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Assessment of the consecutive harmful dinoflagellate blooms during 2015 in the Izmit Bay (the Marmara Sea)

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Abstract

A series of red tides were observed during 2015 in the Izmit Bay (the Marmara Sea) which is located in the most industrialized and populated region of Turkey. Six samplings were carried out in this area following the red tides. Nitrite-N, nitrate-N, ammonia, silica and orthophosphate concentrations were analyzed spectrophotometrically. Physicochemical conditions were measured by CTD probe. Plankton quantification was performed using counting chambers under microscopes. *Prorocentrum micans* was the most abundant species, except on May 14, 2015, when *Noctiluca scintillans* was dominant. The abundance of *P. micans* reached average 18×10^6 ind./L on May 3, 2015 in the Karamürsel station, simultaneously with elevated levels of NH_3 and o-PO_4^{3-}. The sample was also abundant in dead amphipods ((72±12) ind./L) that had been covered by mucilage aggregates produced by *P. micans*. The highest biomass (calculated by carbon) was recorded as (268±26.0) mg/L on May 14 in the Hereke station. Beside the anthropogenic wastewater discharges, unknown sources and resuspensions caused increases in nutrient levels. After long term northeaster gusts (35 km/h for 5 d) an upwelling occurred on November 6, 2015 after wind-induced sediment resuspension. Although nutrient discharges remarkably decreased over 30 years through established wastewater treatment plants, harmful phytoplankton blooms still occur. Comparing the present results with other studies in nearby Mediterranean seas reveals that the most intense harmful dinoflagellate bloom in recent years occurred in the Izmit Bay. Therefore, additional protection measures necessary for a cleaner Izmit Bay. These incidents also demonstrate that contaminants, accumulated in sediment, may have long-lasting effects on enclosed marine ecosystems.

Key words: red tide, harmful algal bloom, phytoplankton, dinoflagellate, Izmit Bay, Marmara Sea, mucilage

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1 Introduction

Marine phytoplankton generates approximately half of the global primary production and affects the fate of sea life, with respect to fishery yields and sustainable marine ecosystems (Boyce et al., 2010). Domestic and industrial discharges cause nutrient enrichment and consequently, alterations of phytoplankton composition in terms of biodiversity and abundance in many coastal areas (Smith et al., 1999). Annual nitrogen and phosphorus input to ecosystems has more than doubled globally during the last fifty years (Falkowski et al., 2000; Smil, 2000). Since the availability of nutrients, necessary for growth and reproduction of phytoplankton, is often lower than the biological demand, additional nutrient supply is necessary for phytoplankton growth (Corbett, 2010). Therefore, in addition to common sources (e.g., terrestrial inflows, airborne particulates) anthropogenic discharges (e.g., industrial and domestic effluents, agricultural runoffs) and sediment resuspensions affect nutrient enrichment, leading to an increase in phytoplankton abundance. Under favorable oceanographic conditions (e.g., temperature and salinity), sufficient nutrient levels may cause excessive phytoplankton reproduction and harmful algal blooms may occur (Heisler et al., 2008). These enrichments mostly happen in coasts near urbanized and industrialized settlements (Ferreira et al., 2011; Heisler et al., 2008). However, biogeochemical structure and environmental pressures may vary for a coastal marine ecosystem and exact mechanisms of algal bloom occurrences remain unknown, as in the Izmit Bay.

The Izmit Bay, one of the most polluted marine ecosystems of Turkey, contains a wide range of contaminants including organic and inorganic compounds such as heavy metals, radionuclides, POPs, and nutrients (Balkis et al., 2007; Ergül et al., 2013a, b; Karademir et al., 2013; Tolun et al., 2006). The bay is located to the northeast of the Marmara Sea (Turkey) and a permanent pycnocline and a two-layered stratification are observed throughout year. Upper layer salinity is generally less than 24.0, and this reaches 36.0 below the halocline layer (Ergül, 2016). There are around 2 million inhabitants living in Kocaeli Province surrounding the Izmit Bay. Population and industrial activities have been increasing rapidly since the 1980s, and the bay receives a considerable amount of domestic and industrial discharge from its drainage basin. Since the 1960s, more than 400 large industrial plants have been built around the bay, including the largest shipyards, metallurgy, paper mill, fertilizer, and petrochemical facilities of Turkey. In addition to 70 shipyards, there are 40 ports located along the coast of the bay. In 2015, the bay received approximately 400 and 80 tonnes of total nitrogen (TN) and phosphorus (TP) discharges from wastewater treatment plants, respectively. Moreover, almost all effluents were dis-

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charged without treatment before 2000, and 41.0% of domestic and industrial discharge has remained untreated until 2010 (ECR, 2013; ISU, 2016). At the current time, there are no available data for untreated TN and TP discharges from other sources (i.e., untreated sewage discharges, agricultural runoffs, etc.) in the Izmit Bay. Eutrophication is also a serious problem (Morkoç et al., 2001; Okay et al., 2001) and phytoplankton blooms were recently reported in the bay (Ergül et al., 2014, 2010).

Phytoplankton blooms, causing brownish-red surface water with bad odor and mucilage formation, drew public attention to domestic and industrial discharges as potential triggering elements. Moreover, meteorological factors likely have an effect on algal bloom occurrence. Therefore, investigation into mechanisms of algal blooms and their harmful effects on the ecosystem will be crucial for evaluating effective environmental factors and solving future problems in an enclosed marine ecosystem, close to a populated and industrialized city, such as the Izmit Bay.

This study evaluated (1) serial phytoplankton blooms with mucilage formation, (2) accompanied physicochemical and meteorological conditions in 2015, (3) evaluation of possible reasons for harmful algal blooms in the Izmit Bay, and (4) a comparison between the present results and remarkable diatom and dinoflagellate blooms around nearby Mediterranean seas.

2 Materials and methods

2.1 *Study area*

The Izmit Bay is a semi-enclosed coastal ecosystem located in the most industrialized area of the Marmara region (Turkey). The 49 km long bay with an area of 300 km^2 has three basins connected by shallow and narrow passages. The eastern basin, the smallest component of the system, is approximately 5.0 km wide, 13 km long, 47.0 km^2 in surface area and relatively shallow with a maximum depth of 40.0 m. The central basin, which is the largest component of the system, is approximately 9.5 km wide, 22 km long, 165 km^2 in surface area and the depth increases up to 208 m in the southern section (Kuşçu et al., 2002). A narrow and shallow area (i.e., 55 m depth) at Dil Creek shore separates the central and western basins which are approximately 8.5 km wide, 14 km long and 47.0 km^2 in surface area. The bottom topography of the western basin slopes downward toward the Marmara Sea and increases to a depth of approximately 100 m near its opening (Fig. 1). Stratification in the water column occurs throughout the year because of salinity differences. Saltier water originates from the Mediterranean Sea in the lower layer, whereas less saline water originates from the Black Sea in the upper layer (Morkoc et al., 1996). Vertical mixing between the two layers is restricted over the Marmara Basin and plays a key role in the principal physicochemical characteristics of the bay (Algan et al., 1999).

2.2 *Sampling and methodology*

Six surveys were carried out when red tides occurred to sample surface water for quantitative analysis of nitrite-N (NO_2^--N), nitrate-N (NO_3^--N), ammonia (NH_3), silica (SiO_2) and orthophosphate (o-PO_4^{3-}) and phytoplankton identification in the Izmit Bay. These were carried out on April 12, April 30, May 3, May 14, July 11, and November 6, 2015 at Ulaşlı, Sekapark, Karamürsel, Hereke, Marina and Marina stations, respectively in the Izmit Bay (Fig. 1). Temperature, salinity, dissolved oxygen (DO), turbidity, pH and chlorophyll *a* (Chl *a*) levels were measured in the water for each samplings. Although phytoplankton blooms were observed throughout the bay, marine water samples were taken at the locations densest in phytoplankton abundance during bloom times.

Composite surface water samples were taken with a Nansen bottle (3 L) twice hanging from the edge of a landing dock approximately 50 m away from the coast at all sites, these were put into amber glass bottles and kept cool for immediate transportation to the laboratory. The first Nansen bottle samples was filtered with a 0.45 μm, 47 mm Whatman filter paper and used for NO_2^--N, NO_3^--N, NH_3, o-PO_4^{3-} and SiO_2 analysis via spectrophotometric methods (Bendschneider and Robinson, 1952; Mullin and Riley, 1955; Murphy and Riley, 1962), while the other Nansen bottle sample (3 L) was used for phytoplankton identification and quantification. A 10 mL tubular plankton chamber was used for pre-identification under an inverted microscope while plankters were alive. Then samples were fixed using 4.00% formaldehyde solution for better identification and quantifying. Before the counting procedure, the samples were kept in a dark room in tubular glass for precipitation. Then phytoplankters were identified and quantified via a Nageotte counting chamber under a light microscope. Counting procedures were repeated at least three times. Results were given as individual per liter.

To estimate the biomass of phytoplankton, dimensions of each taxa were measured under a light microscope equipped with a camera (Olympus BX51). Measured dimensions were used to calculate cell volumes according to calculated values for each species' similar dimensions in a previous study by Olenina et al. (2006) in the Baltic Sea. To calculate cell biomass in terms of organic carbon content as pg C/cell, equations by Menden-Deuer and Lessard (2000), given below, were used:

$$\text{cell}\quad\text{biomass} = 0.288\times\text{biovolume}^{0.811}\quad\text{for Bacillariophycea (diatoms)},$$

$$\text{cell}\quad\text{biomass} = 0.216\times\text{biovolume}^{0.939}\quad\text{for taxons other than diatoms}.$$

The carbon content for each species was multiplied by individual number to calculate the organic carbon mass for each species group. Results were given as mg/L (calculated by carbon) in the text.

A data sonde (Hydrolab DS-5) was used to measure temperature, salinity, dissolved oxygen (DO), turbidity, pH and Chl *a*, in the water (Fig. 1). The device was calibrated before each deployment, waiting at least two minutes for warm up and then it was submerged at a constant speed (i.e., 0.2 m/s) where the water column had enough depth. At least thirty results of measurements were used to calculate average value. The Chl *a* and DO data were tested using a classical acetone extraction and Winkler methods, respectively. Differences between concentrations in classical methods and probe measurements were less than 5.00%. Standard deviations were calculated for statistical sufficiency. Meteorological records were obtained from Turkish State Meteorological Service stations located in Izmit.

Trophic index (TRIX) values were calculated in order to quantify eutrophic state and water quality. To calculate TRIX, concentrations of dissolved inorganic nitrogen (c(DIN), i.e., NO_2^--N + NO_3^--N + NH_3-N), o-PO_4^{3-} (c(P), μg/L), DO deviation from saturation (d(DO), %) and Chl *a* concentration (c(Chl *a*), mg/L) were used in the equation below (Vollenweider et al., 1998):

$$TRIX = \frac{[\log_{10}(c\,(\text{Chl}\ a)\times d\,(\text{DO})\times c\,(\text{DIN})\times c\,(\text{P}))+1.5]}{1.2}.$$

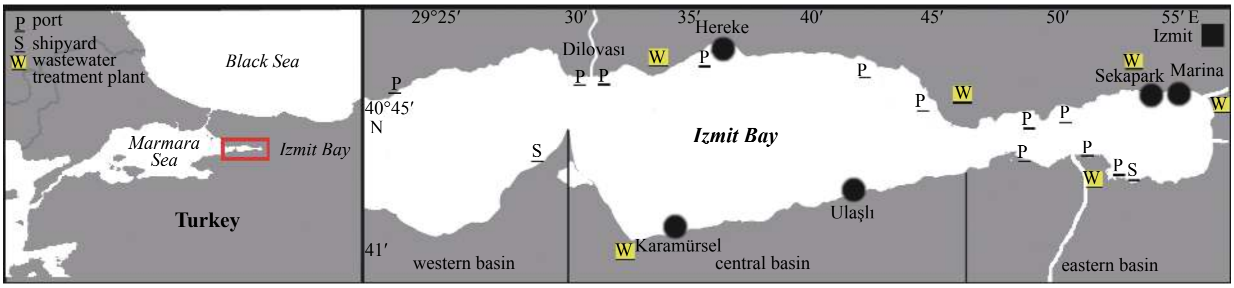

Fig. 1. Map of Izmit Bay and sampling sites. P represents industrial ports, S shipyards, and W wastewater treatment plants.

Pearson correlation coefficients (two-tailed) were calculated among variables to better explain the relationship between parameters and these are used in the text where necessary.

3 Results and discussion

On March 19, 2015, the Izmit Bay which is located at the eastern end of the Marmara Sea, was covered by phytoplankton and a light red appearance was observed over almost all the bay's surface (Fig. 2a). This appearance was disappeared after approximately two days. On April 12, phytoplankton abundance, which was dominated by *Prorocentrum micans* Ehrenberg, 1834, increased, surface water partly reached a brownish-red appearance, and mucilage formation was observed (Fig. 2b).

Six different phytoplankton species (*Achnantes brevipes* C. Agardh, 1824, *Cerataulina pelagica* (Cleve) Hendey, 1937, *Dictyocha speculum* Ehrenberg, 1834, *Melosira* sp., *Navicula* sp., and *Prorocentrum micans*), which belong to three different groups (four Bacillariophyceae, one Dictyochophyceae, and one Dinophyceae) were observed intermittently in the samples on April 12, April 30 and May 3, 2015 with average surface water temperatures of (14±1.0)°C, and salinity of 21±3.0. Regarding abundance, dinoflagellates were dominant with rates of over 99.0% and *P. micans*, which is known as a potential high biomass-forming dinoflagellate (Jeong et al., 2005) was the most abundant species in those three blooms (average 1.10×10^6, 5.40×10^6 and 17.9×10^6 ind./L, respectively; Table 1, Fig. 3a). Mucilage formations occurred in these blooms and a milky light-brown flocculated layer was observed on the sea surface. After the blooms, red appearances and mucilage layers disappeared within a few days from surface waters. In the microscopy slides, mucus aggregates were also observed amongst *P. micans* clusters (Fig. 2c). Therefore, formation of mucilage should be excreted by *P. micans* after their excessive proliferation (Figs 2c, d). On May 3, the mucilage layer was very intense, and dead amphipods, which were coated with amorphous mucus pellets, were observed in the samples in Karamürsel ((72±12) ind./L, Fig. 2d) with the highest surface water turbidity ((287±46.0) NTU) and Chl *a* concentrations ((180±15.0) μg/L, Fig. 4b). Although several phytoplankton blooms have been reported from the Izmit Bay since 1986 (Taş et al., 2016), to our knowledge, this incident was the most intense.

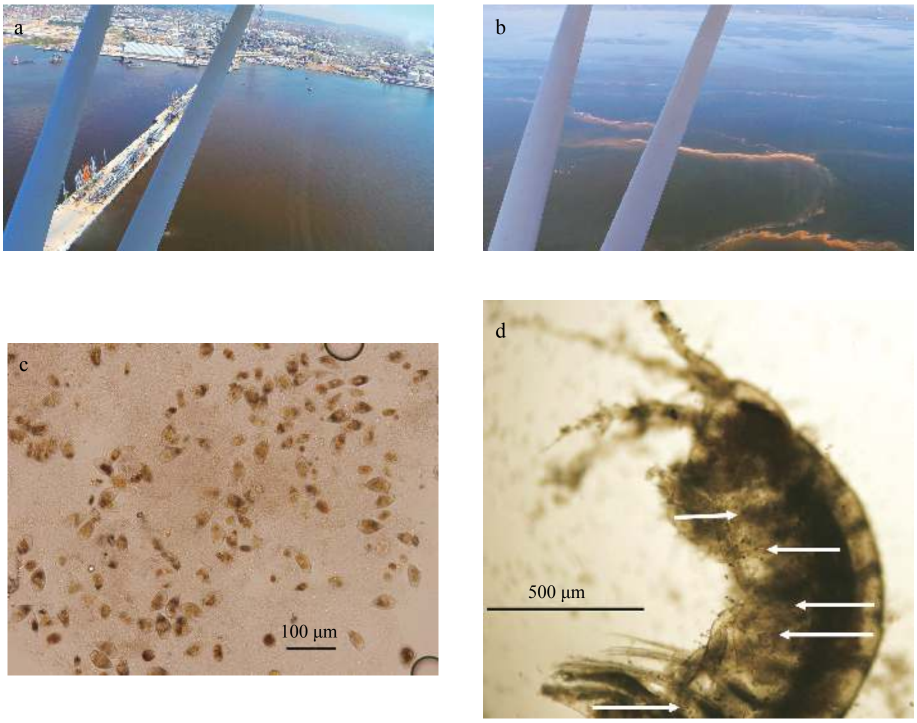

Fig. 2. Pictures of aerial view of the phytoplankton blooms without (a) and with (b) mucilage formations on March 19 and April 12, 2015, respectively; light microscope slides of *P. micans* with mucilage formation, in May 3, 2015 (c); and a dead amphipod individual covered with mucilage layer (d). Arrows indicate pervaded *P. micans* and mucilage clusters on the amphipod body.

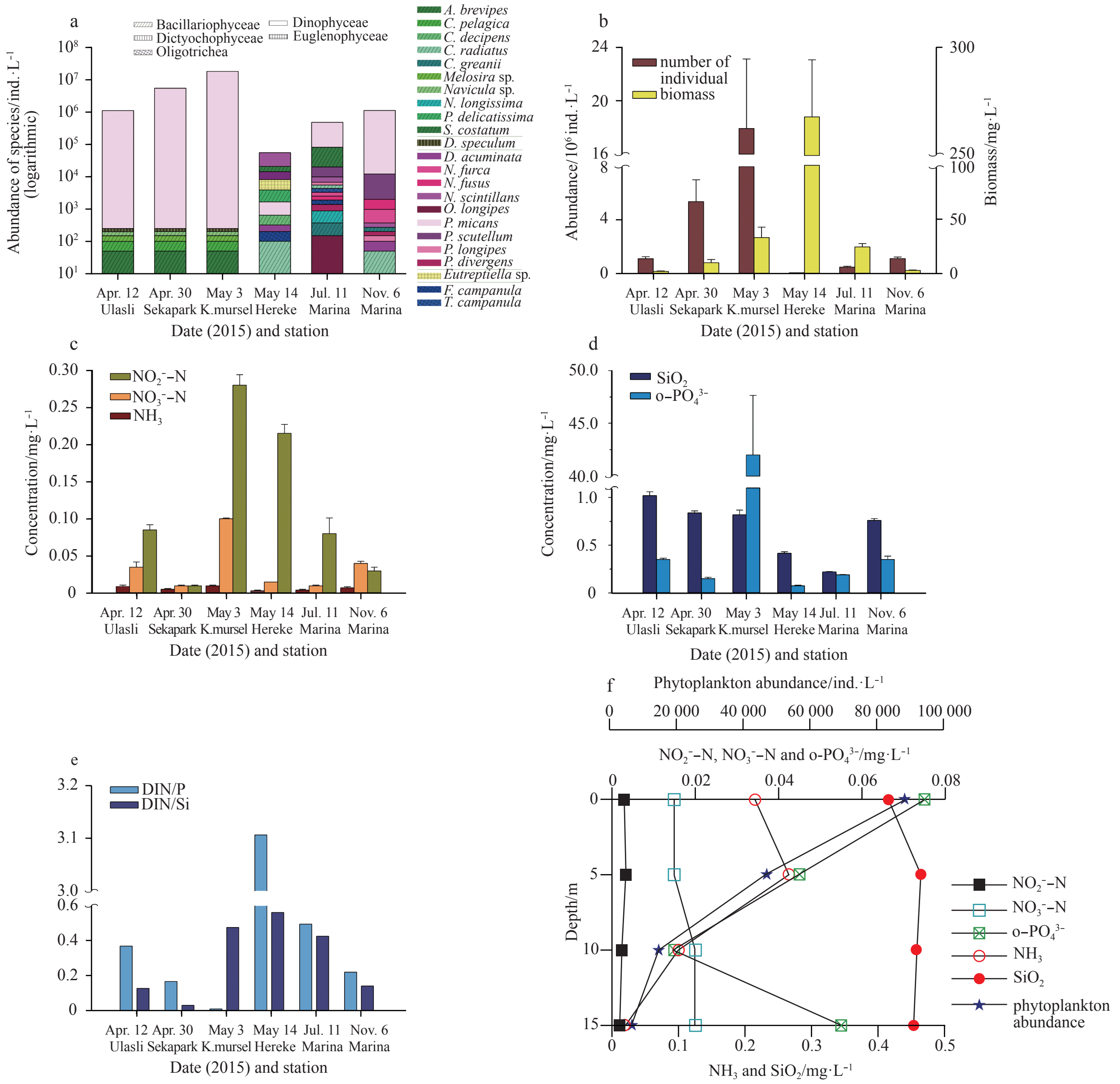

Fig. 3. Phytoplankton diversity and species abundances in logarithmic notation (a); total number of individuals and estimated total biomass of phytoplankton per liter (b); concentrations of NO_2^--N, NO_3^--N, and NH_3 (c), SiO_2 (d) and o-PO_4^{3-} (e) ratios between TIN and P and TIN and Si during the bloom conditions in the surface waters of Izmit Bay; and depth profile of nutrients on May 14, 2015 in the Hereke station (f).

Moreover, the presence of dead amphipods after a phytoplankton bloom was reported for the first time in the present study. Mortis causa of the amphipods was not clear, and there are no (open) scientific literature regarding marine mucilage-dependent amphipod mortality. However, Bruno et al. (1989) reported that farmed and wild fish may also be killed by the smothering of gills due to phytoplankton mucus production. Therefore, given its appearance under stereomicroscope, it is reasonable that mucilage aggregates probably blocked amphipod gills and thus respiration was prevented, leading to their death (Fig. 2d). Also, oxygen concentration decrease may be considered as another reason of amphipod mortality. However we have no simultaneous dissolved oxygen data to prove this claim. Amphipod and copepods are known predators of dinoflagellates including bloom causative species (i.e., *P. micans*), therefore high amphipod abundance in the samples are most likely connected to their feeding behavior (Yi et al., 2017). It should be noted that phytoplankton abundance as well as nutrient concentrations were approximately three fold higher near the shore because of conglomeration after drifting via waves and those values were excluded from further assessment in the present study.

In the bloom conditions on May 3, 2015, nutrient levels remarkably increased, and raised inorganic nitrogen, and excessive o-PO_4^{3-} concentrations were measured (Figs 3c, d). On that day, most intense dinoflagellate bloom occurred concurrently with the highest concentrations of DIN and o-PO_4^{3-} ever recorded during the study. Therefore, the existence of elevated levels of nutrients should have triggered excessive dinoflagellate (i.e., *P. micans*) proliferation and caused excretion (i.e., mucilage formation) from *P. micans* cells, under the favorable meteorological

Table 1. Phytoplankton diversity in the surface water of the Izmit Bay in the bloom conditions during 2015

Species	Classis	Sampling date					
		12 Apr. 2015	30 Apr. 2015	3 May 2015	14 May 2015	11 Jul. 2015	6 Nov. 2015
Achnanthes brevipes C. Agardh, 1824	B	+	+	+	–	–	–
Cerataulina pelagica (Cleve) Hendey, 1937		+	+	+	–	–	–
Chaetoceros decipiens Cleve, 1873		–	–	–	+	–	–
Coscinodiscus radiatus Ehrenberg, 1840		–	–	–	+	+	+
Coscinodiscus granii Gough, 1905		–	–	–	–	+	+
Melosira sp.		+	+	+	–	–	–
Navicula sp.		+	+	+	–	–	–
Nitzschia longissima (Brébisson) Ralfs, 1861		–	–	–	–	+	–
Pseudo-nitzschia delicatissima (Cleve) Heiden, 1928		–	–	–	+	–	–
Skeletonema costatum (Greville) Cleve, 1873		–	–	–	+	+	–
Dictyocha speculum Ehrenberg, 1839	Dc	+	+	+	–	–	–
Dinophysis acuminata Claparède & Lachmann, 1859	Dn	–	–	–	+	–	+
Neoceratium furca (Ehrenberg) F. Gomez, D. Moreira & P. Lopez-Garcia, 2010		–	–	–	–	+	+
Neoceratium fusus (Ehrenberg) F. Gomez, D. Moreira & P. Lopez-Garcia, 2010		–	–	–	–	+	+
Noctiluca scintillans (Macartney) Kofoid & Swezy, 1921		–	–	–	+	+	+
Oxytoxum longiceps Schiller		–	–	–	–	+	–
Prorocentrum micans Ehrenberg, 1834		+	+	+	+	+	+
Prorocentrum scutellum Schröder, 1900		–	–	–	+	+	+
Protoperidinium diabolum longipes (Cleve, 1900) Balech, 1974		–	–	–	–	+	+
Protoperidinium divergens (Ehrenberg, 1840) Balech, 1974		–	–	–	–	+	+
Eutreptiella sp.	E	–	–	–	+	–	–
Favella campanula (Schmidt, 1902) Jörgensen, 1924	O	–	–	–	+	+	–
Tintinnopsis campanula Ehrenberg, 1840		–	–	–	–	+	–

Note: B represents Bacillariophyceae, Dc Dictyochophyceae, Dn Dinophyceae, E Euglenophyceae, and O Oligotrichea.

conditions. It should be noted that, very calm weather and light winds were recorded for two days immediately before the bloom on May 3. Therefore, increased nutrients, which are most likely derived from undesirable or illegal discharges, could not be dispersed by winds, currents, precipitations, etc. from the surface water and finally were consumed by phytoplankton. In fact, the Izmit Bay's renewal capacity is known to be insufficient because of a relatively long residence time of the water masses (Morkoç et al., 2001) and in this incident, light winds, and accordingly weak currents, allowed nutrients accumulation in surface waters. Thus, appropriate conditions occurred for excessive *P. micans* proliferation.

Following phytoplankton increases, which occurred on May 14, July 11 and November 6, 2015 and unlike other blooms, biodiversity reached 10, 14, and 10 species, respectively, during these incidents in the Izmit Bay. During the study, 17 taxa which have not bloom formation were also recorded in different classes (Bacillariophyceae, Dinophyceae, Euglenophyceae, and Oligotrichea) in addition to six bloom taxa (Table 1, Fig. 3a).

On May 14, both DIN and o-PO_4^{3-} concentration decreased compared to May 3 and in this event biodiversity reached ten species (Figs 3a, c, d). The dominant species at this time was *Noctiluca scintillans* (Macartney) Kofoid & Swezy, 1921 (Figs 3a, d) with surface water temperatures of 15.0°C and salinity of 25.9 in the Hereke station, which was the highest salinity level recorded during the study (Fig. 4). Corresponding to individual number, Bacillariophyceae (diatom) was dominant in the second order at a rate of 16.6%, following Dinophyceae (dinoflagellate) (75.5%). Euglenophyceae and Oligotrichea species were also observed in this red tide with rates of 7.70% and <1.00%, respectively. On that day, although the lowest phytoplankton abundance was found (54.0×10^3 ind./L), the highest phytoplankton biomass ((268±26.0) mg/L) was recorded, because of the presence of *N. scintillans* (34.0×10^3 ind./L), which is known for its large cell volume (Figs 3a, b). During the red tide, the *N. scintillans* bloom was also reported from the Marmara Sea on May 17 (NASA, 2015). This organism is not known as toxin producer, but it is, however, able to accumulate toxic levels of ammonia and can thus cause massive mortality to marine organisms because of ammonia excretion into the ambient waters (Faust and Gulledge, 2002; Okaichi and Nishio, 1976). In fact, the highest NH_3 concentration in second order was measured on May 14; however, its concentration was 12 fold higher than the sum of NO_2^--N and NO_3^--N and was the highest rate during the study (Fig. 3c). Therefore, it is reasonable that NO_3^--N and NO_2^--N were indirectly used by *N. scintillans*, and elevated levels of NH_3 were connected with their metabolic activities during the bloom. In addition, phytoplankton abundances and ammonia concentrations remarkably decreased with increased depth in the same station, considering the relationship between decrease in *N. scintillas* abundance and accordingly ammonia excretion (Fig. 3f). In previous studies, *N. scintillans* increase were reported following a *P. micans* bloom in late spring in the Izmit Bay as well as from the Marmara Sea (Aktan et al., 2005; Artüz and Baykut, 1987; Ergül et al., 2014) and those results coincide with the present study.

After late spring, *N. scintillans* was observed in other samplings; however, the following red tides were dominated by *P. micans* in 2015. On July 11, phytoplankton abundance increased again (average 480×10^3 ind./L) and a light red appearance was observed on the sea surface without mucilage forma-

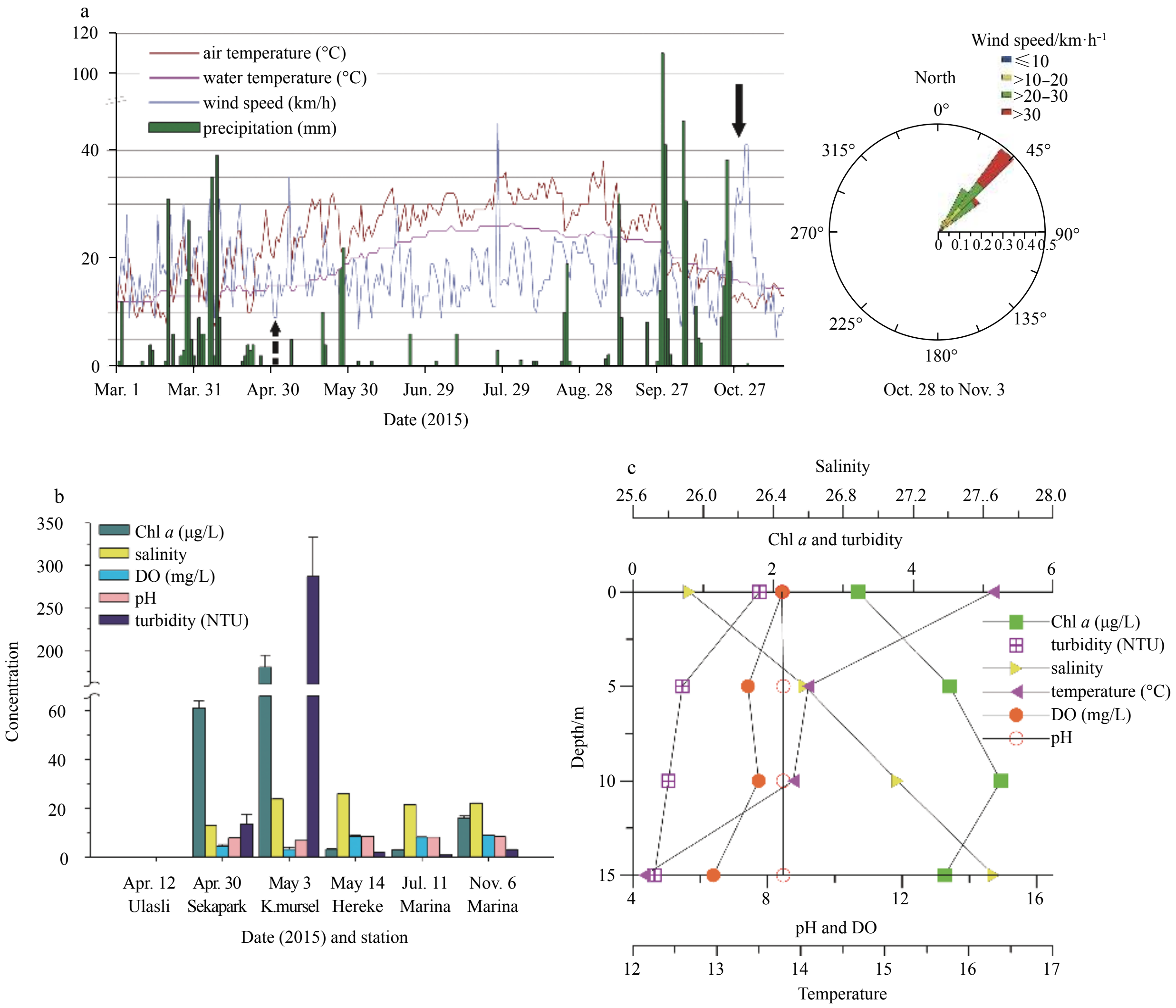

Fig. 4. Daily records of total precipitation, average air and water temperatures, and the highest wind speed (left panel) between March 1 and November 15, 2015 (a) (wind rose diagram (right panel) represents wind speed and directions between October 28 and November 3, 2015; the solid arrow wind rose diagram period; and dashed arrow lower wind speed before the bloom on April 30, 2015); physicochemical parameter levels during the bloom conditions (b); and depth profile of them on May 14, 2015 in the Hereke station (c).

tion, while surface water temperatures of 25.0°C and salinity of 21.4 were recorded at Marina station in the Izmit Bay. With respect to individual count, Dinophyceae was the dominant group, with rates of 86.8%. Bacillariophyceae and Oligotrichea species were also observed with rates of 12.9% and <1.00%, respectively. In this red tide, the highest phytoplankton diversity, with 14 species (eight Dinophyceae, four Bacillariophyceae and two Oligotrichea) were observed simultaneously with the second highest DIN:P ratio as 0.5 (Fig. 3a). Thus, different phytoplankton species found an opportunity to proliferate. Although the highest DIN:P ratio was determined on May 14, as was discussed above, this rate was related to the elevated level of ammonia, which possibly derived from *N. scintillans* excretion. Regarding the increased diatom abundance, the lowest silica concentration ((0.22±0.01) mg/L) was determined on July 11, because of the silica usage in the cell wall of diatoms. On that day, one of the most common diatom species, *Skletonema costatum* reached 60.0×10^3 ind./L which was the highest abundance determined among diatom species throughout the study.

After July 2015, a significant phytoplankton increase was not observed until late autumn in the Izmit Bay. On November 6, a new bloom which was dominated by *P. micans* was observed again, without mucilage formation. Although the incident remained limited and almost no red appearance was observed in the central and western basins, phytoplankton abundance reached 1.10×10^6 ind./L and its diversity reached ten species (eight Dinophyceae and two Bacillariophyceae) in the Marina station located in the eastern basin of the bay. Interestingly, this bloom occurred a few days after a relatively long term strong wind blew. During that time, northeastern gusts, with an average speed of 35 km/h (ranged from 33 to 41 km/h) blew around the Izmit Bay for 5 d between 28 October and 2 November (Fig. 4a-right panel). Over the following days, the wind speed gradually decreased, and on November 6, the last phytoplankton bloom was observed in 2015, when average wind speed was 12 km/h, surface water temperatures were 15°C, and salinity was 22.0 (Fig. 4a). It is well known that the Izmit Bay has been exposed to treated and/or untreated wastewater discharges since the 1960s. Hence, an elevated level of organic and inorganic contaminants including nutrients accumulated in the surface sediment of the bay, is present (Aktan et al., 2005; Ergül et al., 2013a; Karademir et al., 2013; Morkoç et al., 2001). Beside the unknown amount of land based effluents from several sources (e.g., agricultural runoffs, untreated anthropogenic wastewater, underground water), ~400 t total nitrogen and ~100 t total phosphorus were routinely discharged from wastewater treatment plants located around the Izmit Bay in 2015 (Fig. 5). The northern and southern sides of the bay are surrounded by hills, while the eastern and western edges are open from either side. Due to this geographical structure, a corridor occurs in the east-west direction. Therefore, following the ~35 km/h northeastern gusts for 5 d, wind induced sediment resuspensions occurred in the east, the shallowest basin of the Izmit Bay, and ambient water was contaminated with nutrients that were used by phytoplankton for their reproduction. Unlike other cases, NO_3^--N concentration ((0.040±0.003) mg/L) was slightly higher than the NH_3 level ((0.030±0.005) mg/L) in this bloom. This high rate of nitrate may be related to oxidation of resuspended ammonia after nitrification processes in the surface sediment (Fig. 3c). In terms of numbers of individuals, dinoflagellates were dominant with rates of over 99.0%, as in the first three blooms. Therefore, on November 6, 2015, silica content was remained relatively high ((0.76±0.02) mg/L) due to low usage by diatoms (Fig. 3d).

Since DIN:Si ratios ranged between 0.08 and 0.56 (i.e., <1), and DIN:P ratios ranged between 0.03 to 3.11 (i.e., below 16:1) in all samplings, nitrogen was the limiting factor during bloom conditions. It is known that N broadly limits phytoplankton growth in coastal marine ecosystems (Nixon, 1986) and N limitations have been reported from the Izmit Bay during bloom conditions (Aktan and Dede, 2008; Aktan et al., 2005), and other parts of the Marmara Sea (Turkoglu, 2013, 2016; Balkis et al., 2010) as in the present study.

According to previous studies (Morkoç et al., 2001) and Kocaeli Metropolitan Municipality records (ISU, 2016), discharges of total suspended matter (TSM), biological oxygen demand (BOD), total nitrogen (TN) and total phosphorus (TP) into the Izmit Bay gradually decreased 40, 40, 14 and 20 fold, respectively, since 1984, owing to established wastewater treatment plants (Fig. 5). Currently, it is estimated that more than 80.0% of discharge from the wastewater treatment plants has domestic origin, whereas the remaining discharge is from industrial sources. Nevertheless, the present results show that, despite precautions in place to decrease nutrient inputs via these plants, algal blooms still occur, with harmful effects including mucilage presence and invertebrate deaths.

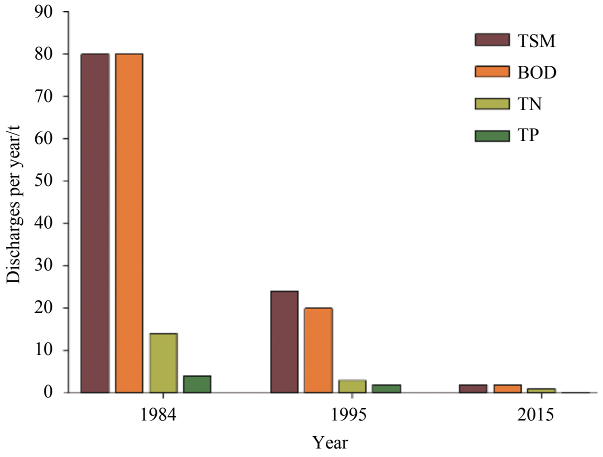

Fig. 5. Daily average domestic and industrial biological oxygen demand (BOD), total suspended matter (TSM), total nitrogen (TN) and total phosphorus (TP) loads (as tonnes) into Izmit Bay between 1984 and 2015.

The trophic index (TRIX), which was developed by Vollenweider et al. (1998) to determine the trophic state and quality of coastal marine ecosystems, ranged from 6.1 to 10 (6.9, 10, 6.3, 6.1, and 6.8 for April 30, May 3, May 14, July 11, and November 6, 2015, respectively). Therefore, based on TRIX values, the eutrophication status of the Izmit Bay was classified as degraded and there was a very high trophic level during the bloom conditions. It should be noted that the eutrophic state of the surface waters of the Izmit Bay fluctuated during the study and in some periods reached good quality and low trophic levels. On the other hand, because of the low current regime and the relatively long residence time of the water masses in the Izmit Bay (Morkoç et al., 2001), nutrient accumulation over certain periods of the year caused deterioration of the trophic state. Also, consecutive high TRIX values may be an important sign for future environmental problems for the bay. In fact, significant correlations between TRIX and total phytoplankton abundance (TPpA) (r=0.98, p<0.01, n=5) indicate that contents of the water encouraged the bloom conditions and following that the water quality of the bay was highly affected by the blooms. Positive significant correlations between o-PO_4^{3-} and TPpA (r=0.96, p<0.01, n=6) and between NO_3^--N and TPpA (r=0.86, p<0.05, n=6) revealed that variations of total phytoplankton density were linked to the nutrient concentrations. Also, a positive significant correlation between total phytoplankton biomass and the DIN:P ratio (r=0.98, p<0.01, n=6) indicates nitrogen limitation. Consequently, despite the improvement work over the last few decades by the municipality of the city around the Izmit Bay, based on adopted legislation in the frame of European Union directives, harmful algal blooms still occur with the abovementioned harmful effects.

In fact, the marine eutrophication issue and phytoplankton blooms, encountered in coastal marine ecosystems of Mediterranean seas, has been addressed in a number of studies since the 1960s (Karydis and Kitsiou, 2012). Through regulation and prevention, based on the Barcelona convention and the directives regarding the European Union policy on eutrophication the Water Framework Directive 2000/60/EC (EC, 2000), Mediterranean countries have modeled their legislation to reduce nutrient inputs to the marine ecosystems (Karydis and Kitsiou, 2012; Saliba, 1995). Nevertheless, despite the precautions, numerous intense phytoplankton blooms have been observed in Mediterranean coastal waters. Hence, we have listed recent intense dinoflagellate and diatom blooms (i.e., >10^6 ind./L) with temperature, salinity and nutrient data in the adjacent seas (i.e., the Marmara and Black Seas) and near Mediterranean coastal waters (i.e., the Aegean, Ionian and Adriatic Seas), in order to evaluate recent cases since 2000 and to compare them with the present results from the Izmit Bay (Table 2).

Temporal distribution of intense diatom and dinoflagellate blooms spread predominantly over three seasons: spring, summer and winter, in the Mediterranean coastal surface waters since 2000. In terms of frequency, the bloom conditions occurred mostly in spring (five diatom and five dinoflagellate blooms) followed by summer (four diatom and two dinoflagellate blooms) and winter (two diatom and three dinoflagellate blooms), whereas intense autumn blooms were recorded less frequently (two diatom and one dinoflagellate blooms). Typically, dinoflagellate blooms occurred in spring and early summer, while diatom blooms occured in winter and early spring.

Table 2. List of the recent most intense dinoflagellate and diatom blooms around Mediterranean coastal surface waters with temperature, salinity and nutrient data, including input sources

Area	Causative species	A_t	Period	T/°C	S	NO_2^--N + NO_3^--N/ mg·L^{-1}	NH_3/ mg·L^{-1}	PO_4^{3-} or TP**/ mg·L^{-1}	SiO_2 or SiO_4^{*}/ mg·L^{-1}	Input
Marmara Sea										
İstanbul Shore1)	*Nitzschia longissima* (Brébisson) Ralfs, 1861	1.3	winter 2000	14	25	–	–	–	–	I, D
Golden Horn Estuary (Istanbul Strait)2), 3)	*Prorocentrum cordatum* (Ostenfeld) J. D. Dodge, 1975 TF	70	summer 2001	23	17	0.03	–	0.34	–	I, D
	Skeletonema marinoi Sarno & Zingone, 2005	54	spring 2010	11	17	0.11	0.07	0.11	0.72	
Çanakkale Strait4), 5)	*Pseudo-nitzschia pungens* (Grunow ex Cleve) G. R. Hasle, 1993	65	summer 2001	23	27	0.01	–	0.03	0.33*	D
	P. pungens	65	winter 2002	8	30	0.04	–	0.04	0.15*	
	Prorocentrum micans Ehrenberg, 1834 *Ceratium* spp.	8.0	E summer 2003	21	22	<0.01	–	0.01	0.12*	
İzmit Bay#	*P. micans*	5.4	spring 2015	15	13	0.02	0.01	0.15	0.84	I, D
	P. micans	18	spring 2015	15	24	0.11	0.28	42.0	0.82	
	P. micans	1.1	autumn 2015	15	22	0.05	0.03	0.35	0.76	
Black Sea										
Trabzon Shore6)	*Scrippsiella trochoidea* (Stein) Loeblich III, 1976	13	winter 2000	9	19	0.40		0.09		D
Constanta Shore7)	*Thalassionema nitzschioides* (Grunow) Mereschkowsky, 1902 *Pseudo-nitzschia delicatissima* (Cleve) Heiden, 1928	8.3	summer 2010	24	11	–	–	–	–	U, R, A
Rize Shore8)	*Noctiluca scintillans* (Macartney) Kofoid & Swezy, 1921 *Melosira* spp.	7.3	spring 2011	11	16	0.18	–	0.01	0.51	D
Aegean and Ionian Sea										
Thermaikos Bay9), 10)	*Skeletonema costatum* (Greville) Cleve, 1873 *Leptocylindrus minimus* Gran, 1915	>6.3	E spring 2000	11	36	0.04	0.02	0.13	0.16*	D, A
	Prorocentrum obtusidens Schiller, 1928	>1, 2	winter 2001	–	–	–	–	–	–	D, A
	Prorocentrum redfieldii Bursa, 1959	>6, 0	winter 2001	–	–	–	–	–	–	D, A
	P. pungens	>1, 8	spring 2002	–	–	–	–	–	–	D, A
	N. scintillans	>5.4	spring 2004	–	–	–	–	–	–	D, A
Amvrakikos Bay9)	*Alexandrium insuetum* Balech, 1985	>2.6	spring 2003	–	–	–	–	–	–	D, R
Kalloni Bay11)	*Pseudo-nitzschia calliantha* Lundholm, Moestrup & Hasle, 2003	>7.3	spring 2005	12	36	0.07	0.01	0.01	0.72	D, R, A
Adriatic Sea										
Trieste Bay12)	*S. marinoi*	2.6	E spring 2000	–	–	–	–	–	–	T, Ir, R, A
	Chaetoceros spp.	4.6	autumn 2000	–	–	–	–	–	–	T, R, A
	Chaetoceros spp.	2.3	E spring 2009	–	–	–	–	–	–	T, R, A
Pesaro–Foglia Coast13)	Diatom species	40	autumn 2000	16	17	0.56	0.01	0.28**	–	R, A, I, D
Kastela Bay14)	*L. minimus*	6.0	E summer 2005	27	36	0.01	0.02	0.01	0.09*	I, D, R
Rovinj Coast15)	*Chaetoceros vixvisibilis* Schiller in Hustedt, 1930	1.4	Summer-2009	26	35	0.47	0.55	0.03	3.42*	U, T, R, A

Note: A_t represents botal abundance, T temperature, S salinity, I industrial, Ir solar irridance, D domestic, R riverine, T terrigenic, U upwelling, A agriculture, and E early. 1) Deniz and Taş (2009), 2) Tas and Okus (2011), 3) Tas and Yilmaz (2015), 4) Turkoglu (2010), 5) Turkoglu (2008), 6) Feyzioğlu and Seyhan (2007), 7) Mihailov et al. (2013), 8) Kopuz et al. (2014), 9) Nikolaidis et al. (2005), 10) Koukaras and Nikolaidis (2004), 11) Spatharis et al. (2007), 12) Cabrini et al. (2012), 13) Penna et al. (2004), 14) Bužančić et al. (2016), 15) Bosak et al. (2016), # the present study, * SiO_4, and **total phosphorus.

However, in some cases, dinoflagellate blooms were reported in winter (*Scrippsiella. trochoidea* (Stein) Loeblich III, 1976, and *Prorocentrum redfeldii* Bursa, 1959 from Trabzon Shore and Thermaikos Bay, respectively) whereas diatom blooms were reported in summer seasons (*Pseudo-nitzschia Pungens* (Grunow ex Cleve) G. R. Hasle, 1993, *Thalassionema nitzschioides* Schiller in Hustedt, 1930, *Leptocylindrus minimus* Gran, 1915 and *Chaetoceros vixvisibilis* Schiller in Hustedt, 1930 from the Çanakkale Stratit, Constanta Bay, Kastela Bay and Rovinj coasts, respectively; Table 2).

Phytoplankton blooms may occur in a wide range of temperatures and salinity (8.00–27.0°C and 11.0–36.0, respectively) and species-specific characters seem to be determined in bloom formations. Nutrient values also had diversified levels (<0.01–0.56, 0.01–0.55, 0.01–42.0 and 0.12–3.42 for NO_2^--N + NO_3^--N, NH_3, PO_4^{3-} and SiO_2 or SiO_4, respectively) and in almost all blooms, the ratios of nitrogen to phosphorus were remarkably different from the classical Redfield ratio (i.e., 16:1; Table 2). Consequently, these data suggest that a combination of nutrients is more important than its levels for diatom and dinoflagellate bloom formations, and termination of proliferation is determined by limiting nutrients. In addition, the blooms are predominantly influenced by temperature and salinity while sufficient light intensity and favorable amounts of nutrients exist in the water.

The spatial distribution of these blooms revealed that dense incidents mainly arose in the coastal waters of the northern parts of the Mediterranean. Although bloom cases have been reported, the abundance of diatoms and dinoflagellates did not exceed 10^6 ind./L in the Levantine Sea. Extreme proliferations of both groups were reported from the Black, Marmara, Aegean and Ionian Seas. However, despite the remarkable dinoflagellate abundance, incidents that exceeded 10^6 ind./L were reported merely for diatoms from the Adriatic Sea since 2000 (Table 2). Because the northern parts of the Mediterranean are richer in nutrients than the southern parts (Ignatiades, 2005), denser phytoplankton blooms are expected in the northern regions. In fact, the densest dinoflagellate bloom (70×10^6 ind./L) was reported from the Golden Horn Estuary, adjacent to the Istanbul Strait as well as the Black and Marmara Seas, in summer 2001 (Taş and Okuş, 2011). Besides being a semi-enclosed basin, the Golden Horn Estuary was known for its polluted water with foul odor because of untreated industrial and domestic discharges. However, the estuary was rehabilitated in the mid-2000s and bloom conditions have not been reported since 2010. Another dense bloom (40×10^6 ind./L) due to proliferation of diatoms was recorded in Pesaro coast in the north Adriatic in autumn 2000. Beside local land-based sources, the Po River was considered as the responsible nutrient carrier for this bloom (Penna et al., 2004). Like above-mentioned blooms, most cases occurred under the land based anthropogenic inputs around the northern Mediterranean. However, legislations, based on EU directives, were set up to prevent further deterioration, and the decreasing bloom densities can be considered possible improvements in many Mediterranean coasts.

A comparison with blooms from nearby coastal surface waters of the Mediterranean seas showed that dinoflagellate blooms in the Izmit Bay were the densest consecutive blooms ever reported in recent years (Table 2). Like other incidents, domestic and industrial inputs were effective in those blooms. Additionally, unlike other incidents, wind-induced sediment resuspension caused a dinoflagellate bloom in the Izmit Bay, and this case indicates that accumulated contaminants in the surface sediment can be a potential nutrient source. This represents long term effects of untreated discharges on an elongated coastal marine ecosystem.

4 Conclusions

Beside low-speed water mass movement and routinely discharged nutrients via wastewater treatment plants from a populated city, various reasons might have contributed to the occurrence of the algal bloom conditions in the Izmit Bay, such as the inadequacy of advanced treatment technology, untreated and/or illegal domestic and industrial discharges, agricultural runoffs, and wind-induced resuspensions. Therefore, the results of the present study suggest that there is a need for increased action to prevent harmful algal blooms, in accordance with the environmental deterioration in the Izmit Bay. This can be achived by improvements to wastewater treatment technology, including nutrient removal capability, prevention of untreated discharges, illegal bilge bailing and controlling agricultural fertilizer usage. Finally, the removal of the upper layer of the eastern basin's surface sediment should be considered to prevent undesirable effects of wind induced resuspensions.

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