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PROOF

Geographic Variation of Picocyanobacteria *Synechococcus* spp. along the Anatolian Coast of the Black Sea during the Late Autumn of 2013

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Abstract

Picoplankton are particularly small phytoplankton with sizes between 0.2 and 2 µm and mostly include chroococcoid cyanobacteria, *Synechococcus* spp. They are major contributors to the total autotrophic biomass in the oceans. The importance of this group has increased in recent years. The aim of the study is to determine the geographic variation of picoplanktonic *Synechoccocus* spp. biomass along the Anatolian coast during late autumn. The sampling program was carried out from October 15 and 11 November 2013. Sample were collected from surface water of 57 station which were located between the coast line and 15 miles off between İğneada-Hopa. Physico-chemical properties of sea water like temperature (°C), salinity (ppt), sigma-t, conductivity (S m^{-1}) and Dissolved Oxygen (mg L^{-1}) were also collected from each station with Sea & Sun Tech M75 CTD profiler. Autotrophic picoplanktonic cells were counted by using ACCURY C6 flowcytometer. Results were given as cells ml^{-1}. Sea surface temperature ranged between 16.2 °C - 18.2° C. Stagnated water was observed from surface to 20 m and thermocline developed between 35-60 m. Our results showed that picoplankton concentrations increased in recent years in the coastal area of Southern Black Sea. The lowest and the highest *Synechococcus* spp. concentrations were found as $7.4x10^3$ cells ml^{-1} and $9.2 x10^4$ ml^{-1} in Şile off and mouth of Kızılırmak River respectively. The results indicate that Şile and Kızılırmak area shown different picoplanktonic structure among the other sampling region.

Keywords: Black Sea, picoplankton, *Synechococcus* spp., flowcytometer.

Başlık

Özet

Pikoplankton 0,2 ile 2 µm arasında boyuta sahip olan fitoplanktonun küçük grubudur ve *Synechococcus* spp. chroococcoid cyanobacteria gruplarını içerir. Okyanuslarda fotosentetik biokütleye büyük katkı yapan grup olarak bilinirler. Son yıllarda bu grubun önemi artmıştır. Bu çalışmanın amacı Anadolu kıyıları boyunca pikoplanktonik *Synechoccocus* spp. gruplarının biyokütlesinin geç sonbahar döneminde coğrafik farklılıklarını belirlemektir. Örnekleme programı 15 Ekim-11 Kasım 2013 tarihleri arasında yürütülmüştür. Örnekler İğneada- Hopa arasında yer alan kıyı ile 15 deniz mili arasında kalan sahada 57 istasyonun yüzey suyundan alınmıştır. Sıcaklık (°C), Tuzluluk (ppt) sigma-t, iletkenlik (S m^{-1}) and Çözünmüş Oksijen (mg L^{-1}) gibi deniz suyunun fiziko-kimyasal özellikleri her istasyondan Sea & Sun Tech M75 CTD profiler kullanılarak toplanmıştır. Ototrof planktonik hücreler ACCURY C6 flowcytometre kullanılarak sayılmıştır. Çalışma döneminde yüzey suyu sıcaklıkları 16.2 °C - 18.2° C arasında değişmiştir. Yüzey ile 20 metre arasındaki sularda tabakalaşma gözlenmemiştir. Termoklin 35 m- 60 m arasında bulunmuştur. Sonuçlarımız son yıllarda pikoplankton biyokütlesinin Güney Karadeniz'in kıyısal bölgelerinde artığını göstermiştir. En düşük ve en yüksek *Synechococcus* spp. yoğunluğu sırasıyla 7.4x103 hücre ml^{-1} ve $9.2 x10^4$ hücre ml-1 olarak Şile ve Kızılırmak nehrinin ağızında bulunmuştur. Sonuçlar Şile ve Kızılırmak alanlarının diğer örnekleme alanlarından farklı bir pikoplanktonik yapıya sahip olduğunu göstermiştir.

Anahtar Kelimeler: Karadeniz, pikoplankton, *Synechococcus* spp., flowcytometre.

Introduction

The Black Sea is largest semi-enclosed marginal sea on the world. It is located between latitudes 40° 55'N to 46° 32'N and longitudes 27° 27'E to 41° 42'E. The main source of salty water for the Black Sea basin is high saline Mediterranean Sea water flowing through the Istanbul Strait. Low salinity level of 17-18 ppt of the basin is obtained through the inflow of rivers like Danube, Dnieper, Dniester,

Sakarya river, Kızılırmak, Yeşilırmak and Çoruh. The vertical stratification of the Black Sea is determined by low-salinity surface waters overlying high-salinity deep waters of Mediterranean origin. (Glazer *et al.*, 2006). The Black Sea is characterized by a relatively shallow euphotic layer located above anoxic deep water mass and this two layer is separated by pycnocline (Uysal, 2006). About 87 % of the Black Sea volume is deprived of oxygen and contaminated with hydrogen sulphide. So no life, except anaerobic bacteria, exists below this level (Kucuksezgin and Pazi, 2003). The major part of the Black Sea water column is unsuitable for life (Zaitsev, 2008). However the surface mixing layer (about 0 to 50m) is well oxygenated (Murray and Izdar., 1989). This part of the water column is inhabited by phytoplankton. Distribution of phytoplankton in the water column is mainly determined by the system of dominating currents in the area. Phytoplanktonic groups are divided four main category as fento-, piko-, nano- and micro-phytoplankton. Majority of oceanic phytoplankton comprises small picoplanktonic algae which is particularly small phytoplankton with sizes between 0.2 and 2 µm and includes mainly chroococcoid cyanobacteria, *Synechococcus* spp. (Suthers and Rissik, 2009; Uysal, 2006). They are major contributors to the total primary production in marine environment. Iturriaga and Mitchell, (1986) indicate that they comprise for 64% of the total photosynthesis in the North Pacific Ocean. Therefore accurate determination of the picoplanktonic biomass is critically important for understanding the marine environment. Abundance of picoplankton has traditionally been analyzed in the laboratory by optical microscopy (Colebrook, 1960). Recently, however, new protocols and electronic instrument like flow cytometer replace the traditional methods. The fluorescence of chlorophyll other photosynthetic pigment makes phytoplankton cells well suited for studies by flow cytometry (Zubcov *et al.*, 2007). Flow cytometer analysis of picoplankton offers several advantages over the traditional approach e.g. it can be performed in a minute on board a ship or in a laboratory while microscopic analysis takes a few hours per sample.

Although numerous studies on phytoplankton in Turkish seas have been conducted, there are few studies on picoplankton distributions in the Black Sea. One major reason for the limited data about this tiny organism is the need for special techniques and instruments as mentioned above. Recently, limited studies on pigments, size, distribution, growth and diurnal variability of *Synechococcus* spp in the Black Sea were performed by several research groups. (Uysal, 2000, 2001, 2006; Kurt, 2002; Feyzioglu *et al.*, 2004). The aim of this study is to determine the abundance distribution of Synechococcus spp. and provide new data set along the Turkish Black Sea coast by using new techniques.

Materials and Methods

Sampling was done between October 15 and 11 November 2013 along the Turkish Black Sea coast. Samples were collected from surface water of 57 stations which were located between the coast line and 15 miles off between İğneada-Hopa (Figure 1). Physico-chemical properties of sea water like temperature (°C), salinity (ppt), sigma-t, conductivity (S m^{-1}) and Dissolved Oxygen (mg L^{-1}) were also collected from each station with Sea & Sun Tech M75 CTD profiler. Water samples for cell counts were collected with Nansen bottles and were transfer into 50 ml, dark-colored glass bottles and fixed with 2% final concentration of glutaraldehyde solution. The samples were kept at -20 °C in deep freeze till the analysis. Autotrophic picoplanktonic cells were counted by using Accury C6 flowcytometer. For analysis 480 nm laser sources were used for pigment activation. After activation chlorophyll-a and Phycoeritrine fluorescence were detected in the orange (FL2: 540-630 nm) and red (FL3: 660-700 nm) cytometric channels, respectively. Results were given as cells ml^{-1}. Regional comparison were done by using Hierarchical Cluster Analysis and Multi-Dimensional Scaling analysis (MDS). During the

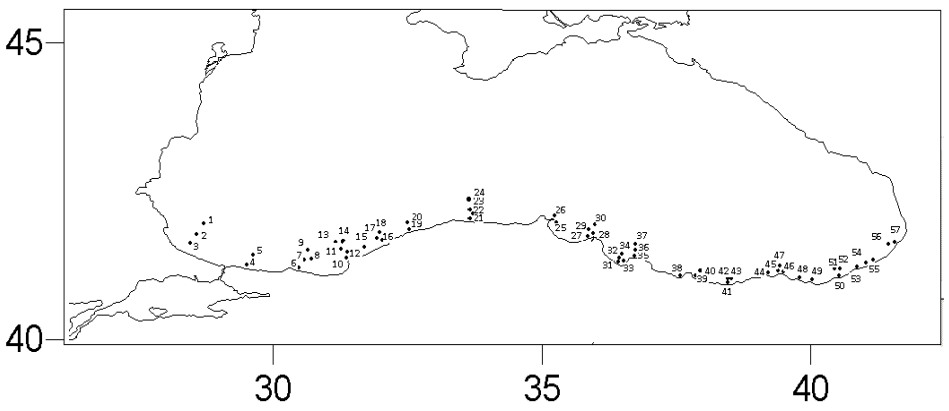


Figure 1. Sampling locations.

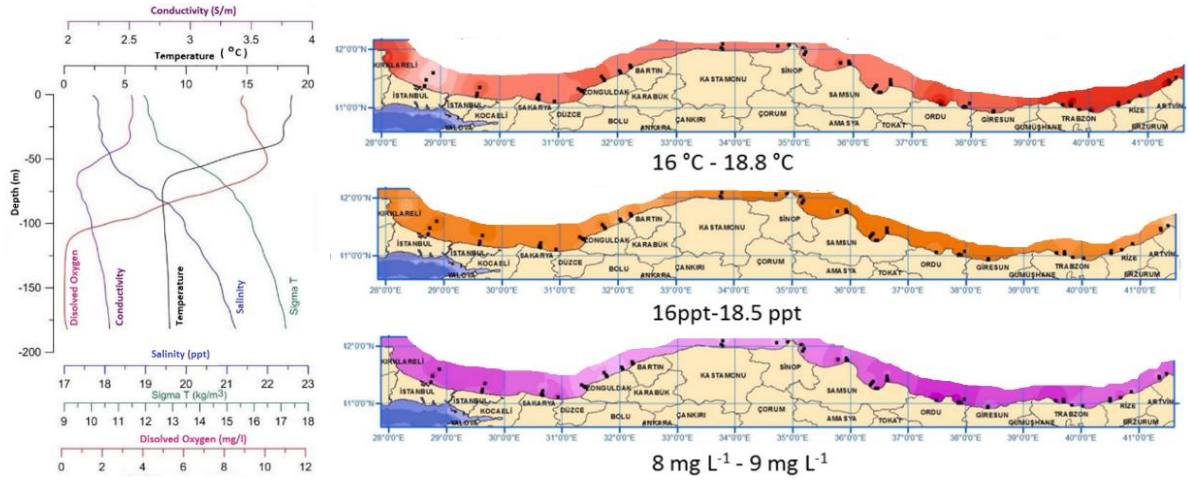
analysis PRIMER 5 for windows software were used.

Results

The data set presented here *was* collected along continental shelf area of the Black Sea Anatolian coast line between October 15 and November 11, 2013. Mean profiles of conductivity (m s^{-1}) , dissolve oxygen (mg L^{-1}) , temperature (°C) , salinity (ppt) , Sigma-t (kg m^{-3}) (Figure 2A) and spatial distribution of sea surface temperature (SST,), salinity and dissolved oxygen is shown in Figure 2B. Our data show that SST range between 16 - 18.8 °C and is almost uniform distribution along the continental shelf area, however western side was colder than eastern part of the Black Sea. Stagnated water was observed from surface to 20 m and thermocline developed between 35-65 m (Figure 2 A). Although salinity values range between 16-18.5 ppt, higher salinity was observed in vicinity of Istanbul strait and continental shelf area between Sinop –Giresun. DO also showed uniform distribution but relatively lower concentrations were measured between Zonguldak and Samsun continental shelf areas.

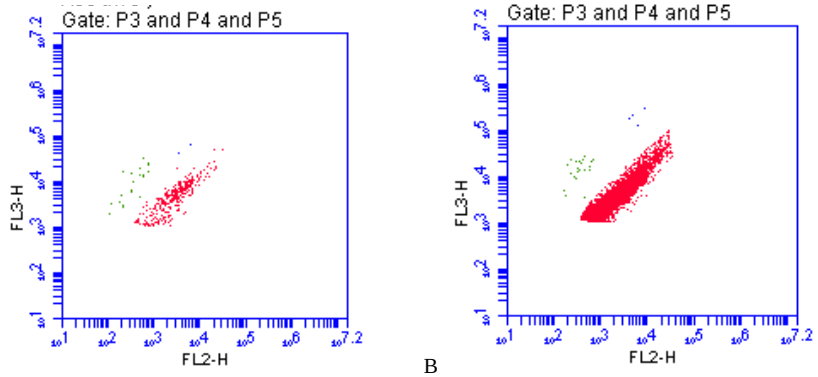
Sampling stations were investigated under 20 regional groups. Those groups include Istanbul–Karacaköy, Şile, Sakarya-Karasu, Eregli, Zonguldak, Bartın, İnebolu, Sinop, Kızılırmak, Samsun, Yeşilırmak, Fatsa, Ordu, Giresun, Trabzon, Araklı, Rize, Çayeli-Pazar and Hopa. The flow cytometric cytogram of picoplanktonic cell population at Şile and Kızılırmak stations were shown in Figure 3. The mean cell concentrations in surface waters were $3.63x10^4$ cells/ml in the whole sampling area. However the minimum and maximum cell concentrations were observed as $7.4x10^3$ cells/ml and $9,2x10^4$ cells/ml at station BLS 5 in Şile off in and the western at station BLS 27 which is located at mouth of Kızılırmak respectively (Figure 3 and Figure 4). Second highest cell concentrations were at the Yeşilırmak and Cayeli-Pazar station (near the Fırtına River).

Hierarchical Cluster Analyses and MDS analyses results were shown in Figure 5. According to analysis result, the similarity of the sampling region



(A) (B)

Figure 2. (A) Mean profiles of conductivity (m s^{-1}) , Dissolve Oxygen (mg L^{-1}) , temperature (°C) , Salinity (ppt) , Sigma-t (kg m-3) and (B) spatial distribution of sea surface temperature (SST)(°C), salinity (ppt) and dissolved oxygen (mgL^{-1}) . (Dark and light colors represent higher and lower values respectively).



A B

Figure 3. The flow cytometric cytogram of cell population for Şile (A) and Kızılırmak (B) Stations.

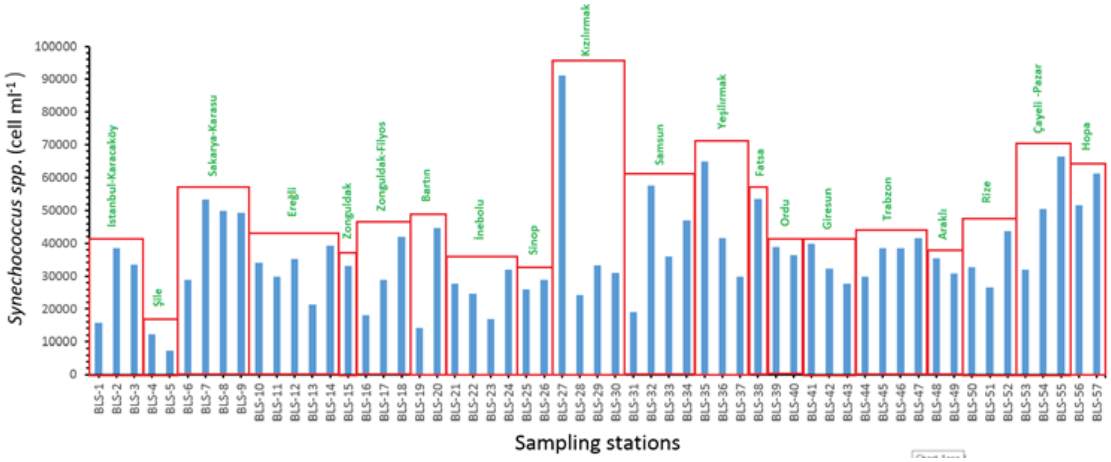
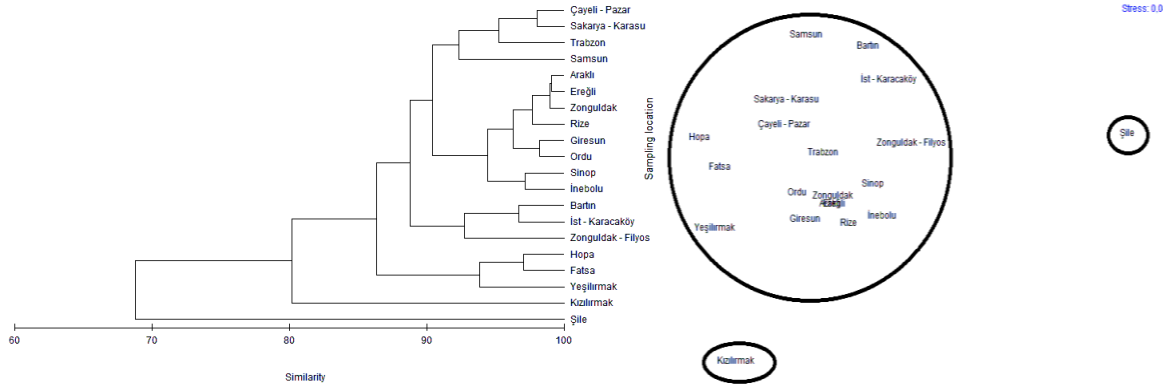


Figure 4. *Synechococcus* spp. cells concentration per ml in sampling station.



A. B.

Figure 5. A) Regional Hierarchical Similarity Cluster Analyses and B) MDS analyses results for *Synechococcus* spp.

were higher than 85%. Among the sampling sites Kızılırmak and Şile were shown different picoplankton structure. Şile station was the most specific site along the Anatolian coast. Because *Synechococcus* spp. biomass was the lowest level. Therefore Şile showed high differences among the sampling areas.

Discussion

Although limited studies related to picoplankton were conducted especially *Synechococcus* spp. in the Black Sea, all investigators have stressed the importance of this group in the Black Sea ecosystem (Uysal, 2000, 2001; Kurt, 2002; Uysal, 2006; Feyzioglu *et al*., 2004; Kopuz *et al*., 2012; Kopuz, 2012). According to Uysal (2006), the mean cells concentrations in surface waters were $1.09x10^5$ cells/ml and the highest concentration was $2.1x10^5$ cells/ml at station near Kızılırmak River in the October 1996. In this study *Synechococcus* spp. biomass was also found to be highest near the Kızılırmak river, supporting Uysal (2006). The *Synechococcus* spp. cell concentrations seem to be low in this study when compared to the Uysal (2006) results. This may be due to the change in the water regime of the river. Our finding and Uysal's results show that Kızılırmak is an important area for picoplanktonic organism along the Anatolian coast of the Black sea. Although some stations have high cell concentration western part of the Anatolian coast, in general *Synechococcuus* spp. cell consecrations were higher at eastern part than western part of the study area.

Cell size is important parameter for carbon biomass. Carbon content of the cells is the function of the cell size (Mullin *et al.*, 1966). According to previous studies cell size is two times larger at deep water than surface. In addition to this the largest cell size were found at late autumn. It can be said that cell size and the carbon biomass were the high during our sampling period along the year. Beside those findings Kopuz (2012) indicated that maximum cell number were observed at surface water in October along 2011 and the maximum cell concentration were between 20-50 m depth along the water column. Due to the

water samples were taken from only surface water during the sampling period, our observations do not present the maximum cells concentration along the water column. But probably the results reflect surface maxima for 2013.

The lowest cell number was observed at Şile stations. This may be the combination effect of Istanbul Strait and western cyclonic gyro. Because of the combine effect Şile station was different from other stations. Picoplanktonic organisms have high production rate and causes elongation of the food chain, should be monitored for understanding the process of ecosystem

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