



## Distribution and abundance of modern dinoflagellate cysts from Marmara, Aegean and Eastern Seas of Turkey

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### Abstract

Twenty-one surface sediment samples were collected from coastal areas of Turkey to determine horizontal distribution and abundance of the cysts. A total of 40 dinoflagellate cyst types were identified and recorded in the range of 34 and 31532 cyst g<sup>-1</sup> d.wt. in the sediments. The concentration of *Lingulodinium machaerophorum*, *Polykrikos kofoidii*, *Quinquecuspis concreta*, *Dubridinium caperatum* and *Spiniferites bulloideus* cysts dominated over other species. Although cysts of potentially toxic species of *Alexandrium affine* type and *A. catenella/tamarensis* complex were found only in Izmir Bay. *A. minutum* type, *Lingulodinium machaerophorum* and *Operculodinium centrocarpum* were observed in the surface sediments of Marmara Sea and Fethiye Bay. The present study provides a database on the distribution and composition of dinoflagellate cysts in the Eastern Mediterranean sea coastal waters of Turkey where modern dinoflagellate cysts have been little studied.

### Key words

Dinoflagellate cysts, Izmir Bay, Marmara Sea, Surface sediment, Toxic species

### Introduction

Dinoflagellates are a diverse and important group of marine microphytoplankton (Ribeiro and Amorim, 2008). This group also includes the largest number of harmful species (Smayda and Reynold, 2003). In some bloom-forming species, resting cysts are the key to bloom initiation as well as a tool for species maintenance when conditions become unfavorable (Estrada *et al.*, 2010). Bravo *et al.* (2006) studied the efficacy of benthic stages on the resting cysts of *Alexandrium* genus in several semi-enclosed environments (harbors, beaches, and embayments) in the Western Mediterranean. The cyst stage of dinoflagellate life cycle plays an important role in both initiation and decline of toxic blooms and gives information regarding the number and identity of cysts in the sediment. They also provide an integrated record of the presence of toxic species in an area. If the blooms are small, short-lived or otherwise go undetected, vegetative cells may not be observed in the water column, but the cysts can be found any time during the year in the sediments (Joyce *et al.*, 2005).

The main environmental cyst signals recognized so far include the coastal/oceanic transition, climatic signal, salinity signal, eutrophication signal and productivity signal (Dale *et al.*, 1999; Marret and Zonneveld, 2003; Ribeiro and Amorim, 2008). Recently, modern dinoflagellate cysts are important for studying of dinoflagellate ecology and biogeography, occurrence of toxic and harmful algal blooms and for taxonomy and phylogeny of living species (Orlova *et al.*, 2004).

Mudie *et al.* (2004) reported the distribution and assemblage of cysts with palynological records from Marmara, Aegean and Mediterranean Seas. However, investigations on the distribution, concentration and assemblage of modern dinoflagellate cysts are meagre (Uzar *et al.*, 2010; Aydın *et al.*, 2011). In view of the above, the present work was undertaken to investigate the recent distribution, abundance and composition of dinoflagellate cysts in the sediments of Marmara, Aegean and Mediterranean Seas.

### Materials and Methods

**Study Area :** Izmir Bay is one of the largest embayments in the eastern Aegean Sea in the Eastern Mediterranean. It has limited freshwater input and typical tropic-subtropical characteristics with highly disturbed environment due to rapid increase in population and industrial developments. The main source of pollution are untreated domestic and industrial wastes, agricultural run-offs, atmospheric pollution and shipping. The Marmara Sea is a semi-enclosed basin located between Europe and Asia. It is connected to the Black Sea through the strait of Bosphorus in the north-east and to the Aegean sea through the strait of Dardenelles in the south-west. Fethiye Bay is one of the important natural bays in the Mediterranean Sea and is affected by many tourist activities like boat trips and cruise ships.

**Collection of sediments :** In this study, out of 21 surface sediment samples collected during 2002-2009, 17 samples were from Izmir Bay, 1 from Fethiye Bay and 3 from Marmara Sea respectively. Sediment samples were collected with a hand gravity corer (30 cm long and 3 cm in diameter tube).

**Preparation of samples :** For analysis of dinoflagellate cysts, the samples were treated following the palynological method suggested by Matsuoka and Fukuyo (2000). To prevent cyst germination, the upper 2 cm of cores were cut and immediately preserved in dark at 4°C. Sediments were processed in 10% HCl and 47% HF acid to remove calcium carbonate and silicate

materials. The samples were then repeatedly washed with distilled water to remove acid until the pH value was 7. The chemically treated samples were sonicated for 30 sec and then successively sieved through two different stainless steel meshes of 125 and 20  $\mu\text{m}$  pore sizes. The sediments retained on a latter screen were transferred into a plastic tube containing 10 ml of distilled water. A 1 ml aliquot of refined sample was observed for identification and counting of cysts under an inverted microscope (Olympus IX71) equipped with a camera of 100, 200 and 400 magnification. The terminology used for describing the dinoflagellate cysts essentially followed the works of Wall and Dale (1968), Matsuoka and Fukuyo (2000) and Matsuoka *et al.* (2004). Cyst concentration in each sediment sample was calculated by the following formula:  $N/W(1-R)$ , where N is the cyst number obtained by multiplying the counted cyst number by a coefficient to make 10 ml refined sample; W is the weight of sediment sample under wet condition and R is the rate of water content of the sediment sample. The result of cysts concentration was expressed in cysts  $\text{g}^{-1}$  d.wt. sediment.

### Results and Discussion

A total of 38 cysts types were recorded in the sediment sample out of which 35 cyst types were identified up to species level and 3 up to genus level. Two cyst types could not be identified and were reported in the present study as cyst type A and B. Besides 18 autotrophic cyst types, 20 heterotrophic cyst groups were also recorded.

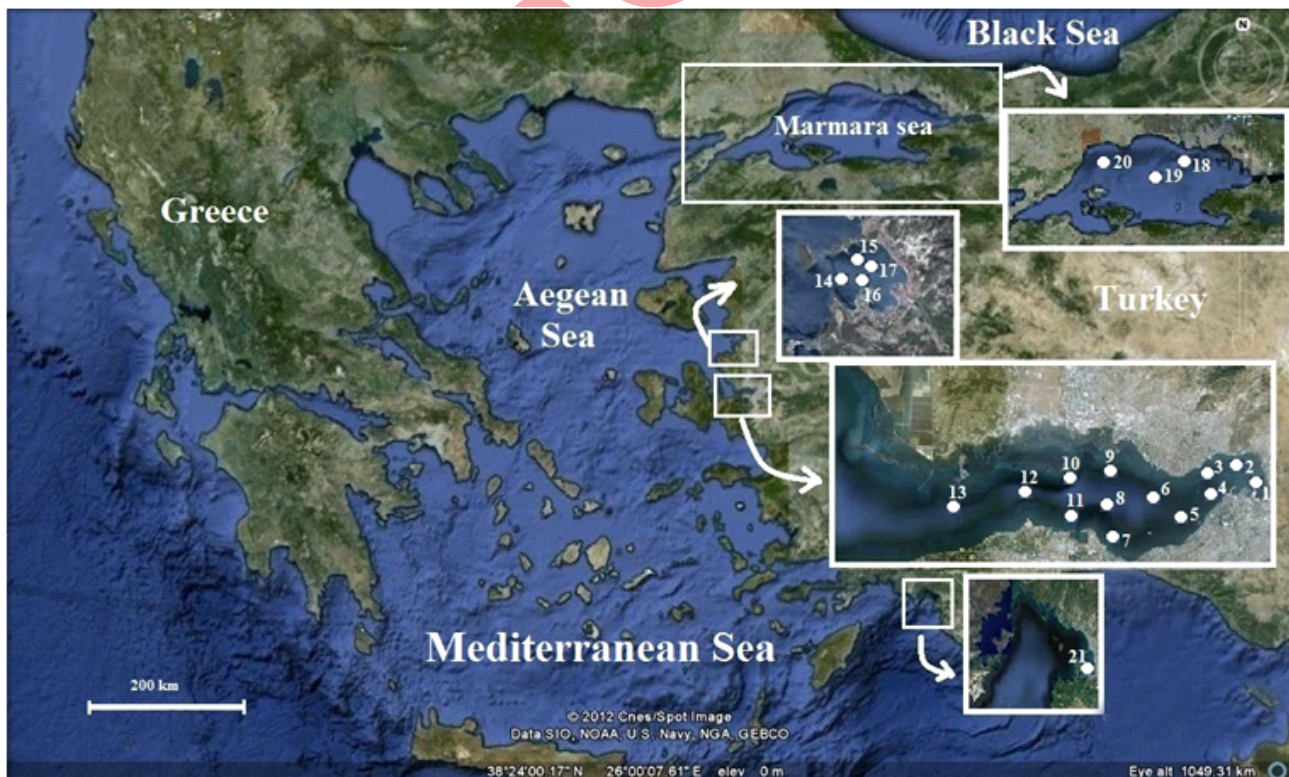


Fig. 1 : Location of sampling stations

In the present study, 16 cyst types identified belonged to order Gonyaulacales, 20 cysts belonged to order Peridinales and 2 cysts belonged to Gymnodinales respectively. It was found that Spiniferites species was most abundant and belonged to Gonyaulacoid group. Six different Spiniferites species identified were *Spiniferites belerius*, *S. bentorii*, *S. bulloideus*, *S. cruciformis*, *S. cf. delicatus*, *S. hyperacanthus*, *S. mirabilis* and *S. ramosus*, respectively. Protoperidinoid cyst type (16 species) was the most abundant group among Peridinales.

Potentially toxic cysts of *L. machaerophorum* and *Operculodinium centrocarpum* showed cosmopolit distribution at each sampling point. *Tuberculodinium vancompoae*, *Spiniferites cruciformis*, *Stelladinium abei*, *S. reinii*, and *S. robustum* cyst types were only observed in Marmara Sea. Other cyst types were found at most of the stations. Although station 8 had the highest cyst diversity with 28 cyst types (Izmir Bay, Aegean Sea), the lowest cyst diversity, with 6 cyst types was observed at station 17 (Aegean Sea) and 21 (Fethiye Bay, Mediterranean Sea).

In the present study, composition of modern dinoflagellate cyst assemblages and abundance were investigated in the sediments of Turkish coastal water. Most of the cysts were found living and empty. Cyst abundance in the stations ranged from 34 (station 21) to 31532 (station 7) cyst g<sup>-1</sup> d.wt. sediment. As a whole, autotrophic cyst types were dominant with a total of 86492 cyst g<sup>-1</sup> d.wt. sediment (60.15 %) whereas heterotrophic cyst types were less dominant with a total of 57297 cyst g<sup>-1</sup> d.wt. sediment (39.85%).

*Lingulodinium machaerophorum*, with a total of 59773 cyst g<sup>-1</sup> d.wt. sediment concentration (41.56 %) was the most abundant cyst species in the study area. *L. machaerophorum* cyst ranged between 7 (station 16) and 17936 cyst g<sup>-1</sup> d.wt. sediment (station 7). Besides, cysts of *Polykrikos kofoidii* (a total of 15106 cyst g<sup>-1</sup> d.wt. sediment), *Quinquecuspis concreta* (a total of 14802 cyst g<sup>-1</sup> d.wt. sediment) and *Dubridinium caperatum* (a total of 12434 cyst g<sup>-1</sup> d.wt. sediment) were also found in high concentrations in the study area.

Uzar *et al.* (2010) and Aydin *et al.* (2011) reported 29 dinoflagellate cyst types and 36 cyst types in Izmir Bay. In addition, different cyst types of *Spiniferites cruciformis*, *Nematospheropsis sp.*, *Tuberculodinium vancompoae*, *Stelladinium abei*, *S. reidii*, *S. robustum*, *Trinovantedinium capitatum* were identified only at the stations located in the Marmara Sea.

The concentration of dinoflagellate cysts reported in the present study (16-31532 cyst g<sup>-1</sup> d.wt. sediment) is in confirmation with the earlier investigations carried out in other seas. Sangiorgi *et al.* (2005) and Rubino *et al.* (2010) reported dinoflagellate cysts in the range of 3000-120000 cyst g<sup>-1</sup> d.wt. and 43-828 cyst g<sup>-1</sup> d.wt. in the surface sediments of Adriatic Sea and Syracuse Bay

respectively. Cyst concentrations were found between 1250 and 10780 cyst g<sup>-1</sup> d.wt. in the sediments of Celtic Sea, Ireland (Marret and Scorse, 2002). Persson *et al.* (2000) reported 250-1689 cyst g<sup>-1</sup> d.wt. in recent coastal sediment from Swedish west coast. In an another study, Cho and Matsuoka (2001) studied the distribution of dinoflagellate cysts and found cyst concentration varying between 0-7566 cyst g<sup>-1</sup> d.wt. in the surface sediment samples of Yellow Sea and East China Sea. Godhe and McQuoid (2003) reported density of cysts varying between 5000-101000 cyst g<sup>-1</sup> d.wt. in the surface sediment samples from the northern part of the Swedish West coasts. Shin *et al.* (2007) found cyst concentrations ranging between 528 to 2834 cyst g<sup>-1</sup> d.wt. in the sediment of Eastern Part of Geoje Island, Korea. Kim *et al.* (2009) studied dinoflagellate cyst (115 and 2188 cyst g<sup>-1</sup> d.wt.) in the sediments of Gwangyang Bay of South Korea Sea. Recently Aydin *et al.* (2011) reported cyst concentration ranging between 41 and 3292 cyst g<sup>-1</sup> d.wt. in the sediments of Izmir Bay. Comparing the above mentioned data, concentration of cysts recorded in the present study were much higher than those reported by other researches.

Cyst diversity varied between 6 and 28 cyst types at the sampling stations. Cysts were found to be less diverse at the outer stations ( 14, 15, 16 and 17) of Izmir Bay and Fethiye bay (21) than stations located at inner and middle part of Izmir Bay (between station 1 and 13) and Marmara sea (station 18, 19 and 20) respectively. Sayin (2003) studied the physical-features of Izmir Bay and found Izmir outer bay water similar to Marmara Sea. Further it was reported that the physical-features of Izmir outer bay water was also affected by water of the Black Sea and five different water masses were found in the Izmir Bay seawater.

The distribution of cysts within Izmir bay appears to correspond well with the sedimentology of the bay. The highest concentration of cysts at the inner and middle bay stations corresponded to higher percentage of mud in these areas, and the lower cyst concentration around the edges of the bay corresponded to sediments comprising mainly of sandy deposits. The abundance of cyst was found highest (131504 cyst g<sup>-1</sup> d.wt. sediment) at inner bay followed by middle bay (9827 cyst g<sup>-1</sup> d.wt. sediment) and lowest (577 cyst g<sup>-1</sup> d.wt. sediment) at outer bay.

*L. machaerophorum* was found most abundant cyst type at most of the stations. Marret and Zonneveld (2003) reported that *L. machaerophorum* cysts were mainly found in coastal and neritic environments and were broadly distributed in different salinity range. This cyst type was found in several eutrophic areas and is known as an indicator of eutrophication (Dale *et al.*, 1999; Matsuoka, 1999; Marret and Zonneveld, 2003). Eutrophication of the inner İzmir Bay is a serious problem throughout the year and red tide events are becoming more frequent. Kucuksezgin *et al.* (2006) found that nutrient level was much higher in the middle and inner bay than in the Adriatic Sea, the Mediterranean and the Aegean Sea.

Table 1 : Dinoflagellate cyst concentrations (cyst g<sup>-1</sup> d. wt. sediment) in study areas

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
<b>Gonyaulacales</b>																					
<i>Alexandrium affine</i> type	0	0	0	0	0	0	0	0	0	0	0	0	0	28	14	7	4	0	0	0	0
<i>Alexandrium catenella</i> /	2	0	2	8	0	0	0	0	0	0	0	0	0	33	14	15	4	0	0	0	0
<i>tamarense</i> complex																					
<i>Alexandrium minutum</i> type	0	0	0	0	0	0	0	3007	0	0	0	0	0	6	3	0	0	2	0	2	0
<i>Lingulodinium machaerophorum</i>	1163	226	6144	6584	6586	1119	17936	8607	4867	1793	1543	324	2316	94	37	7	21	24	174	196	12
<i>Operculodinium centrocarpum</i>	67	35	267	48	232	79	437	489	347	55	57	40	124	17	3	0	0	22	184	132	2
<i>Operculodinium israelianum</i>	0	4	0	0	37	14	37	74	116	41	4	22	28	0	0	0	0	0	2	6	0
<i>Spiniferites belerius</i>	63	11	67	123	65	4	52	37	13	0	8	0	24	6	0	0	0	0	6	0	0
<i>Spiniferites bentorii</i>	258	88	623	58	248	30	1289	141	307	70	57	31	128	0	3	0	0	4	8	10	0
<i>Spiniferites bulloideus</i>	279	54	1087	183	476	593	829	711	67	2097	514	67	928	89	3	22	17	3	80	66	8
<i>Spiniferites cruciformis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	18	0
<i>Spiniferites cf. delicatus</i>	22	4	73	82	71	12	37	7	40	48	37	13	36	6	0	0	4	2	2	4	0
<i>Spiniferites hyperacanthus</i>	26	21	211	32	118	123	281	252	84	297	73	13	236	44	3	7	0	8	32	16	4
<i>Spiniferites mirabilis</i>	10	0	12	16	31	4	15	30	22	28	8	9	56	6	0	7	0	14	217	104	6
<i>Spiniferites ramosus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	0
<i>Nematosphaeropsis</i> sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	22	14	0
<i>Tuberculodinium vancompoae</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
<b>Peridinales</b>																					
<i>Scrippsiella</i> sp1	1027	324	917	34	51	167	74	156	231	234	469	27	1540	0	0	0	0	0	0	0	0
<i>Scrippsiella</i> sp2	0	2	150	20	16	46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Autotrophic cyst concentration</b>	2917	769	9553	7188	7931	2191	20987	13511	6094	4663	2770	546	5416	329	80	65	50	79	421	540	32
<b>Gymnodiniales</b>																					
<i>Polykrikos kofoidii</i>	594	431	1515	56	749	591	3799	3778	427	1979	576	173	356	6	0	0	0	54	22	0	0
<i>Polykrikos schwartzii</i>	10	2	31	30	39	8	44	74	31	14	4	4	0	0	0	0	0	0	0	0	0
<b>Peridinales</b>																					
<i>Brigantedinium asymmetricum</i>	0	4	25	10	20	26	141	148	58	28	37	107	60	0	0	0	0	0	0	2	0
<i>Brigantedinium irregulare</i>	0	4	27	4	114	44	178	119	31	103	24	44	40	0	0	0	0	0	2	2	0
<i>Brigantedinium simplex</i>	0	0	0	0	0	0	0	7	13	55	0	9	0	0	0	7	0	2	6	4	0
<i>Diplopelta parva</i>	0	0	644	14	303	77	570	881	347	179	188	80	124	0	0	7	0	2	2	0	0
<i>Dubridinium caperatum</i>	12	89	297	20	319	1304	444	6304	618	2138	220	409	260	0	0	0	0	0	0	0	0
<i>Protoperdinium nudum</i>	35	60	190	6	718	341	978	1015	156	379	94	40	128	0	0	7	0	0	2	10	0
<i>Protoperdinium obtusum</i>	0	0	0	0	55	6	311	511	71	117	41	27	40	6	0	0	0	0	2	0	0

<i>Quinquecupis concreta</i>	61	441	1083	50	997	601	2792	3733	791	1821	816	982	584	6	0	0	0	0	0	32	12	0
<i>Selenopemphix nephroides</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	2	18
<i>Selenopemphix quanta</i>	63	53	169	4	234	113	518	341	71	228	106	76	192	6	0	0	0	0	0	10	4	0
<i>Stelladinium abei</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0
<i>Stelladinium reidii</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0
<i>Stelladinium rubostum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
<i>Stelladinium stellatum</i>	2	1	13	4	16	20	193	74	22	14	37	18	28	0	0	0	4	2	14	24	24	0
<i>Trinovantedinium capitatum</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0
<i>Votadinium calvum</i>	6	2	33	18	51	2	44	119	53	62	20	18	20	0	0	0	0	0	0	0	0	2
<i>Votadinium spinosum</i>	2	4	21	14	51	26	81	119	53	48	20	22	24	0	0	0	0	0	0	6	0	0
<i>Xandarodinium xanthum</i>	4	12	50	4	22	2	452	178	0	0	0	0	0	0	0	4	0	0	4	2	0	0
<b>Heterotrophic cyst concentration</b>	789	1103	4098	234	3687	3161	10545	17223	2742	7165	2183	2009	1856	24	0	21	8	64	110	94	2	2
<b>Total cyst concentration</b>	3706	1872	13651	7422	11618	5352	31532	30734	8836	11828	4953	2555	7272	353	80	58	143	531	634	34	634	34

Matsuoka (1999) and Kim *et al.* (2009) reported that in some bays with high diatoms biomass, the concentration of heterotrophic cysts were found to be higher than the autotrophic cysts due to increased nutrient enrichment, which was considered as a good indicator of eutrophication. Buyukisik *et al.* (1994) and Gençay and Büyükişik (2004) reported diatoms and dinoflagellate species as the most abundant phytoplanktonic groups in the Izmir Bay. However, in the present study concentration of autotrophic cysts were found on the higher side than heterotrophic cyst concentration.

*S. quanta* has been identified as an indicator of industrial pollution and primer production in areas with high primer production and low autotrophic cyst concentration (Vink *et al.*, 2000; Sangiorgi and Donders, 2004). In this study, low concentration of *S. quanta* cysts were found in the sediments of Izmir Bay and autotrophic cyst concentration was reported to be on higher side than heterotrophic cyst concentration. This indicates that Izmir Bay was least affected by industrial waste and discharge of streams and hundreds of small domestic wastes into the bay led to pollution. The inner bay was heavily polluted by nutrients and organic material, but metals were not found in high concentration due to establishment of waste water treatment plants in early 2000 (Kucuksezgin *et al.*, 2006).

*Alexandrium minutum* type, *A. catenella/tamarensis* complex, *A. affine* type, *Lingulodinium machaerophorum* and *Operculodinium centrocarpum* were potentially toxic cyst species observed in the sediments of Izmir Bay. Vegetative forms of *A. minutum* were reported from Turkish coastal water (Balkıs, 2004; Tüfekçi *et al.*, 2010). In this study, limited distribution of *A. minutum* cyst was recorded at the study areas (stations 8, 14, 15, 18 and 20). However, cyst concentration of 3007 cyst g<sup>-1</sup> d.wt. at inner Izmir Bay (station 8) indicated seed bank for future blooms. *A. affine* was found at the outer Izmir Bay (station 14, 15, 16 and 17). Although, *A. catenella/tamarensis* complex was found in high abundance at outer bay stations, this cyst complex distribution was more limited at the inner bay stations (station 1, 3 and 4). Aydın *et al.* (2011) observed *A. catenella/tamarensis* complex only at Izmir outer bay station. *A. affine* type cyst was found in most side of Izmir bay stations (Aydın *et al.*, 2011). Vegetative forms of these two cysts have not been recorded earlier in Turkish coast (Feyzioglu and Ogut, 2006). Vegetative form of *L. machaerophorum* (*Lingulodinium polyedrum*) and *S. hyperacanthus/S. mirabilis* (*Gonyaulax spinifera*) have been recorded in many harmful algal bloom events (Koray, 2004). Sabancı and Koray (2005) reported that diversity of dinoflagellate species increased between 1998-2001 in the Izmir Bay. Identification of few gonyaulacoid species could be difficult due to their vegetative forms and this study of cyst might be useful to prepare data for species check list and monitoring studies in local areas such as Izmir Bay.

The results of the present study conclude that concentration of dinoflagellate cysts were found to be more in

Izmir Bay than Marmara Sea and Fethiye Bay stations. Although some cyst types were observed at all sampling stations, it is obvious that cyst assemblages showed significant difference in Marmara Sea. Autotrophic cyst concentration was also found higher than heterotrophic cyst concentration most stations. Potentially toxic cysts of *A. minutum* type, *L. machaerophorum* and *O. centrocarpum* were identified at all the study areas. Further, more studies are required to understand the distribution and assemblages of dinoflagellate cysts in relation to environmental parameters in different seas of Turkey.

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