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REVIEW

What is known about Cyanoprokaryota and the algal blooms along the Bulgarian Black Sea coast: an overview

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ABSTRACT

This article presents a summary of the research related to the taxonomic composition, algal blooms and toxic potential of representatives from Cyanoprokaryota (Cyanobacteria, Cyanophyta) in the coastal waters of the Bulgarian Black Sea area. Main strands of the algal research, including Cyanoprokaryota, are presented in a chronological order. The taxonomic composition of cyanoprokaryotic microorganisms, their importance as a part from the algal flora of the Bulgarian Black Sea coast and the main problems caused by this group organisms are discussed. This information could be used as a basis for future investigations related to the taxonomy, diversity, distribution and evaluation of the biological activity of cyanoprokaryotic species in the Black Sea and particularly in the Bulgarian coast area.

Key words: Cyanoprokaryota, Cyanobacteria, abundance, Black Sea, algal blooms

Introduction

Marine waters, including estuaries, coastal zones and the open waters, usually are rich in planktonic cyanoprokaryotes. Most of the cyanoprokaryotic groups are presented in the sea plankton, but the specific environmental factors determine the prevalence of some or other representatives. All methods used in phytoplankton investigations (microscopic, flowcytometric and fluorescent) determine the simplest unicellular forms of Cyanoprokaryota as the most common in the marine basins. These are the non-nitrogen-fixing cyanobacterial genera *Synechococcus*, *Chroococcus*, *Prochlorococcus*, *Synechocystis* and some recently described nitrogen-fixing genera such as *Crocosphaera* (Moisander et al., 2010; Paerl, 2012). Marine planktonic cyanoprokaryotes are very diverse in their morphology, physiology and ecology, and often are considered as the dominant phytoplankton fraction associated with the production of primary biomass (Paerl, 2000). Those of them, which under certain environmental conditions (eutrophication, high temperatures, lack of vertical mixing of water) can reach blooming concentrations, are of high interest. Algal blooms have significant biochemical and ecological importance. Such concentration of phytoplankton biomass inevitably has an impact on the carbon cycle, nutrients (N, P, Fe, trace elements) and the oxygen levels. Some cyanoprokaryotic species are identified as hazardous (CyanoHABs), because during blooming adversely affect the water quality and change the habitat conditions producing toxins, enhancing the hypoxia and altering the food chains. Production of cyanotoxins is the reason that provokes the interest of the scientists to these organisms from many years. Cyanotoxins are a large group of alkaloids, low molecular weight cyclic peptides or secondary metabolites, with negative effects on the humans, animals, plants and eukaryotic microorganisms. Traditionally, the studies related to the cyanotoxins are focused on two major groups - hepatotoxins and neurotoxins.

In recent years, the algal blooms (including potentially toxic species) in the Sea of Azov and Black Sea are considered as most urgent issues in comparison with other environmental problems. Cyanotoxins are associated usually with the freshwater blooms, but during the last years more cyanobacterial blooms are occurring in the estuaries and marine waters (Sivonen et al., 1990; Paerl, 2000; 2011; Miller et al., 2010).

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REVIEW

As a consequence of the pollution over the last 30 years, the cyanoprokaryotic blooms are common in many places in the coastal area of the Black Sea. They often result in death of a huge number of fish and other marine organisms.

Black Sea is a closed continental sea, with weak inflow of bottom-hugging Mediterranean waters and huge river inflow (Don, Dnieper, Dniester and Danube rivers), low salinity and low temperature. These features put the Black Sea on the top places in the list of the most endangered from ecological disaster seas in the world. One of the main forms of pollution in Black Sea is the high degree of eutrophication, which significantly increases the levels of organic matter, both in the water and on the bottom. The process of eutrophication is one of the main causes of algal blooms. In last two decades, cyanoprokaryotic blooms with high intensity have been registered along the Black Sea coastal area of Russia, Ukraine, Romania (Bodeanu et al., 1998; Bodeanu, 1993, 1995, 2002) and Turkey (Uysal et al., 1998; Koray, 2004; Oguz, 2005).

The information about toxic species and toxic potential of Cyanoprokaryota in the Bulgarian Black Sea coast is still very limited. There is only one report for a cyanoprokaryotic bloom of *Oscillatoria sp*. (Moncheva et al., 2001). Although there are several reports about the species composition of Cyanoprokaryota in Black Sea, the information related to the toxic species and their toxic potential is incomplete, which is due mainly to the limited detection methods that are based primarily on the morphological identification by light microscopy. This article aims to summarize the information related to the algal blooms in the Bulgarian Black Sea coast and to focus our knowledge on the species composition of Cyanoprokaryota and those of them that are considered as a potential threat. Despite the lack of mass cyanoprokaryotic blooms in the Black Sea coast, this problem should not be underestimated.

Studies on the phytoplankton in the Bulgarian Black Sea coast

The first studies on the algae in Bulgaria are from 1890. Within the programme "National Strategy on the Biodiversity" Vodenicharov et al. (1993) summarized the information about the algal flora in Bulgaria for almost a 100-years period (1890-1993). For the period 1993-2003, the information related to the biodiversity of algae from natural pools and habitats, including the Black Sea, was updated by Temniskova et al. (2005).
According to them, the composition of the phytoplankton in the Bulgarian Black Sea coast consists of 302 species, varieties and forms belonging to 125 genera (Table 1). Of these, 13 species from 9 genera belong to division Cyanoprokaryota (Temniskova et al., 2005).

The phytoplankton in Black Sea consists mainly Dinophyta and Bacillariophyta, as well as some representatives of Chrysophyta, Chlorophyta, Cyanoprokaryota and Euglenophyta (Temniskova et al., 2005). Dinophyta algae are dominant (41.7%) followed by the diatoms (Bacillariophyta) (36.4%). During the last 50 years, Dinophyta gradually shifted the diatoms, which is due to the progressive eutrophication (natural and anthropogenic). Along with the taxonomic biodiversity of the phytoplankton, most studies until 2003 are directed to investigate the functional characteristics of the algae such as biological cycles, ecophysiological characteristics of massive species, chemical composition including methods for monitoring and analysis.

Table 1. *Biodiversity of the phytoplankton in the Bulgarian Black Sea coast (according to Temniskova et al., 2005).*

Division	Genera	Species	Varieties and forms
Cyanoprokaryota	9	13	-
Chrysophyta (incl. *Primnesiophyta*)	18	22	-
Xanthophyta	1	1	-
Bacillariophyta	47	103	7
Craspedophyceae	1	1	-
Cryptophyta	3	4	
Dinophyta	31	122	4
Euglenophyta	4	10	-
Chlorophyta (incl. *Prasinophyta*)	11	15	-
Total:	**125**	**291**	**11**

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Table 2. *List of the cyanoprokaryotic species (including potentially toxic species, PTS) found in the Bulgarian Black Sea coastal waters during the last 50 years.*

Species	Toxic/toxins	Bibliographic source
Anabaenopsis arnoldii Apetk		Moncheva S., 2010; Konsulov A., 1998; Petrova V., 1963
Aphanizomenon flos-aquae (Linnaeus) Ralfs ex Bornet & Flahault, 1888	PTS, Anatoxin-a, Anatoxin-a(s), Microcystins	Konsulov A., 1998
Gloeocapsopsis crepidinum (Thuret) Geitler ex Komárek, 1993		Moncheva S., 2010
Nodularia spumigena Mertens in Jürgens, 1822	PTS, Nodularin	Moncheva S., 2010; Konsulov A., 1998
Lyngbya aestuarii (Mertens) Liebman ex Gomont, 1892		Moncheva S., 2010
Microcystis aeruginosa (Kützing) Kützing, 1846	PTS, Microcystins	Moncheva S., 2010; Konsulov A., 1998
Merismopedia elegans A. Braun in Kützing, 1849		Moncheva S., 2010
Merismopedia glauca (Ehrenberg) Kützing, 1845		Moncheva S., 2010; Konsulov A., 1998
Merismopedia minima Beck, 1897		Konsulov A., 1998
Micromonas pusilla (Butcher) Manton & Parke		Moncheva S., 2010
Merismopedia punctate Meyen, 1939		Konsulov A., 1998
Merismopedia tenuissima Lemmermann, 1898		Moncheva S., 2010; Konsulov A., 1998
Oscillatoria angusta Koppe 1924		Moncheva S., 2010
Oscillatoria princeps Vaucher ex Gomont, 1892		Moncheva S., 2010
Phormidium limosum (Dillwyn) P.C. Silva, 1996		Konsulov A., 1998; Moncheva S., 2010
Spirulina tenuissima Kützing		Moncheva S., 2010
Spirulina major Kützing ex Gomont, 1892		Konsulov A., 1998; Moncheva S., 2010
Synechococcus elongates (Nägeli) Nägeli 1849		Moncheva S., 2010; Konsulov A., 1998

An important stage in the phytoplankton research is the given priority of studies focused on the environmental factors.

As part of the report "State of the Environment of the Black Sea 2001-2006/7", drafted by the Commission on the Protection of the Black Sea Against Pollution (Black Sea Commission), whose members are Bulgaria, Georgia, Romania, Russia, Turkey and Ukraine , Neshterova et al. (2008) present a summary of the performed research including information on the phytoplankton in Black Sea.

In 2010, with the active participation of Prof. Moncheva (Institute of Oceanology, BAS) it was created a Black Sea Phytoplankton checklist (http://phyto.bss.ibss.org.ua/wiki/Main_Page) and a regional oceanographic database for Black Sea. According to to this database until 2010 in the Bulgarian Black Sea coast are identified 17 cyanoprokaryotic species assigned to 11 genera (Table 2).

Within the National Marine Strategy and in pursuance of Article 8, Article 9 and Article 10 of the European Marine Strategy Framework Directive, Moncheva & Todorova (2013) published “Initial assessment of the state of the marine environment, in accordance with Article 8 of the Regulation on the Protection of the Environment in Marine Waters” (RPEMW). The assessment of the phytoplankton was performed by using data covering the period 2006-2011, and currently this is the most complete study of the Bulgarian Black Sea phytoplankton.

Studies on the algal blooms in the Bulgarian Black Sea coast

The algal blooms in the Bulgarian Black Sea coast, the importance of the interaction between abiotic and biotic factors in the initiation and maintenance of these blooms, as well as their role as a key factor for the destruction of the biocenoses, are subject of study of a number of authors until 2003 (Moncheva, 1991; Konsulova et al., 1991; Moncheva et al., 1995; 2001; Velikova et al., 1998, 1999). Summarizing the known data, Moncheva et al. (2001) determined that the climate changes and pollutions have highest impact on the changes of the species diversity and frequency of the algal blooms in Black Sea.

Recent data showed that in the Bulgarian coastal waters there is a tendency to reduce the frequency, duration and

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intensity of the algal blooms that are typical for other areas of the Black Sea (Nesterova et al, 2008; Moncheva & Todorova, 2013). The species that have been registered in bloom concentrations are: *Cerataulina pelagica* (during the summer in 2008; 1761.7 mg/m^3), *Pseudonitschia delicatissima* (in April 2009; 4 821 600 cells/L), *Proboscia alata* (during the spring in 2009; 1452 mg/m^3), *Skeletonema costatum* (1 004 508 cells/L), *Chaetoceros diadema* (1 451 980 mg/m^3), *Gymnodinium najadeum* (1000 mg/m^3) during the spring in 2011 and *Thalassiosira nordenskioeldii* (during the summer in 2011; 3 196 044 cells/L). Blooms of *Prorocentrum minimum*, an indicator species during the intensive eutrophication, are associated with phenomena of mass extinction of demersal organisms observed almost every year. The maximal concentration of 280×10^6 cells/L is measured in the 80s and early 90s of the last century (Moncheva et al, 1995; Velikova et al., 1999; Moncheva et al., 2001). During the period 2006 – 2011 its concentrations are lower (1×10^6 cells/L), whereas the frequency of the *Emiliania. huxleyi* blooms increase (Monceva & Todorova, 2013).

In the composition of the phytoplankton communities are present species with well-expressed toxic potential in other regions of the Black Sea. Such species are *Alexandrium tamarense*, *Alexandrium minutum*, *Lingulodinium polyedrum*, *Pseudonitzschia calliantha*, and also cyanoprokaryotes as *Nodularia spumigena* (Vershinin & Moruchkov, 2003; Vershinin & Morton, 2005; Vershinin & Orlova, 2008; Vershinin et al., 2005; Besiktepe et al., 2008; Alexandrov et al., 2010).

Although, in the Bulgarian coast, manifestations of toxicity are not registered, the potential risk should be taken in account. This remark is very important for the assessment of the state of the Bulgarian Black Sea coast. As already mentioned above, the monitoring of the Cyanoprokaryota in Black Sea, currently is being performed by methods of the traditional botanical approach based on the morphological identification by microscopy.

Cyanoprokaryota are a complex group of organisms that are difficult to recognize only by using morphological and cytological criteria. Moreover, the identification of the cyanoprokaryotic species by morphological criteria does not show the potential for production of cyanotoxins. Therefore, to achieve better monitoring and control of the pollution in the coastal waters of the Black Sea, it is necessary to improve the detection systems of Cyanoprokaryota and their toxins by applying a combination of modern research methods such as highly sensitive immunoassays (ELISA), analytical methods (HPLC) and molecular methods. By combining these methods with analysis of the environmental factors and microscopic analysis of the phytoplankton, we could determine which cyanoprokaryotic species cause blooms, the factors contributing to the development of toxic Cyanoprokaryota and what cause the massive proliferation of these organisms in the Black Sea coast, and in particular in the Bulgarian coast. Probably the lack of such monitoring system is the reason for gaps in registering the presence of certain types Cyanoprokaryota along the Bulgarian coast and cyanoprokaryotic blooms. There are several initiatives to overcome this problem. An example of this is the launched three years ago an international research project (MARCY, BS-ERA.NET) with participation of Bulgarian researchers. Its purpose is to implement new tools and methods for obtaining more accurate and complete information about Cyanoprokaryota and produced toxins in the Romanian, Bulgarian and Turkish Black Sea coast. The intent is by using combination of molecular, genetic, immunological and physicochemical methods to create a system for detection of cyanoprokaryotes and their toxins aiming better monitoring and control of the pollution.

Within the aforementioned project, during the summer months (May-September) of 2012 we have identified 7 cyanoprokaryotic species in the Bulgarian Black Sea coast: *Aphanocapsa incerta*, *Coelomoron pusillum*, *Komvophoron constrictum*, *Phormidium tergestinum*, *Pseudoanabaena limnetica*, *Romeria gracilis* and *Snowella lacustris*. These species are still not included in the taxonomic list of Cyanoprokaryta species occurring in the Bulgarian Black Sea. *Romeria gracilis*, collected from the Bay of Varna in July 2012 was in bloom concentrations. The performed immunological tests for presence of hepatotoxins and neurotoxins in the water samples were negative. The obtained results show the need from further investigations in this direction.

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REVIEW

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