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Short Communication

Excessive growth of *Cladophora laetevirens* (Dillwyn) Kutzing and enteric bacteria in mats in the Southwestern Istanbul coast, Sea of Marmara

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Abstract

In the summer of 2010, the excessive growth of *Cladophora laetevirens* along the southwestern shoreline of Istanbul, which carries great importance for tourism, drew major attention. The aim of this study was to investigate the excessive growth of *Cladophora laetevirens*, some ecological parameters, and the number of total coliform bacteria of surface waters in this region during the summer of 2010. During this period, this species multiplied and mucilage like environment was formed and the total coliform bacteria values were found to be between 10^5 and 10^8 MPN/100 mL. Due to the lack of available evidence prior to the sampling, it is possible that the excessive mucilage formation may have been trigged by *Cladophora laetevirens*. *Cladophora* samples were collected on different sampling dates during the 2010 summer (July and August). On these sampling days, the temperature, salinity and dissolved oxygen levels of the seawater ranged between 26.6-28°C, 14-33 ppt, and 8.2-9.7 mg/L, respectively. Nitrate+nitrite-N (0.21-0.26 µg-at/L), Ammonium-N (0.43-0.94 µg-at/L), Phosphate-P (0.53-3.50 µg-at/L), Silicate-Si (9.63-15.30 µg-at/L) and Chlorophyll *a* (3.68-14.11 µg/L) concentrations were measured.

Marmara Denizi'nin Güneybatı İstanbul Sahilinde *Cladophora laetevirens* (Dillwyn) Kutzing ve enterik bakterilerin ani kitlesel artışı

Özet

Turizm için büyük önem taşıyan İstanbul Güneybatı sahilinde, 2010 yılı yazında, *Cladophora laetevirens*'in aşırı büyümesi oldukça dikkat çekmiştir. Bu çalışmanın amacı, 2010 yılı yaz aylarında gözlenen artışın etkenleri arasında bulunan *Cladophora laetevirens* türünü, ortamın bazı ekolojik özellikleri ve yüzey sularında total koliform bakteri sayısını araştırmaktır. Örnekleme döneminde, bu tür aşırı çoğalarak musilaj benzeri bir yapı oluşturmuş ve o dönem toplam koliform bakteri değerleri de 10^5 ve 10^8 MPN/100 mL olarak tespit edilmiştir. Örnekleme dönemi öncesinde bu yapı ortamda gözlenmediğinden, aşırı musilaj oluşumundan bu türün sorumlu olduğu düşünülmüştür. 2010 yılının yaz aylarında farklı tarihlerde (Temmuz ve Ağustos) *Cladophora* örnekleri toplanmış ve bu esnada, deniz suyu sıcaklığı, tuzluluk ve çözünmüş oksijen değerleri sırasıyla 26,6-28 °C, 14-33 ppt ve 8,2-9,7 mg/L olarak ölçülmüştür. Ayrıca, Nitrit + nitrat-N (0,21-0,26 µg-at/L), Amonyum-N (0,43-0,94 µg-at/L), Fosfat-P (0,53-3,50 µg-at/L), Silikat-Si (9,63-15,30 µg-at/L) ve Klorofil *a* (3,68-14,11 µg/L) konsantrasyonları da belirlenmiştir.

Anahtar Kelimeler: *Cladophora* kitleleri, total koliform bakteri, musilaj olayı, Marmara Denizi.

Introduction

The genus *Cladophora* is a branching, filamentous, green alga (Chlorophyta, Cladophoraceae) that is found in both fresh and marine waters (Dodds and Gudder 1992). It is cosmopolitan in temperate and tropical regions in freshwater, brackish and marine habitats (Marks and Cummings 1996; John 2002). *Cladophora* is predominantly benthic, but it can be found as floating mats or as loose masses on soft substrates. Its metabolism and morphology are related to hydrodynamic conditions such as water temperatures in the range of 10-25 ºC, adequate light and nutrients (Dodds and Gudder 1992; Harris 2004). This filamentous green alga can reach nuisance levels as a result of cultural eutrophication. Nitrogen and phosphorus are the most commonly reported limiting nutrients (Dodds and Gudder 1992).

According to Dodds and Gudder (1992), describing the hydrodynamic conditions associated with the growth is very difficult because such conditions may vary over time in both fresh water and marine habitats. Also, the morphology of this genus has also been linked to hydrodynamic factors. The branching of marine *Cladophora* may become more pronounced with the increased wave energy (Van den Hoek 1982). However the mechanisms involved in the termination or collapses of macroalgal blooms are not well understood. There may be several mechanisms -grazing, nitrate toxicity, physiological responses, all previously mentioned- that might be responsible for ending macroalgal blooms. The simplest explanation might be what we articulated in relation to the conceptual loading/residence time model -increasing nutrient supply stimulates phytoplankton growth and this in turn increases light interception in the water column and shading of macrophytes below (Valiela et al. 1997).

Green tides, accumulations of green macroalgal biomass, occur under suitable hydrographic conditions in eutrophicated areas (Blomster et al. 2002; Raven and Taylor 2003). Especially, blooms of two genera of the Ulvophyceae, *Ulva* and *Enteromorpha* are reported in studies that have demonstrated the effects of eutrophication (Fletcher 1996; Sivri et al. 2002, 2005). In our working area, there is no record of the excessive growth of macroalgal and mucilage. Only the effect of phytoplankton and bacteria are observed in the records of the formation of mucilage (Aktan et al. 2008; Tüfekçi et al. 2010; Balkıs et al. 2011). With regards to the studies of excessive growth of microalgae and mucilage, Balkıs et al. (2011) indicated that in that time the active role of bacteria and some of the phytoplanktonic organisms. In this study, the mucilage structure that resulted from the excessive increase of *Cladophora laetevirens* is presented for the first time. Studies done in the Sea of Marmara report the existence of 5 different *Cladophora* species (Güner and Aysel 1987; Aysel et al. 2006). These types are *Cladophora albida* (Nees) Kützing, *Cladophora laetevirens* (Dillwyn) Kützing, *Cladophora pellucida* (Hudson) Kützing, *Cladophora prolifera* (Roth) Kützing and *Cladophora rupestris* (Linnaeus) Kützing. In the summer of 2010, excessive growth of *C. laetevirens* along the southwestern shoreline of Istanbul, which carries great importance for tourism, has drawn major attention.

In terms of hygiene and natural balance, priority should be give to issues like reducing the risk of being infected by infectious diseases for people who use the sea for recreational purposes and protecting the number and quality of marine life. For people to actualize these goals, quality standards regarding sea water is necessary (Kemper 2008). It is difficult to exclusively detect each individual pathogenic microorganism that leads to microbiological pollution during water pollution checks. For this reason, detecting bacteriological pollution will not only determine the existence of pathogenic microorganism but also determine the coliform bacteria count as a baseline indicator. (Cingilli-Vural and Akçin 2011). The existence of enteric microorganisms in sea water is the result of the interaction between the physical, biological, and biochemical processes (Kelsey et al. 2003). The Preto River, northwest of São Paulo State, with regards the occurrence and abundance of macroalgae, the Rhodophyta were found only in unpolluted or weakly polluted sites, whereas Cyanophyta occurred mostly under high pollution load; the Chlorophyta species were observed under a wide range of conditions. In the same area, the total coliform bacteria levels and relationship between macroalgal populations were observed to be very high. (Necchi Júnior et al. 1994). Recently, *Cladophora glomerata* (L.) from several Lake Michigan beaches was shown to harbor not only high densities of *Escherichia coli* and enterococci but also potential human pathogens such as *Salmonella* and *Campylobacter* spp. (Whitman and Nevers 2003).

The aim of this study was to investigate the excessive growth of *C. laetevirens* and some ecological parameters with enteric bacteria density of surface waters in the southwestern coast of Istanbul during the summer of 2010.

Materials and methods

Cladophora samples were collected from surface waters in Kumburgaz, which is located along the southwestern coast of Istanbul (Fig. 1)

Samples were collected on different sampling dates in summer period of 2010 by hand, placed in Whirl-Pak bags, and transported to the laboratory at 4°C. The material was fixed with a neutral 4% formaldehyde solution and observed under an "Olympus CH30" light microscope and "Olympus CK2" inverted phase contrast microscope equipped with a microphotosystem. *C. laetevirens* was identified according to Söderström (1963).

Figure 1. Satellite location of sampling site, Kumburgaz Istanbul.

In addition to the risks of chemical pollutants, the EPA (2003) draws attention to the potential pathogenic risks that may be neglected. Therefore, this study aimed to place extra emphasis on the microbiological and pathogenic criteria in waters used for recreational purposes. For this reason, in this study, Multiple-Tube Fermentation Technique for Members of the Coliform Group [most probable numbers (MPN) technique, Standart Methods, 9221 B] was used for finding enteric bacteria (Fig. 2) (APHA 1999). The seawater samples were collected at the surface level, approximately 10 cm below the water surface. All water samples were collected in volumes of 150 mL in sterile flasks and immediately put in a 4°C cooling box, transported on ice to the Microbiology Laboratory and processed within 6 h after collection.

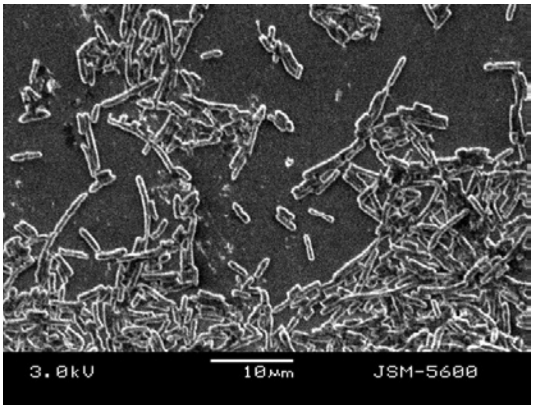

Figure 2. SEM for enteric bacteria (JSM 5600-SEM)

Measurements of temperature (°C), salinity (ppt), and dissolved oxygen (mg/L) were performed insitu at the sampling station with the Mohr-Knudsen method (Ivanoff 1972), which was used for measuring the salinity values and the Winkler (1888) method was used for dissolved oxygen (DO).

Water samples for the determination of nutrients were collected in 100-mL polyethylene bottles and continuously kept frozen (–20°C) until their analysis in the laboratory. Nitrate+nitrite (NO_3+NO_2-N) concentrations were analyzed by cadmium reduction method on a Seal Analytical continuous-flow AutoAnalyzer 3 (APHA 1999). Phosphate (PO_4-P), silicate (SiO_4-Si) and chlorophyll *a* (Chl *a*) analyses were conducted by the methods described by Parsons et al. (1984) and ammonium (NH_4-N) determination with the indophenol blue method (Harwood and Kuhn 1970). Chlorophyll *a* was measured after filtering 1 liter of the sample through Whatman GF/F filters. 1 mL of a 1% suspension of $MgCO_3$ was added to the sample prior to filtration. Samples were stored in a freezer and pigments were extracted in a 90% acetone solution and measured with a spectrophotometer.

Figure 3. Masses of *Cladophora laetevirens* in the study area

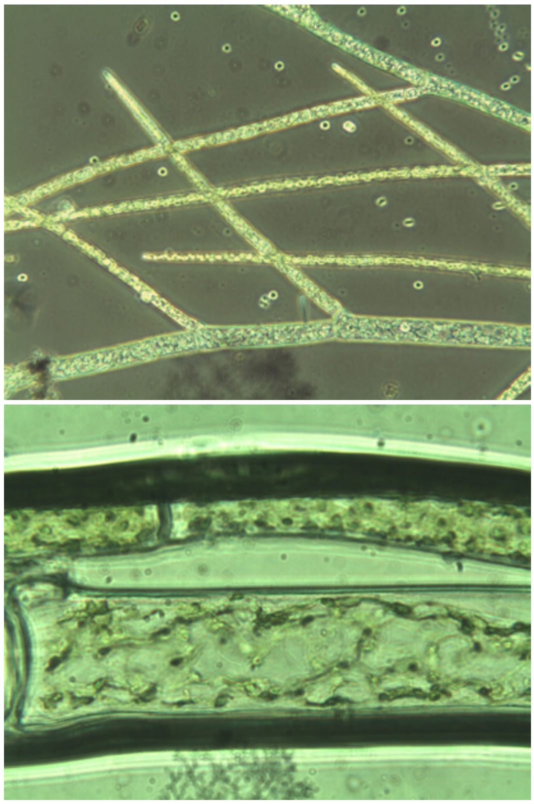

Figure 4. *Cladophora laetevirens* identified from study area.

Results

The temperature, salinity and dissolved oxygen levels of the seawater ranged between 26-29 °C, 14-33 ppt, and 8.2-9.7 mg/L, respectively. Of nutrients NO_3+NO_2-N values ranged between 0.21 µg-at/L and 0.26 µg-at/L, NH_4-N between 0.43 µg-at/L and 0.94 µg-at/L, PO_4-P between 0.53 µg-at/L and 3.5 µg-at/L and SiO_4-Si between 9.63 µg-at/L and 15.30 µg-at/L, and also chlorophyll *a* values ranged between 3.68 µg/L and 14.11 µg/L. The ratios of N:P (1.02-2.17), N:Si (0.04-0.14), Si:P (4.27-28.87) and P:Chl *a* (0.14-0.25) were recorded on each sampling occasion.

In Figure 3 and 4, are the masses of *C. laetevirens* observed in the study area. Total coliform bacteria values were found to be between 10^5 and 10^8 MPN/100 mL. These high levels of total coliform bacteria are associated with the presence of these masses. The bacterial values were transformed on log10- data to meet parametric assumptions, between 5.2-8.3 log MPN/100 mL.

Discussion

In coastal areas, *Cladophora* mats can provide a suitable environment for many organisms and microbial communities. Olapade et al. (2006) mentioned that *Cladophora* prolonged the survival of *E. coli* and enteric bacteria, which is the origin of the indicator microorganisms, where this might be not only fecal but also *Cladophora* mats may serve as a secondary habitat. Also, it provides a niche for pathogenic bacteria and nutrient source for survival and possibly growth of *E. coli* and enterococci (Whitman et al. 2003). In addition, there are some specific interactions between macroalgae and bacteria (Goecke et al. 2010).

Cladophora mats along the shorelines are an environmental source indicative of bacteria near the shoreline, which can potentially affect beach quality and increase the health risks to both wildlife and the public who access these beaches. While the values vary from country to country, the maximum accepted total coliform bacteria value is between 10^2-10^3 MPN/100 mL. Legal regulations stipulate the maximum allowable numbers for these bacteria. If fecal coliform counts are high (over 200 colonies per 100 mL of water sample) in the sea or stream, there is a greater chance that pathogenic organisms are also present. Despite the fact that they cannot be linked directly to contamination by human sewage, fecal coliform bacteria counts are often used to regulate surface waters for recreational use. Diseases and illnesses such as typhoid fever, hepatitis, and gastroenteritis, dysentery, and ear infections can be contracted in waters with high fecal coliform counts.

In this study, the total coliform bacteria were found to be between 5.2-8.3 log MPN/100 mL. By obtaining high levels of total coliform bacteria, our findings suggest the presence of the other pathogenic bacteria (*Salmonella, Shigella,* etc.). Large masses of *Cladophora* accumulations not only create mats but also negatively affect recreational areas and influence the water quality. *Cladophora* was determined as a secondary habitat, which provides nutrients and suitable environment against to environmental stress such as desiccation, predation, and harmful radiation (Malinsky-Rushansky and Legrand 1996; Byappanahalli et al. 2003). The general assumption that traditional fecal indicators (e.g., *E. coli* and enterococci) do not occur in natural environments (soil or water) has recently been challenged. These bacteria occur in soils and perhaps as epiphytic microflora on terrestrial plants. Observations of these indicators living on aquatic plants, such as algae, are lacking; however, such associations would be significant since aquatic macrophytes have the potential to harbor, shed, and possibly support the growth of these indicator bacteria. The presence of indicators associated with aquatic macrophytes may lead to the misinterpretation of water quality tests or misidentification of the source of indicator bacteria (Whitman et al. 2003; Abd-Elnaby 2010). Findings from our study are consistent with the literature.

These situations suggest that *Cladophora* has a suitable potential for the survival of pathogenic bacteria that are released from point and nonpoint sources (Ishii et al. 2006). The excessive growth of algae in the Great Lakes was associated with an increase in phosphorus (Olapade et al. 2006). In this study, N:P ratios were below the normal value of 16:1, and N was a limiting nutrient and PO_4-P concentration ranged between 0.53 and 3.5 µg-at/L. It is known that P stress is common in freshwater systems, whereas N stress is found in marine systems (Ryther and Dunstan 1971). Robinson and Hawkes (1986) mentioned that the growth rate of *Cladophora* would be maximal at eutrophic areas where PO_4-P concentrations were higher than 2 mg/L. Lapointe and O'Connell (1989) concluded that growth of *Cladophora* was limited by N and P oligotrophic inshore surface waters in Bermuda. Additionally, *Cladophora* was described as a fast-growing, opportunistic algal species with a high nitrogen demand by Pedersen and Borum (1996). In this study, the most striking case in terms of ecological conditions in which excessive increase in the amount of Chl *a* (14.11 µg/L) observation of a significant increase in the amount of phosphorus (3.5 µg-at/L) in the August and in the same time, salinity value is much higher than the others (33 ppt). However, depending on the algal activity increased chlorophyll *a* values. Using phosphorus by this organisms and the algal activity caused to decrease the ratio of P/Chl *a* (0.14-0.25). In a study of the Sea of Marmara (Gulf of Bandırma) showed the same results of the high value of Chl *a* in August (Balkıs et al. 2012). This result emphasized that phosphorus value in the surface layer was higher than bottom layer. In addition, during the formation of mucilage in the same area, the Chl *a* values are reached to 22 µg/L (Tüfekçi et al. 2010). The reason for the high amount of oxygen in the surface layer, with the increase in algal photosynthetic activity is higher here.

The excessive algal growth, which is promoted by eutrophication, is the result of human activities even if they are carried out in restricted coastal areas, might pose a risk to human health and the environment. Due to the fact that unwanted microorganisms harboured within the *Cladophora* mats, it is possible that they might also be diffused and dispersed to adjacent waters by both wind and wave action (Englebert et al. 2008).

Findings from this study indicate that there is a reduction of water quality in recreational areas also suggests a potential increase in pathogenic threats. Also it is possible that the periodic monitoring efforts, to determine water quality in coastal area parameters, may be insufficient. With this in mind, if the *Cladophora* mats, with increased stability, are created and aged, then the survival and replication ability of *E. coli* will be simplified.

Another finding suggests that Phosphorus, which plays a critical role in the protection and improvement of water quality, needs to be reduced at its source as an overload of nutrients may result in eutrophication problem that can reduce the water quality. There are several methods for reducing the phosphorus concentrations in the water source. Methods include chemical phosphorus removal, limiting the use of phosphorus based detergents and other cleaning chemicals, controlling the use of the land, and treating the surface water bodies in the reservoirs before it reaches the receiving water body. Approaches regarding controlling land use are inclusive of implementing legal regulations as these activities limit the production of necessary nutrients. In addition, controlling diffused pollutant resources such as agricultural and urban pollution is also another inevitable concept for water quality management.

Cladophora accumulations in recreational areas may have public health and economic consequences; therefore, in order to increase the economic values and reduce health risks, we must improve the overall water qualities at our public beaches. Also, in terms of preserving both public health and seafood, we need to ensure that future generations have the opportunity to benefit from them in a healthy manner. In order to ensure and actualize this, the government needs to decide whether if these sites are to be used and maintained as recreational areas benefiting tourism in the long run or if they are to be used as a disposal dump area.

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