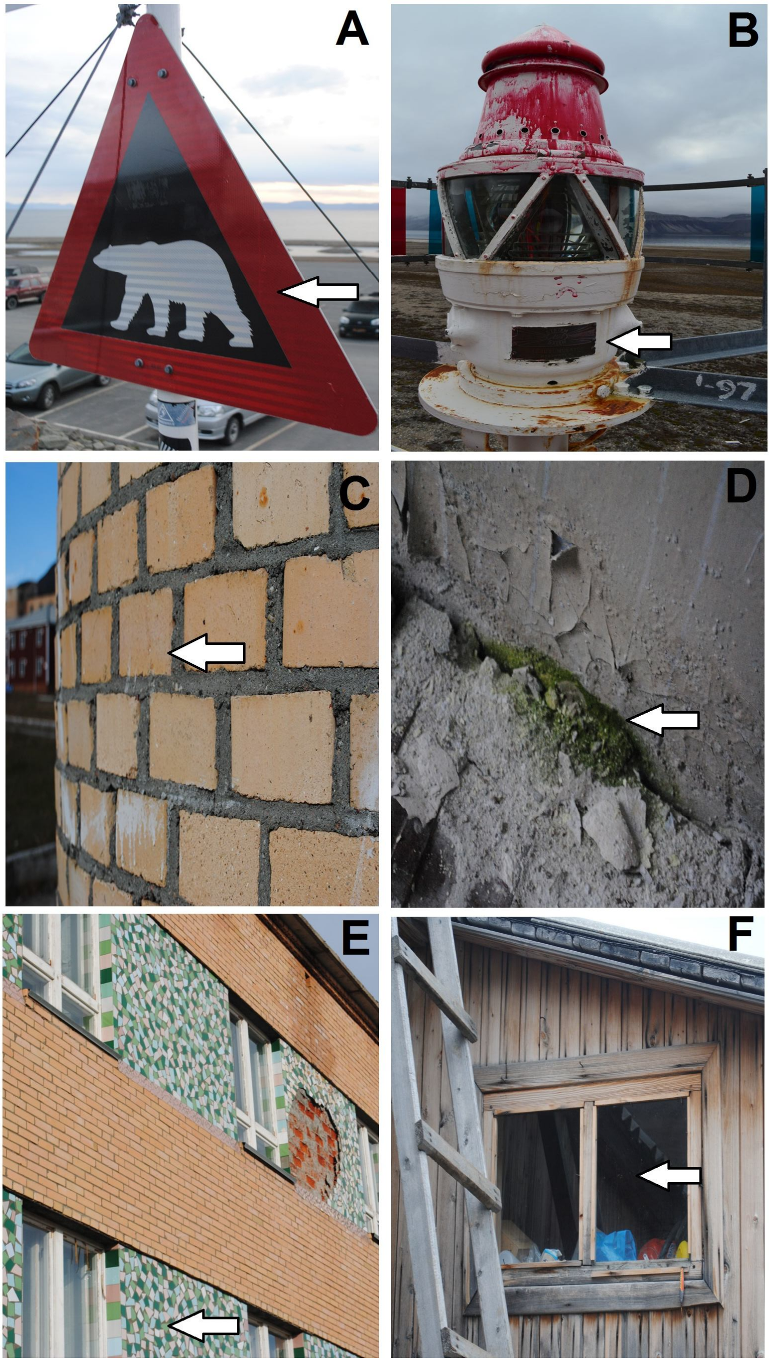

Fig. 1. The different man-made origin substrates at the central part of Svalbard: A – iron made road sign, Longyerbyen B – Mathiensondalen, lighthouse, C – Pyramiden, brick wall, D – Pyramiden, Swedish House, inside masonry, E – Pyramiden, kindergarden house, ceramic tile, F – Petuniabukta, Czech Station hut, wood wall.

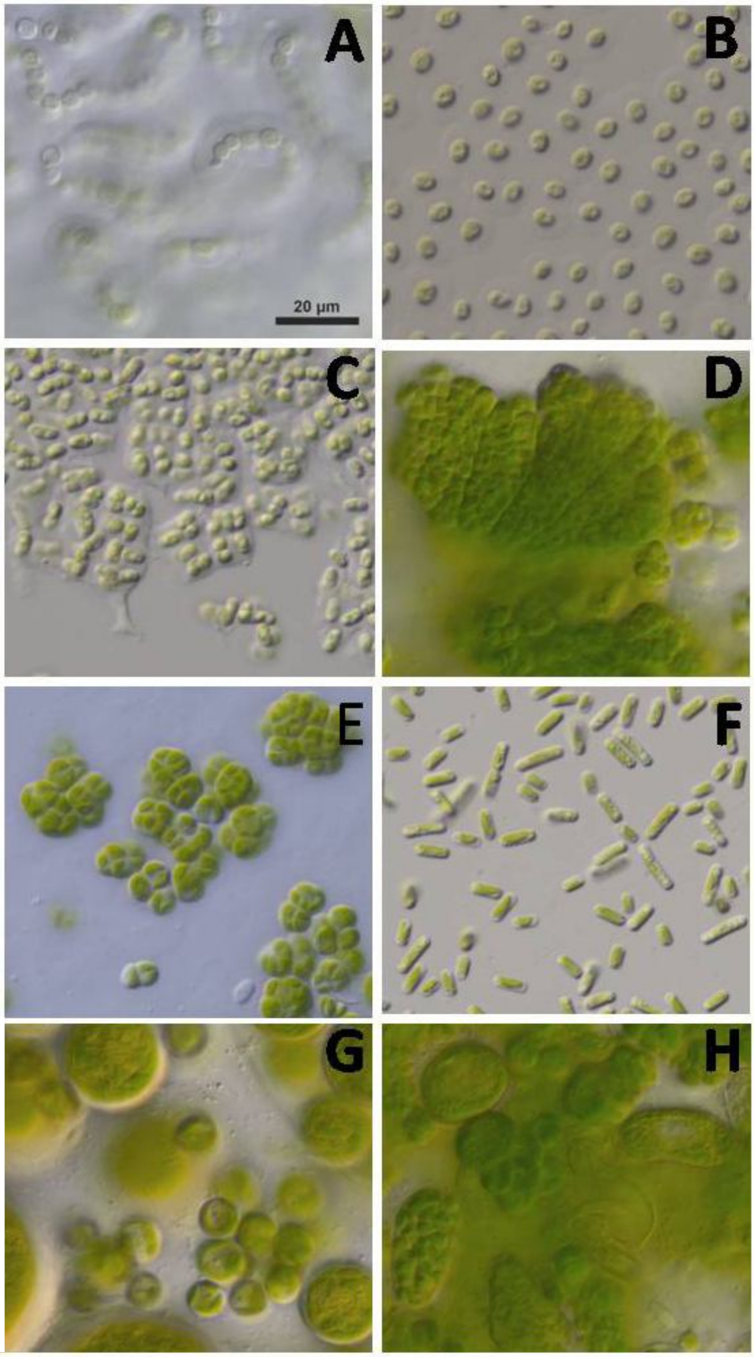

Fig. 2. Illustrative photomicrographs of different taxa described from studied anthropogenic substrates: A – *Nostoc commune*, B – *Sphaerococcomyxa olivacea*, C – *Fottea stichococcoides,* D – *Polysphaera composita*, E – *Diplosphaera chodatii,* F– *Stichococcus bacillaris*, G – *Bracteacoccus minor*, H – *Coleochlamys oleifera.*

Discussion

It was observed that the algal mats in polar region contained unicellular and filamentous algae and filamentous cyanobacteria. Unlike the ceramic substrates and glazed wall tiles in non-polar regions of the globe, which were mostly colonized by *Chlorella*, the similar substrates in Svalbard showed a dominance of *Stichococcu*s sp., *Diplospahaera chodatii* and *Bracteacoccus minutus* and the widespread cyanobacterial genus *Nostoc*. In comparison to other natural habitats in Petuniabukta bay, the cyanobacterial population is comparatively lower in anthropogenic substrates. Komárek et al. (2012) documented 85 cyanobacterial morphospecies, specifying the dominance of different taxa different biotopes. Davydov (2014) also surveyed the Pyramiden area and reported 73 cyanobacterial taxa with *Nostoc commune*, *Microcoleus autumnalis* and *Calothrix parietina* as the most abundant species. Aero-terrestrial cyanobacteria and algal communities may develop in all climate zones of the Earth (Nowicka-Krawczyk et al. 2014). In temperate and tropical zones this type of organisms are well known, from building facade and historical monuments (Samad et Adhikary 2008, Coutinho et al 2015). In temperate zone these communities are largely formed by unicellular and filamentous forms of green algae, while in warmer tropical climate, the communities are dominated by cyanobacteria that produce various kinds of mucilaginous envelopes protecting them from excessive drying (Samad et Adhikary 2008, Nowicka-Krawczyk et al. 2014).

Despite a long history of interest in terrestrial algae in polar environments the substrates of man-made origin are not included in ecological studies generally. This review is focused primarily on aero-terrestrial cyanobacteria and algae colonizing these substrates in Svalbard Archipelago and to understand if the actual local economic activities in Arctic have impact on its biodiversity.

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