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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Some potentially toxic dinoflagellate cysts in recent sediments from İzmir Bay

İzmir Körfezi yüzey sedimentinde olası toksik dinoflagellat kistleri

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Özet: Ötrofikasyon ve aşırı çoğalma olaylarıyla tanınan İzmir Körfezi'nde 2003-2011 yılları arasında potansiyel toksik dinoflagellat kistlerinin dağılımı ve bolluğu incelendi. *Lingulodinium machaerophorum*, *Operculodinium centrocarpum, Alexandrium affine* tip, *Alexandrium catenella/tamarense* kompleks, *Alexandrium minutum* tip potansiyel toksik kistler olarak bulundu. *Lingulodinium machaerophorum* en yüksek konsantrasyonuna (toplam 24872 kist g^{-1} kuru ağırlık) 2008-2009 yılında ulaştı. Bu çalışma ayrıca son on yıl için İzmir Körfezi yüzey sedimentinde potansiyel toksik dinoflagellat kistlerinin dağılımı ve konsantrasyonlarının kısa bir derlemesini içermektedir.

***Anahtar kelimeler*:** Toksik dinoflagellat kistleri, yüzey sedimenti, *Alexandrium spp., Lingulodinium machaerophorum, Operculodinium centrocarpum.*

Abstract: The distribution and abundance of potentially toxic dinoflagellate cysts were investigated from the surface sediments in İzmir Bay which is famous for red-tide and eutrophication between 2003-2011. *Lingulodinium machaerophorum, Operculodinium centrocarpum, Alexandrium affine* type, *Alexandrium catenella/tamarense* complex, *Alexandrium minutum* type were found as potentially toxic cysts. *Lingulodinium machaerophorum* reached to their highest concentrations (total 24872 cyst g^{-1} dry weight) in 2008-2009. This study also includes a short review of distribution and concentration of potentially toxic cyst types in recent sediment of İzmir Bay for last decade.

***Keywords*:** Toxic dinoflagellate cysts, surface sediment, *Alexandrium* spp., *Lingulodinium machaerophorum, Operculodinium centrocarpum.*

INTRODUCTION

Dinoflagellates contain the largest number of harmful species in phytoplankton and can be extremely toxic (Taylor, 1979; Smayda, 1990; Hallegraef, 1993). Some dinoflagellate species produce a resting cyst in their life cycle. These resistant cysts can germinate in optimal environments after a certain resting period and vegetative cells are regarded as seeds for future blooms (Anderson and Wall, 1978).

Marine microplankton and bloom events have been searched in Turkish coastal water since 1955. Moreover interspecific relations and physicochemical parameters connected with phytoplankton species have been investigated as a part of phytoplankton studies in different areas. Since red-tides and other noxious algal blooms were observed almost every year mainly due to progressive eutrophication from terrestrial inputs (Koray and Buyukışık, 1988; Koray, 1990, 1992, 1994, 2002; Koray *et al.*, 1996). Phytoplankton check list of Turkish coastal waters was published by Koray (2001). Harmful algal bloom events according to areas for Turkish Coastal waters and potentially toxic species list were published in another study (Koray, 2004).

Recently, modern dinoflagellate cyst studies have become a very important topic for some studies such as dinoflagellate life cycle, harmful algal bloom, aquaculture and monitoring of water quality (Uzar *et al.*, 2010; Aydın *et al.*, 2011). Cyst studies also help to observe distribution and abundance of dinoflagellate species. Modern dinoflagellate cyst studies are important in studies of toxic and harmful algal blooms of dinoflagellates (Orlova *et al.*, 2004; Matsuoka *et al.*, 2009).

The main aim of this study is to observe the distribution and concentration of potentially toxic cyst types in recent sediment of İzmir Bay from 2003 to 2011 and to discuss compared to other locations of Mediterranean Seas.

MATERIALS AND METHODS

The study area is one of the largest bays in the Eastern Mediterranean. İzmir Bay is limited freshwater inputs and has typical tropic-subtropical characteristics (Sayın, 2003). The bay is a highly disturbed environment due to the rapid increase of the population and development of industry (Kontas *et al.*, 2004; Küçüksezgin *et al.*, 2006).

In this study, 20 surface sediment samples were collected at different stations from İzmir Bay between 2003 and 2011 (Figure 1).

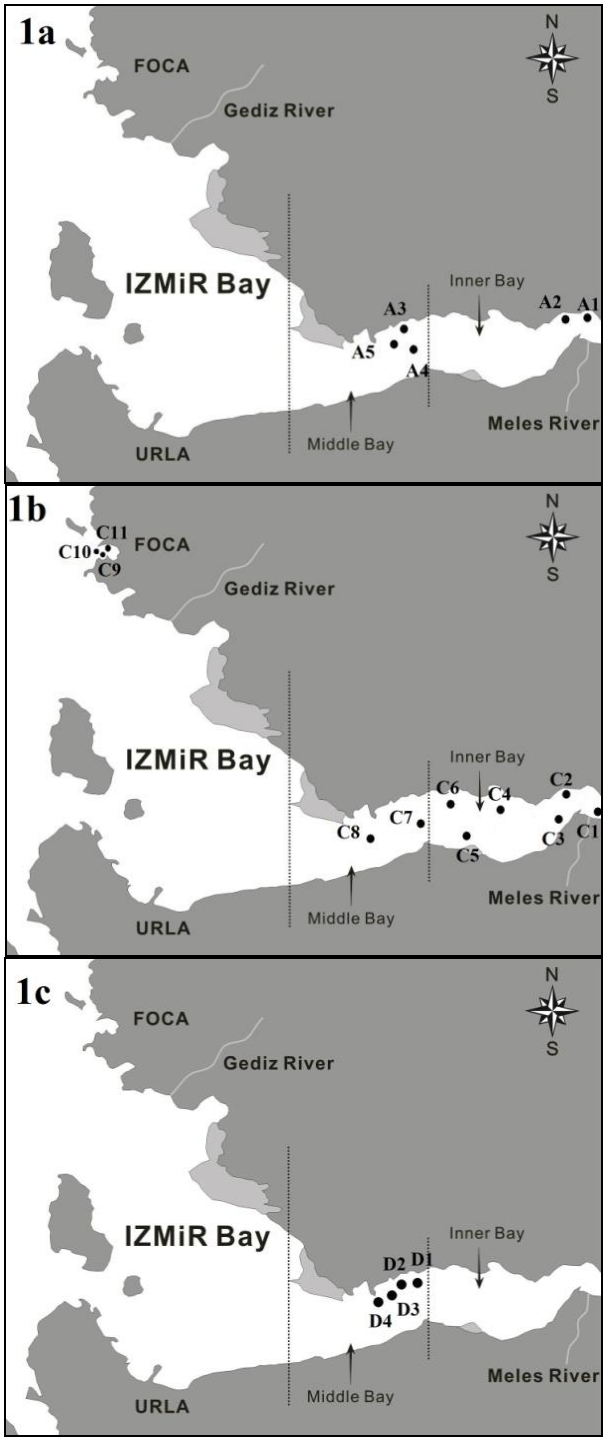

Fig. 1. Location of sampling stations in the outer, middle and inner parts of İzmir Bay, Aegean Sea (**1a.** Sampling stations in 2003, **1b.** Sampling stations in 2008-2009, **1c.** Sampling station in 2011).

Sediment samples were collected with the TFO (Tokyo University Fisheries and Oceanography Laboratory Gravity Corer) corer. The samples for dinoflagellate cyst analyses were treated using standard palynological methods (Matsuoka and Fukuyo, 2000). The upper 2 cm of cores were cut and immediately preserved in a refrigerator in the dark at 4.00 °C to prevent cyst germination. In the palynological procedure, sediments were processed with ca. 10% HCl (Hydrochloric acid) and ca. 47% HF (Hydrofluoric acid) to remove calcium carbonate and silicate materials. The samples were then repeatedly washed with distilled water to remove acid until the pH values were almost 7.00. The chemically treated samples were sonicated for 30s and then successively sieved through two different stainless steel meshes with 125 and 20 µm opening sizes. The sediments retained on a latter screen were transferred into a plastic tube and suspended in 10 ml distilled water and kept in a vial. Observations and counting carried out on 1-0.5 ml aliquot of processed sample with Sedgewick-Rafter chamber under an inverted microscope (Olympus IX71 with DIC) at magnifications 100, 200 and 400 times. *Alexandrium* spp. cysts were also observed with an Olympus BX51 fitted with a filter arrangement for ultraviolet excitation (330-385 nm) following primuline-staining method of Yamaguchi *et al.* (1995). Primuline-stained *Alexandrium* spp. cysts exhibit an intense yellow-green fluorescence under a fluorescence microscope with blue-light excitation. The terminology used for describing the dinoflagellate cysts essentially followed the works of Wall and Dale (1968), Matsuoka and Fukuyo (2000), Cho and Matsuoka (2001), Matsuoka *et al.* (2004), Wang *et al.* (2004), Bravo *et al.* (2006), Touzet *et al.* (2008), Satta *et al.* (2010) and *Aydın et al.* (2011). Because of the morphologically resembling species with *A. tamarense, A. catenella* and *A. fundyense*, they are recently grouped within *A. catenella/tamarense* species complex (Anderson *et al.*, 2012). Because of the fact that we reported these cysts as type or complex in this study. Although *Alexandrium* spp. cyst types were found in processed samples, live cyst types were also checked in row samples by sieving technique.

Cyst concentration in each sample was calculated as cysts g^{-1} dry weight of sediment. In the literature, cyst concentration is expressed as cysts per gram of dry sediment weight. Surface sediments inevitably contain large and variable water content. Calculating a cyst concentration based on sediment volume, rather than dry weight, therefore, introduced a variable factor (amount of water content) that will hinder comparison with other studies. In order to facilitate comparisons between our stations, as well as with other studies, we have presented all our results as cysts per gram dry weight.

RESULTS

Qualitative and quantitative analyses of potentially toxic cyst types in surface sediment samples collected from İzmir Bay, Eastern Aegean Sea and their descriptive statistical analyses are shown in Table 1, 2 and 3. Moreover, various photo micrographs of these toxic dinoflagellate cysts are presented in Figure 2.

Five potentially toxic cyst types were found in surface sediment samples collected from İzmir Bay. They are *Alexandrium affine* (Inoue et Fukuyo) Balech 1995 type, *Alexandrium catenella/tamarense* complex, *Alexandrium minutum* Halim 1960 type, *Lingulodinium machaerophorum* (Defl. et Cook) Wall 1967 and *Operculodinium centrocarpum* (Defl. et Cook) Wall 1967 (Table 1 and Figure 2.)

The total potentially toxic dinoflagellate cyst concentration according to sampling years is given Table 1. *A. affine* cyst type was recorded range from 0 to 156 cysts g^{-1} dry weight sediment between 2003 and 2011. *A. catenella/tamarense* complex concentrations were counted between 0 and 33 cysts g^{-1} dry weight sediment between same years. *A. minutum* type concentration was recorded between 0 and 84 cyst g^{-1} dry weight sediment between 2003 and 2011. *L. machaerophorum* concentration was recorded from 7 to 6584 cysts g^{-1} dry weight sediment between 2003 and 2011. Concentration of *O. centrocarpum* was ranged between 0 and 347 cysts g^{-1} dry weight sediment between 2003 and 2011. *L. machaerophorum* concentration was highest in 2008-2009 (24872 cysts g^{-1} dry weight sediment) compared to other cyst types and common in all stations. *O. centrocarpum* was observed in most of stations. *A. minutum, A. catenella/tamarense* complex and *A. affine* type were in very lower concentrations than any other cyst types between 2003 and 2011 (Table 2).

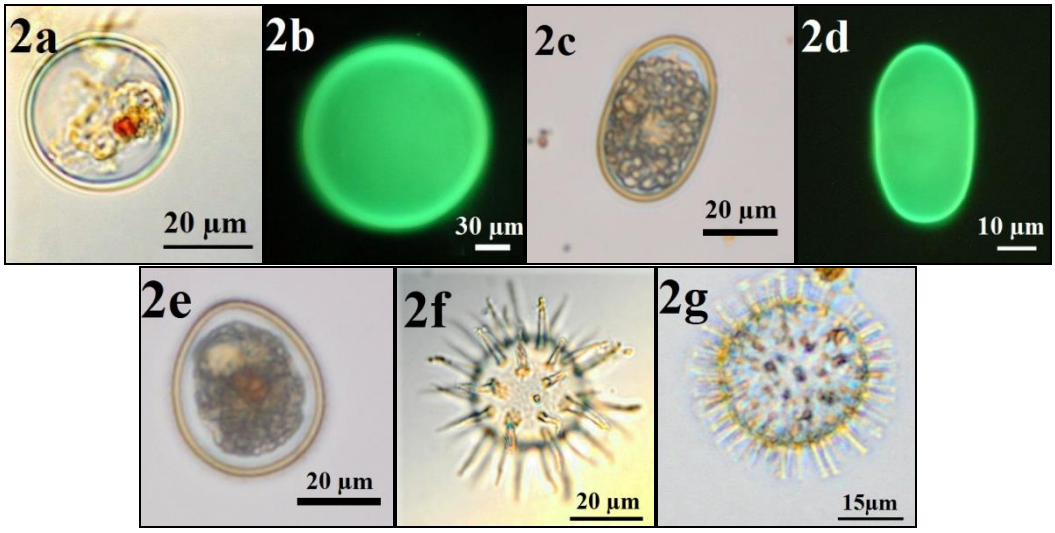

Figure 2. Photomicrographs of potentially toxic dinoflagellate cysts collected in surface sediments from the İzmir Bay, (**2a:** *Alexandrium affine* type cyst type under normal light, **2b.** *Alexandrium affine* type cyst type under UV light, **2c.** *Alexandrium catenella/tamarense* complex under normal light, **2d.** *Alexandrium catenella/tamarense* complex under UV light **2e.** *Alexandrium minutum* type cyst **2f.** *Lingulodinium machaerophorum*, **2g.** *Operculodinium centrocarpum*).

Table 1. Total concentration of potentially toxic cysts between 2003, 2008-2009 and 2011.

Biological Name	Paleontological Name	2003	2008-2009	2011
Alexandrium affine type	None	118	49	200
Alexandrium catenella/tamarense complex	None	0	74	70
Alexandrium minutum type	None	73	9	180
Lingulodinium polyedrum	*Lingulodinium machaerophorum*	2633	24872	1635
Protoceratium reticulatum	*Operculodinium centrocarpum*	148	1025	518

Table 2. Qualitative and quantitative analyses of potentially toxic cyst types in surface sediment samples collected from Izmir Bay, eastern Aegean Sea and their descriptive statistical analyses in different periods of 2003 – 2011 (cyst g^{-1} dry weight sediment).

	2003				2008-2009				2011			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
***Alexandrium affine* type**	20	42	23.60	15.96	0	28	4.49	9.00	0	156	50.00	72.21
***Alexandrium catenella/tamarense* complex**	0	0	0.00	0.00	0	33	6.73	10.41	0	25	17.50	11.79
***Alexandrium minutum* type**	10	51	14.60	21.09	0	6	0.82	1.94	17	84	45.00	28.15
Lingulodinium machaerophorum	202	897	526.60	329.92	7	6584	2261.09	2469.31	239	529	408.75	144.18
Operculodinium centrocarpum	5	123	29.60	52.85	0	93.18	112.48	158.72	77	240	129.50	76.73

Table 3. Qualitative and quantitative analyses of potentially toxic cyst types in surface sediment samples collected from İzmir Bay, Eastern Aegean Sea and their descriptive statistical analyses in full study period (cyst g^{-1} dry weight sediment).

	Min	Max	Mean	SD
***Alexandrium affine* type**	0	156	18.35	35.35
***Alexandrium catenella/tamarense* complex**	0	33	7.20	10.73
***Alexandrium minutum* type**	0	84	13.10	22.87
Lingulodinium machaerophorum	7	6584	1457.00	2017.14
Operculodinium centrocarpum	0	347	84.55	97.16

DISCUSSION

The results of this study showed that İzmir Bay was one of the regions in the Aegean Sea where the potentially toxic dinoflagellate cysts were accumulated. *A. minutum* and *A. catenella* are well-known Paralytic Shellfish Poisoning (PSP) toxins producers and have received increasing importance and attention in the last few decades because of the impact on aquaculture and human health (Bravo *et al.*, 2006, 2008). As is inner part of İzmir Bay, it is known that harbours are excellent sheltered habitats for at least these two *Alexandrium* species, since low dispersion aids bloom formation and cyst accumulation (Bravo *et al.*, 2008).

A. minutum is widespread along the Mediterranean coasts. This species is frequently detected year-round at low concentrations and it proliferates recurrently in some Mediterranean localities. However, A. catenella is currently under going a major increase in distribution (Penna *et al.*, 2005). *A. minutum* was first described species in the Mediterranean Sea forming a red water discoloration in Alexandria harbour, Egypt (Halim, 1960) and it has since then been widely reported from the Mediterranean (Koray and Buyukışık, 1988; Delgado *et al.*, 1990; Montresor *et al.*, 1990; Honsell, 1993; Giacobbe *et al.*, 1996; Forteza *et al.*, 1998; Daly Yahia-Kefi *et al.*, 2001; Vila *et al.*, 2001; Tahri-Joutei *et al.*, 2003; Koray, 2004). These *A. minutum* blooms seem to be restricted to nutrient-enriched coastal sites, particularly harbours, estuaries and lagoons (Giacobbe *et al.*, 1996; Vila *et al.*, 2005).

A. catenella had never been observed in the Mediterranean before 1983 and was reported as a rare species by Margalef and Estrada (1987). The first known bloom in the Mediterranean Sea occurred in 1994 in Valencia harbour (Spain) and from 1996 to 2000 recurrent blooms were reported more to the north, along the Catalan coast (Vila *et al.*, 2001). In the last few years, the expansion of this species has also been documented in all over the NW Mediterranean Sea, from the Thau Lagoon (France) (Lilly *et al.*, 2002), the North Lake and Channel of Tunis (Tunisia) (Turki and Balti, 2005) and Olbia harbour (Italy) (Luglie´ *et al.*, 2003; Satta *et al.*, 2010). Most of these events were related to harbours or confined water areas.

In this study, potentially toxic cysts of *A. minutum, A. catenella/tamarense, A. affine, L. machaerophorum, O. centrocarpum* were observed in the surface sediments of the İzmir Bay. Although vegetative form of *A. minutum* was reported first time in the İzmir Bay by Koray and Buyukışık (1988) and the vegetative forms of *A. affine, A. catenella* or *A. tamarense* had not previously recorded from İzmir Bay (Koray, 2001, Koray *et al.*, 1992, Sabancı and Koray, 2001, 2005, 2012). But three species were first recorded as a cyst type in the surface sediments by Uzar *et al.* (2010) and Aydın *et al.* (2011).

A. minutum type was found very limited distribution and concentration ranged between 0-84 cysts g^{-1} dry weight concentration in sampling stations. *A. minutum* was reported ca. 278 cysts g^{-1} dry weight in Alexandria Harbour, Egypt by Ismael and Khadr (2003). On the other hand, this cyst type was found 3270 cysts g^{-1} dry weight from Arenys de Mar harbour in Spain (Garces *et al.*, 2004). Bravo *et al.* (2006) recorded this cyst type between 110 and 3270 cysts cm^{-3} wet weight from Western Mediterranean coast. Rubino *et al.* (2010) found *A. minutum* types ca. 207 cyst g^{-1} dry weight in Ionian Sea, Mediterranean. Comperasion of the above cyst concentrations, İzmir Bay has lower cyst concentrations than other Mediterranean ecosystems.

A. catenella/tamarense complex concentration ranged from 0 to 33 cysts g^{-1} dry weight from İzmir Bay stations between 2003 and 2011 while *A. tamarense* cysts were counted as 212 cysts ml^{-1} wet sediment from Scotland by Joyce (2004), They were counted as 130 cysts g^{-1} dry weight by Genovesi-Giunti *et al.* (2005). However *A. catenella* cysts varied from 105 to 3480 cysts cm^{-3} wet weight in Mediterranean waters (Bravo *et al.*, 2006). *A. tamarense* cysts were 200 cysts g^{-1} dry weight in Irish coastal waters (Touzet *et al.*, 2008). Rubino *et al.* (2010) observed that *A. cf. tamarense* cysts were 3 cysts g^{-1} dry weight in Western Ionian Sea.

L. machaerophorum was recorded in very high concentration rates and varied between 7 and 6584 cysts g^{-1} dry weight compared to other cyst types during the study (Table 1). *L. polyedrum* was very important bloom species recorded in many harmful algal bloom events (Koray *et al.*, 1992; 1996; Koray, 2004). This cyst type was found in many eutrophic areas (Dale *et al.*, 1999; Matsuoka, 1999; Marret and Zonneveld, 2003). Eutrophication of the inner bay of İzmir was a serious problem throughout the year and red tide events were common (Kontas *et al.*, 2004; Küçüksezgin *et al.*, 2006). This study showed that cyst concentration values of *L. machaerophorum* increased for last decades in İzmir Bay.

Protoceratium reticulatum (Claparade & Lachmann) Butschli, 1885 (vegetative form of *O. centrocarpum* cyst) was recorded in many studies from the sampling area (Koray *et al.*, 1992, 1996; Koray, 2001, 2004; Sabancı and Koray, 2001). *P. reticulatum* is known to produce yessotoxins (Satake *et al.*, 1997). *O. centrocarpum* has a cosmopolitan distribution and very broad tolerance in temperature and salinity (Marret and Zonneveld, 2003). This study showed that the *O. centrocarpum* had a widely distribution in İzmir bay between 2003 and 2011.

Accumulation of potentially toxic cysts is very important for some bay, harbours and enclosed coast like İzmir Bay. As a result of this study, some potentially cyst types in İzmir Bay might work as a seedbed in sediment and may cause harmful algal blooms when environmental conditions are adequate to support to bloom. Our survey suggests that more intensive cyst surveys are needed to determine possible bloom and toxin production where the cysts are located.

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