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Modern Dinoflagellate Cyst Assemblages of Aliağa and Nemrut Bay: Influence of Industrial Pollution

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Abstract

The spatial distribution of modern dinoflagellate cysts was studied, with the purpose of understanding the impact from industrial pollution and sediment characteristics. Eight surface sediment samples were collected to analysis of the spatial distribution of dinoflagellate cysts from two industrially polluted areas: Aliağa and Nemrut Bay (Eastern Mediterranean). A total of 42 dinoflagellate cyst morphotype were identified and total cyst abundance ranged between 11 and 2543 cyst g^{-1} dry weight sediment at sampling points. The cyst assemblages were represented by cyst of *Gymnodinium nolleri*, Alexandrium affine type and *Lingulodiniumma chaerophorum*. The higher cyst concentration was recorded at mostly Aliağa Bay stations however the higher cyst diversity was found at Nemrut Bay stations. Total cyst concentration and autotrophic cyst concentration indicated metal pollution in the sampling areas whereas total number of cysts had no correlation with any metal levels in the sediment except Cr. The distribution of dinoflagellate cysts also showed correlation with sediment structure in Aliağa and Nemrut Bay.

Keywords: Resting cyst, heavy metals, sediment, Eastern Mediterranean.

Endüstriyel Kirliliğin Etkisindeki Aliağa ve Nemrut Körfezi Modern Dinoflagellat Kist Topluluğu

Özet

Aliağa ve Nemrut Körfezi'nde (Doğu Akdeniz) endüstriyel kirlilik ve sediment karakteristiklerinin etkisini anlayabilmek amacı ile modern dinoflagellat kistlerinin uzamsal dağılımı çalışıldı. Sekiz yüzey sediment örneği endüstriyel kirlilik içeren bu alanlardan toplanarak dinoflagellat kistlerin uzamsal dağılımları analiz edildi. Örnekleme noktalarında toplam 42 dinoflagellat kist morfotipi belirlendi ve toplam kist bolluğu 11 ile 2543 kist g^{-1} kuru sediment ağırlığı olarak bulundu. Kist topluluğu *Gymnodinium nolleri*, Alexandrium affine tip ve Lingulodinium machaerophorum tarafından temsil edildi. Aliağa Körfezi istasyonlarında kist konsantrasyonu daha yüksek iken Nemrut Körfezi istasyonlarında kist çeşitliliği daha yüksek kaydedildi. Toplam kist konsantrasyonu ve ototrofik kist konsantrasyonu örnekleme alanında metal kirliliğini işaret etmesine rağmen toplam kist sayısı Cr hariç herhangi bir metalle bağlantılı değildir. Bununla birlikte dinoflagellat kist dağılımının Aliağa ve Nemrut Körfezi sediment yapısı ile ilişkili olduğu gözlenmiştir.

Anahtar Kelimeler: Kalıcı kist, ağır metal, sediment, Doğu Akdeniz.

Introduction

Industrial pollution in coastal areas with causing rapid environmental changes and threating ecosystem diversity become more significant due to the urbanization and industrialization (Lim *et al.*, 2012). Many metals occur naturally in soils, rocks, sediments, waters and microorganisms with natural background concentrations and these metal concentrations rise higher with anthropogenic release into the environments (Mohammed *et al.*, 2011). The presence of these metals in the environment may be poisonous or toxic and cause harm for living organisms in the ecosystem (Duruibe *et al.*, 2007). More importantly, metal exposure results a potential threat to natural ecosystems, even death in aquatic organisms. Many metals are bio-accumulated into the organisms via the food chain and it can either give damages to ecosystem or induce serious human health problems.

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Phytoplankton is an important autotrophic component of the aquatic ecosystem, affects the structure and efficiency of the food web and has a part of the global biogeochemical cycles. Phytoplankton is often the first target of the anthropogenic impact, resulting in dramatic changes in species composition, abundance and biomass, seasonal dynamics and succession (Jing *et al.*, 2011). Although decreasing of some metals such as Fe, Cu, Cd and Mn can limit phytoplankton growth in the ocean, whereas high levels may cause toxic effects on phytoplankton species (Bruland *et al.*, 1991).

Dinoflagellates are a diverse group of marine phytoplankton and some dinoflagellate species produce a resting cyst under the stress as a survival strategy (Okamoto *et al.*, 1999). Dinoflagellate cysts recently have received more attention and different from many other marine protists the cysts deposit into sediments under a wide range of environmental conditions. The cysts are increasingly used as environmental bioindicator especially in polluted areas with their sensitivity to environmental factors may be expressed by a change in the composition of assemblages (Rochon *et al.*, 2008). Dinoflagellate cysts respond to anthropogenic changes and during the last years few works have tried to related dinoflagellate cyst distribution pattern to different pollution types (Pospelova *et al.*, 2002; Dale, 2009). Most of researches have focused on the effect of nutrient enrichment and eutrophication on the dinoflagellate cysts (Marret and Zonneveld, 2003; Pospelova and Kim, 2010; Kim *et al.*, 2012; Zonneveld *et al.*, 2013). The effects of industrial pollution on dinoflagellate cysts assemblages have been studied only in a few areas (Dale, 2001; Pospelova *et al.*, 2005; Liu *et al.*, 2012).

Aliağa is located 50 km northwest of Izmir and one of the growing industrial areas on Aegean Sea Coastline of Turkey. The Aliağa Bay is one of the most important ship recycling zone of the Mediterranean together shipping activity, shipbreaking industry, steel works and petrochemical complexes (Neşer *et al.*, 2008).There are iron-steel factories, coal storage yards, fuel storage yards, fertilizer factory, natural gas power plant, electrical substation, small industrial areas and other medium and small establishments in region which cause heavy metal pollution (Sponza and Karaoğlu, 2002; Esen *et al.*, 2010). The Aliağa Bay is contaminated by heavy metal, polycyclic aromatic and aliphatic hydrocarbons (PAHs) (Neşer *et al.*, 2012a, 2012b). TheNemrut Bay, the neighboring marine environment of Aliağa town, is a subsystem within the Çandarlı Bay ecosystem and connects to the northern Aegean Sea.The Nemrut Bay is considered with anthropogenic pollution and sediments from the Nemrut Bay is highly contaminated by Hg, Pb, As and Zn (Esen *et al.*, 2010). Pollution of the Aliağa and Nemrut Bay has studied by many researchers whereas there is limited biological records from the area. Gençay and Büyükışık (2006) investigate phytoplankton composition and effects of environmental parameters on phytoplankton in the Çandarlı Bay where is located north west of the Nemrut and Aliağa Bay.

We present here a detailed study on distribution and abundance of modern dinoflagellate cysts in surface sediments from the Aliağa and Nemrut Bay depend on heavy metal concentration and sediment structure.

Materials and Methods

Surface sediments were collected with a Van Veen grab sampler for sediment grain size analysis, detection of metal and organic carbon content and determination of dinoflagellate cysts at 8 stations from two industrially polluted area the Aliağa and Nemrut Bay, Aegean Sea in July 2013 (Figure 1 and Table 1).

Sediment Characteristics

Sediment Grain Size

For grain size analysis, sediment samples were collected with a Van Veen grab in same stations. Sediment grain sizes were analyzed by wet sieving method (Folk, 1980). Wet sediments were weighted and dried 70 °C in the oven a few days. The sediments were weighted for calculation and each samples were transferred a 100 ml beaker. Dry sediments were suspended with 200 ml distilled water then the samples were sieved through 1.2 mm, 500 µm, 250 µm, 100 µm, 63 µm, 23 µm, 10 µm opening stainless meshes respectively. Each sediment part above the sieves was transferred in a weighted aluminium pan and was dried in the oven a few days. The dried sediments are weighted again and grain sizes of sediment are calculated as a percentage of dry sediment. Textural classification of the sediment samples was based on the relative percentages of mud (<63 µm) and sand (>63 µm).

Geochemistry

Sediment samples were preserved in -20 °C until analysis. The samples were freeze-dried, sieved through 63 µm stainless steel sieve and homogenized. The samples were digested in a microwave digested with HNO_3-HF-$HClO_4$-HCl acid mixture solutions in microwave digestion system (UNEP, 1982, 1985). Metal concentrations were measured by a Varian Atomic Absorption Spectrophotometer and performed by flame (Pb, Cr, Cu and Zn), cold vapor (Hg) and graphite furnace (Cd). The amount of organic carbon was determined by spectrophotometrically in dried sediment samples following the sulfochromic oxidation method. The accuracy of this method is

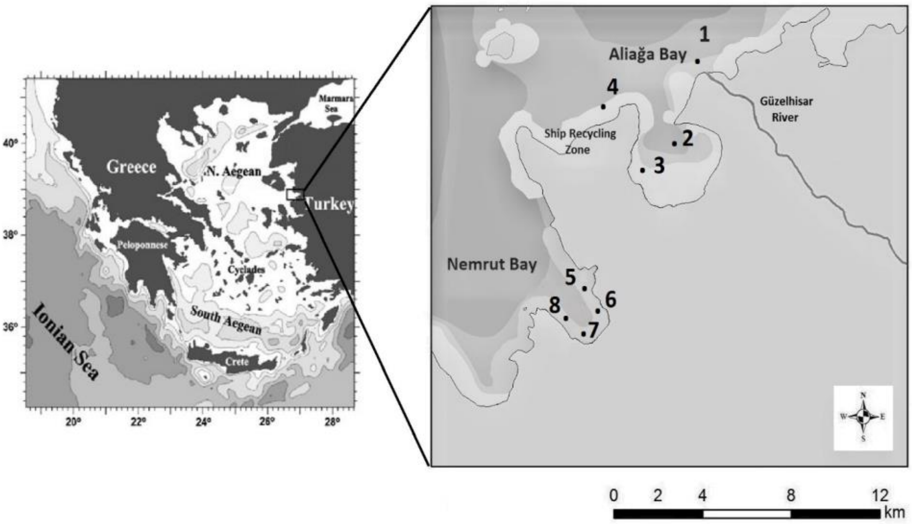

Figure 1. Location of the sampling points in Nemrut and Aliağa Bay (Eastern Aegean Sea).

Table 1. Sampling locations together with water depth (m), relative proportions of sediment grain size (%): mud and sand in surface sediments

Station no	Location	Latitude (N)	Longitude (E)	Water depth (m)	% Sand	% Mud
1	Aliağa	38°51'21"	26°58'31"	23	94.66	5.34
2	Aliağa	38°49'643"	26°57'754"	23	42.58	57.42
3	Aliağa	38°49'398"	26°57'097"	24	23.92	76.08
4	Aliağa	38°49'778"	26°55'279"	23	25.29	74.71
5	Nemrut	38°46'638"	26°55'216"	33	41.63	58.37
6	Nemrut	38°46'285"	26°55'508"	24	44.07	55.93
7	Nemrut	38°45'784"	26°55'932"	17	68.68	31.32
8	Nemrut	38°46'016"	26°55'077"	34	86.11	13.89

0.017% organic matter (Hach, 1988). The detection limits for heavy metals are Hg: 0.05 µg kg^{-1}, Cd: 0.10 µg kg^{-1}, Pb: 0.10 µg kg^{-1}, Cr: 0.10 µg kg^{-1}, Cu: 0.03 mg kg^{-1}, Zn: 0.01 mg kg^{-1}. The samples were controlled by using intercalibration sediment (IAEA-433) sample (from the International Laboratory of Marine Radioactivity, IAEA) for the analytical methods. The values obtained for the analysis of six replicates of this sample (certified: observed values in µg g^{-1} dry weight (dw)±standard deviation) were as follow Hg, 0.168±0.017: 0.167±0.012 Cd, 0.153±0.033: 0.140±0.025 Cr, 136±10: 136.7±2.0 Cu, 30.8±2.6: 30.8±2.3 Pb, 26.0±2.7: 27.0±3.1 Zn, 101±8.0: 103±1.9.

Analysis of Dinoflagellate Cyst

Top 2 cm of sediment samples were used and stored at 4°C in the dark until cyst analysis. For the observation of dinoflagellate cyst, the sediment samples were processed using standard palynological methods followed by Matsuoka and Fukuyo (2000). The subsamples were treated by room temperature HCl (10%) and HF (40%) and rinsed with distilled water respectively. The residue was sonicated 30s in ultrasonic bath and sieved through 125 µm and 20 µm pore size mesh stainless steel screen. The residue above 20 µm pore sized were used and transferred into a plastic tube. Identification and enumeration of dinoflagellate cyst was carried out on a 1 ml sub sample of 10 ml aliquots in Sedgewick-Rafter counting chamber under a DIC attachment inverted microscope (Olympus IX71). Quantitative data were reported both living and empty as cyst g^{-1} dry weight of sediment (dws). The paleontological name of cyst taxa were used in the text except some cyst taxa which are only known with their biological name. The followed nomenclatures for identification was given in Table 2. *Alexandrium* spp. cysts were named by type or complex because different *Alexandrium* species can produce morphologically same cyst types (Matsuoka and Fukuyo, 2000). Some cyst types were also identified genus level due to the difficulties on observation cyst orientation and their preservation.

All cyst concentrations are reported as cysts g^{-1} dry weight sediment (dws) and concentration in each sample was calculated by the formula: N/W(1-R), where N is the cyst obtained by multiplying the counted cyst number by coefficient to make 10 ml refined sediment, W is the weight of wet sediment sample, R is the rate of sediment sample water content. The water content of the sediment sub-samples was measured by comparing the weights in wet condition and after they were dried in an oven at 60°C for 24 h.

Statistical Analysis

Statistical analyses were done on sediment properties to determine whether there were dry correlations between sediment characteristics (grain size, heavy metals and total organic carbon) and cyst abundance. Some cyst taxa were grouped together for the statistical analysis: *Spiniferites* spp. includes all *Spiniferites* species observed in the study area. Statistical relationships between these data were tested using Pearson's correlation coefficient r along with corresponding p-values and statistical analysis was performed by using STATISTICA software package. In all case, the level of significant was set at P<0.005. The diversity of dinoflagellate cyst was monitored in this study by using the Shannon-Weiner

Table 2. Palynological name of dinoflagellate cysts taxa and their biological affinities according to references: Wall and Dale (1968), Head (1996), Rochon *et al.* (1999, 2009), Marret and Zonneveld (2003), Matsuoka and Fukuyo (2000), Radi *et al.* (2013)

Paleontological name	Biological name (affinity)
Autotrophic species	
-	*Alexandrium affine* type
-	*Alexandrium catenella/tamarense* complex
-	*Alexandrium minutum* type
Ataxodinium choane	*Gonyaulax* sp.
Impagidinium spp.	*Gonyaulax* sp.indet
Lingulodinium machaerophorum	*Lingulodinium polyedrum*
Operculodinium centrocarpum	*Protoceratium reticulatum*
Operculodinium israelianum	*Protoceratium reticulatum?*
	Gonyaulax scrippsae
Spiniferites bentorii	*Gonyaulax digitale*
Spiniferites bulloideus	*Gonyaulax spinifera complex*
Spiniferites delicatus	*Gonyaulax* sp.
Spiniferites hyperacanthus	*Gonyaulax spinifera complex*
Spiniferites mirabilis	*Gonyaulax spinifera complex*
Spiniferites ramosus	*Gonyaulax spinifera complex*
Spiniferites sp.	*Gonyaulax spinifera complex*
Tectatodinium pellitum	*Gonyaulax spinifera complex*
-	*Gymnodinium nolleri*
Heterotrophic species	
Brigantedinium asymmetricum	*Protoperidinium* sp.
Brigantedinium cariacoence	*Protoperidinium avellanum*
Brigantedinium irregulare	*Protoperidinium denticulatum*
Brigantedinium simplex	*Protoperidinium conicoides*
Brigantedinium spp.	*Protoperidinium* spp.
Echinidinium sp.	*Protoperidinium* sp.
-	*Protoperidinium* sp1
-	*Protoperidinium* sp3
-	*Protoperidinium* sp4
-	*Protoperidinium minutum*
-	*Protoperidinium nudum*
-	*Protoperidinium obtusum*
Quinquecuspis concreta	*Protoperidinium leonis*
Selenopemphix nephroides	*Protoperidinium subinerme*
Selenopemphix quanta	*Protoperidinium conicum*
Stelladinium stellatum	*Protoperidinium compressum*
Trinovantedinium capitatum	*Protoperidinium pentagonum*
Trinovantedinium pallidifurvum	*Protoperidinium* sp.
Votadinium calvum	*Protoperidinium oblongum*
Votadinium spinosum	*Protoperidinium claudicans*
Dubridinium caperatum	*Preperidinium meunieri*
Dubridinium sp.	-
-	*Oblea acanthocysta*

diversity index of species (Shannon and Weaver, 1949). Shannon-Weiner Index (H′) was calculated for each station based on the proportion of each cyst taxon in the sample and total number of cyst taxa. PAST (2.17) was used to make all diversity calculations for this study (Hammer *et al.*, 2001).

Results

Characterization of Sediments

Grain Size

A total eight surface sediment samples from the Aliağa and Nemrut Bay have been analyzed. The general features of the sediment samples are given in Table 1. Grain size analysis reveals a predominance of the fine mud fraction at the stations in the Aliağa and Nemrut Bay. Station 1, 7 and 8 is noted for mainly sand while other stations covered by muddy sediment. Sand sized material fractions vary in the range of 23.92-94.66% and fine grained (mud) sized material fractions change between 5.34% and 76.08% in the study area.

Metal concentrations and Total Organic Content

The concentration of metal and organic carbon were given in Table 3. The lowest concentration of metals and organic carbon were detected at station 1 in the Aliağa Bay. The highest concentrations of Pb, Cu and Zn were found at station 4 in the Aliağa Bay where the recycling ship zone is located. The maximum concenrations of Cr and Org C were recorded at station 7 in the Nemrut Bay, while the highest Hg and Cd concentration were measured at station 5 and 8 in the Nemrut Bay respectively. The concentrations of heavy metals and organic carbon were ranged between Hg: 0.09-12.79, Cd: 0.061-1.048, Pb: 48.2-1277.0, Cr: 20.0-66.8, Cu: 25.7-337.0, Zn: 67.2-660.0 μg g^{-1} and Org C: 0.67-6.37 (%).

Dinoflagellate Cyst Assemblage and Abundance

A total of42 dinoflagellate cyst morphotype were identified from eight surface sediment samples and classified into four taxonomic groups: gonyaulacoid (17 taxa), gymnodinoid (1 taxa), protoperidinoid (20 taxa) and diplopsalid (3 taxa) in Table 4. The identified cysts were divided into 18 autotrophic and 23 heterotrophic cysts (Figure 2-29). One unknown cyst type was identified and classified as unidentified cyst in the text. The cyst richness was distributed unevenly at different sites with a range of 2-27 cysts (at station 1 and 2 respectively). The highest number of cyst taxa was found at station 2 in the Aliağa Bay and the lowest number of cyst taxa was recorded at station 1where the Güzelhisar River flows to the Aliağa Bay. Cyst of *Gymnodinium nolleri*, *Operculodinium centrocarpum* and cyst of *Protoperidinium minutum* were recorded at all stations, while *Tectatodinium pellitum*, *Brigantedinium irregulare*, *Protoperidinium* sp1, *P. obtusum*, *Trinovantedinium capitatum* and *Dubridinium caperatum* showed very limited distributions in the sampling points. Shannon Weiner and Fisher's diversity index were varied between 0.33-2.67 and 0.72-5.30 respectively. The maximum value of Shannon Weiner and Fisher's index were calculated for station 6. The minimum Fisher's index value were found for station 1, whereas the lowest Shannon Weiner index value were calculated for station 4.

Dinoflagellate cyst concentration ranged between 11 and 2543cyst g^{-1} dws. The highest concentration was observed at station 4 and the lowest concentration was recorded at station 1 in the Aliağa Bay. The total autotrophic cyst concentration were observed higher at all stations and the total concentration of heterotrophic taxa were quite low compare to autotrophic cyst concentration. In the study areas, the cyst concentration was mainly dominated by Gymnodinoid and Gonyaulacoid group. Cyst of *G.nolleri* had maximum abundance following cyst type of *Alexandrium affine*, *Lingulodinium machaerophorum and O.centrocarpum*.

Correlation between Cyst Abundance and Sediment Parameters

Pearson's correlation results between dinoflagellate cyst concentration and sediment type were given in Table 5. There is significant correlation between sediment type and the total cyst concentration. The fine grain sized sediment were

Table 3. Concentration of metals data (μg g^{-1}dws) and total organic carbon content (%) in the sampling stations

Stations	Hg	Cd	Pb	Cr	Cu	Zn	%Org
1	0.09	0.061	48.2	20.0	25.7	67.1	0.67
2	0.37	0.076	60.0	49.8	37.9	107.0	3.12
3	0.55	0.069	66.5	49.5	43.9	125.0	3.99
4	0.64	0.892	1277.0	58.2	337.0	660.0	6.18
5	12.72	0.287	105.0	61.7	42.2	212.0	3.55
6	9.78	0.287	76.2	45.1	37.7	142.0	5.67
7	6.03	0.585	396.0	66.8	61.9	335.0	6.37
8	11.84	1.048	236.0	49.2	36.1	336.0	5.46

Table 4. Dinoflagellate cyst concentrations (cyst g^{-1}dws) in the surface sediments of Aliağa and Nemrut Bay

	Stations							
Dinoflagellate Cyst Taxa	1	2	3	4	5	6	7	8
Alexandrium affine type	0	51	15	24	59	0	17	0
Alexandrium catenella/tamarense complex	0	10	10	6	0	4	6	0
Alexandrium minutum type	0	54	15	12	11	0	23	5
Ataxiodinoum choane	0	3	10	0	0	4	6	3
Impagidinium sp	0	0	0	0	0	0	6	3
Lingulodinium machaerophorum	0	14	20	0	30	38	57	8
Operculodinium centrocarpum	0	20	5	18	19	30	45	3
Operculodinium israelianum	0	10	5	6	0	0	0	0
Spinifertes belerius	0	0	0	6	0	4	0	3
Spiniferites bentroii	0	0	0	0	0	26	6	0
Spiniferites bulloideus	0	24	10	0	15	38	34	5
Spiniferites delicatus	0	7	10	0	11	9	0	0
Spiniferites hyperacanthus	0	3	5	0	15	4	0	3
Spiniferites mirabilis	0	10	5	0	7	9	28	0
Spiniferites ramosus	0	0	10	0	4	17	0	0
Spiniferites sp.	0	0	0	0	7	4	11	0
Tectatodinium pellitum	0	0	5	0	0	0	0	0
Gymnodinium nolleri	8	1555	1444	2411	845	68	419	440
Brigantedinium asymmetricum	0	24	0	6	4	0	0	0
Brigantedinium cariacoence	0	10	0	0	0	0	6	0
Brigantedinium irregulare	0	7	0	0	0	0	0	0
Brigantedinium simplex	0	0	0	6	4	4	0	0
Brigantedinium sp.	0	7	20	0	0	0	28	3
Echinidinium sp.	0	3	20	0	0	17	11	0
Protoperidinium sp1	0	3	0	0	0	0	0	0
Protoperidinium sp3	0	0	0	0	0	9	0	3
Protoperidinium sp4	0	0	0	0	0	0	6	3
Protoperidinium minutum	3	17	25	0	11	13	6	3
Protoperidinium nudum	0	3	0	0	7	13	17	3
Protoperidinium obtusum	0	0	0	0	4	0	0	0
Quinquecuspis concreta	0	7	5	0	4	0	0	0
Selenopemphix nephroides	0	14	10	0	4	4	6	0
Selenopemphix quanta	0	7	0	0	0	9	11	0
Stelladinium stellatum	0	3	5	0	0	0	0	0
Trinovantedinium capitatum	0	3	10	18	19	4	0	5
Trinovantedinium pallidifurvum	0	0	0	12	0	0	0	0
Votadinium calvum	0	3	0	6	0	0	0	5
Votadinium spinosum	0	0	5	0	0	0	11	0
Dubridinium caperatum	0	0	0	0	0	0	6	0
Dubridinium sp.	0	27	25	12	15	0	40	3
Oblea acanthochysta	0	0	0	0	0	0	6	5
Unidentified cyst	0	0	5	0	4	4	0	0
Total autotrophic cyst concentration	8	1761	1569	2483	1023	255	658	473
Total heterotrophic cyst concentration	3	138	125	60	72	73	154	33
Total cyst concentration	11	1899	1699	2543	1099	332	812	506
Total cyst number	2	27	24	13	21	22	24	18
Shannon Weiner	0.59	0.99	0.87	0.33	1.15	2.67	2.06	0.75
Fisher's Alpha	0.72	4.46	3.96	1.79	3.68	5.30	4.64	3.64

significantly and positively correlated with total cyst concentration, while percentage of sand had negative significant correlation with total cyst concentration. Fine grain sediment were positively correlated with total number of cyst. There is a positive significant correlation between total cyst concentration, total autotrophic cyst concentration and level of Pb and Cu. However no significant relation was found total heterotrophic cyst concentration and any sediment characteristics except level of Cr. A positive significant correlation was also found between some cyst taxa and heavy metal levels in the sediment (Table 5). Concentration of *Votadinium calvum* had positive correlation with level of Cd, Cu, Pb and Zn. Concentration of *Trinovantedinium pallidifurvum* linked to level of Pb, Cu and Zn. There are also correlation between cyst of *G. nolleri* concentration and level of Pb and Cu. Total organic content (carbon) had a significant positive correlation with *Operculodinium centrocarpum* concentration. There are some negative correlations between levels of metal and cyst concentrations. Hg level in sediment

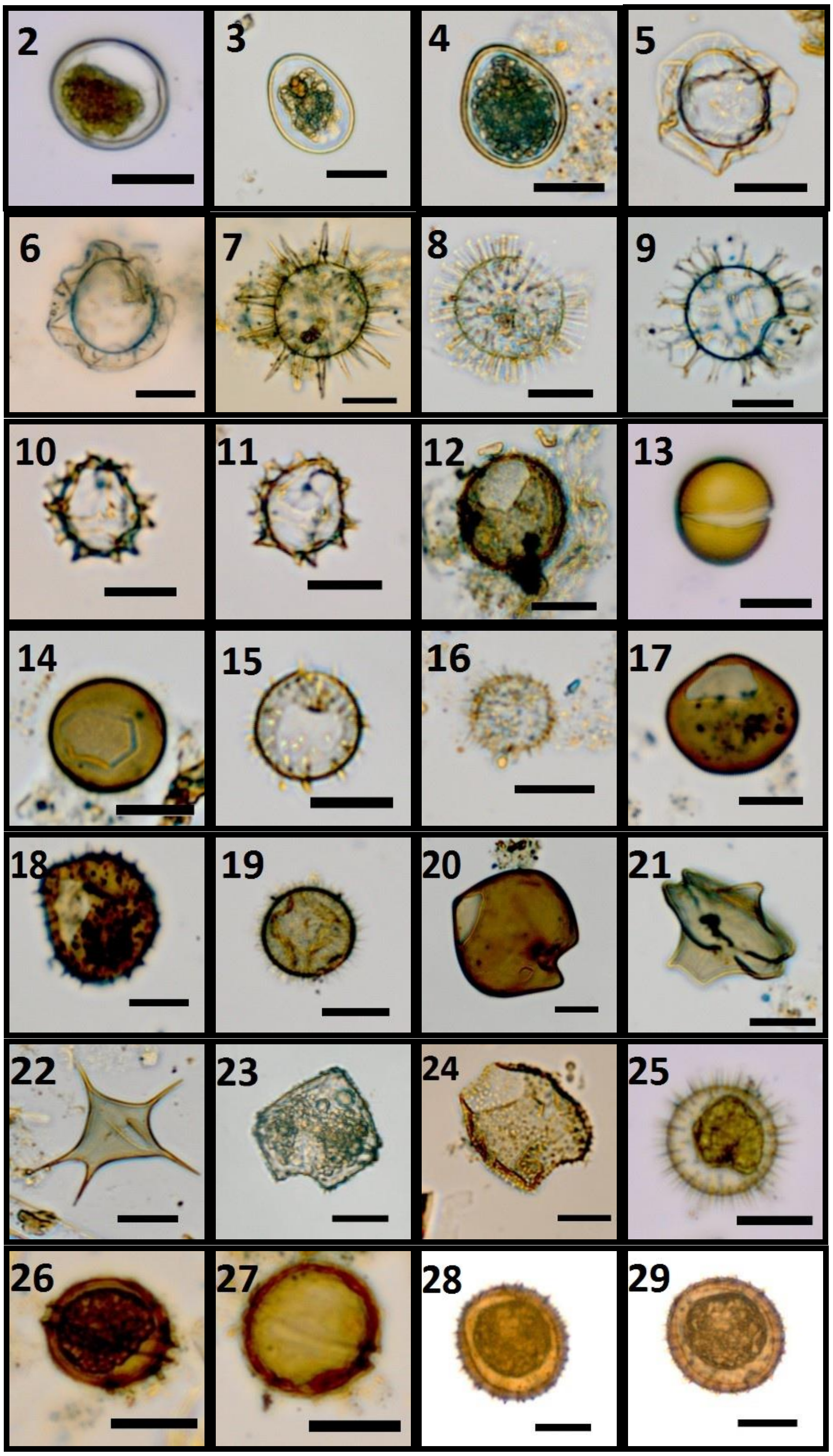

Figure 2-29. Photomicrographs of selected dinoflagellate cysts found in surface sediments from Aliağa and Nemrut Bay: (2) *Alexandrium affine* type, (3) *Alexandrium catenella/tamarense* complex, (4) *Alexandrium minutum* type, (5-6) *Ataxodinium choane*, (7) *Lingulodinium machaerophorum*, (8) *Operculodinium centrocarpum*, (9) *Spiniferites hyperacanthus*, (10-11) *Impagidinium* sp., (12) *Tectatodinium pellitum*, (13) cyst of *Gymnodinium nolleri*, (14) *Brigantedinium cariacoence*, (15) *Echinidinium* sp., (16) *Islandinium* sp., (17) cyst of *Protoperidinium* sp1, (18) cyst of *Protoperidinium* sp2, (19) cyst of *Protoperidinium minutum*, (20) cyst of *Protoperidinium obtusum*, (21) *Selenopemphix nephroideus*, (22) *Stelladinium stellatum*, (23) *Trinovantedinium pallidifurvum*, (24) *Votadinium spinosum*,(25) cyst of *Oblea acanthocysta*,(26-27) *Dubridinium* sp., (28-29) Unidentified cyst, (Black scale bar is 20 micrometer).

Operculodinium israelianum.

Discussion

Spatial distribution and composition of dinoflagellate cysts were studied with the structure of sediment and the impact of industrial pollution in surface sediments from two different locations the Aliağa and Nemrut Bay (Eastern Mediterranean). This study provides a detailed data on distribution and abundance of dinoflagellate cysts in industrially polluted areas from Eastern Mediterranean and the results were compared to other marine coastal areas. A total of 42 cyst morphotype (including the unidentified cyst) were recorded in the sampling areas. Most of the cyst taxa have been reported from other Turkish coastal areas in previous studies (Uzar *et al.*, 2010; Aydin *et al.*, 2011; 2014; Aydin and Uzar, 2014). However *Ataxodinium choane*, *Impagidinium* sp., *Dubridinium* sp., were recorded for the first time from the Turkish coastal areas. This study does not only provide data for dinoflagellate cyst studies for Turkish coastal waters but also contribute distribution of dinoflagellate species in Turkish marine waters. According to our knowledge phytoplankton composition of the Aliağa and Nemrut Bay has not been studied previously. Phytoplankton composition were studied and only three dinoflagellate cyst taxa including cyst producer species *Gonyaulax* sp. were reported in Dem Harbour, Çandarlı Bay where is the closest location to the study areas (Gençay and Büyükışık,2006).

Dinoflagellate cysts concentration ranged between 11 and 2543g^{-1} dry weight sediment in the sampling area. The higher total cysts concentration were observed at stations in the Aliağa Bay than station in the Nemrut Bay. In comparison, the total cyst concentration were found higher than Sishili Bay, China (Liu *et al.*, 2012), Visakhapatnam Harbour, India (D'Silvia *et al.*, 2013), whereas the lower than Izmir Bay, Turkey (Aydin *et al.*, 2011), Thermaikos Gulf, Greece (Giannakourou *et al.*, 2005) and Buzzards Bay, USA (Pospelova *et al.*, 2005). Cysts behave like fine particles and accumulate into the sediment. Sandy sediment is not suitable for dinoflagellate cyst to deposit and local currents might help to cyst transfer from one to another area(Dale, 1983; Matsuoka *et al.*, 2003). In the study area, the Aliağa Bay sediment was found muddy except station 1which is located in the river flows. The Nemrut Bay, in station 5 and 6 were mainly muddy, station 7 and 8 contains mostly sand and these result showed similarities with Esen *et al.* (2010). The higher total cyst abundance observed where the sediment contains mud and dinoflagellate cysts abundance changed depends on the sediment type for the Aliağa and Nemrut Bay. Pearson's correlation also showed significant relation between sediment grain size and total cyst abundance. The most of taxa had significantly links to sediment structure.

The Aliağa and Nemrut Bay are very important area in the Eastern Mediterranean and pollution of the bays has been searched by several authors (Sponza and Karaoğlu, 2002; Esen *et al.*, 2010; Neşer *et al.*, 2008; 2012a; 2012b). The level of metals in surface sediments were compared with the other areas from world oceans (Table 6). The level of Zn and Pb were higher than other areas. Zn, Pb and Cu levels were found higher in the sampling areas except Buzzards Bay, USA and previous the Aliağa Bay records. The level of Cr are lower than other areas except Thau Lagoon, France, while Cd level were found lower from other areas except Izmir Bay, Nemrut Bay (Turkey) and Thau Lagoon.

In this study, the highest total cyst abundance and the highest level of most metals were determined at station 4 in the Aliağa Bay where the ship recycling zone is located. The Nemrut Bay had the higher metal levels compared to the Aliağa Bay stations. According to Pearson's correlation in Table 5, the total cyst concentration significantly and positively correlated with Cu level in sediment and level of Pb, Cr and Zn had little correlations with the total cyst concentration. However level of Hg in surface sediments showed a negative correlation with the total cyst concentration. Different from these results, Horner *et al.* (2011) reported positive correlation between Cd level and cyst abundance from Puget Sound, USA. As micronutrients, trace elements are important as well as nutrients for phytoplankton growth due to the physiological needs and the high metal concentration can help for phytoplankton bloom in water column. On the other hand, the high metal concentration can cause toxicity and stress to phytoplankton and these unfavorable conditions might triggered encystment for cyst producer dinoflagellate species. Furthermore, high metal toxic environments let to dinoflagellate stay in cyst stage and cyst could germinate difficultly under metal stress conditions (Liu *et al.*, 2012; Okomato and Colepicolo, 1998; Pinto *et al.*, 2003). There were also correlations between dinoflagellate cyst taxa and metal concentrations in the surface sediments from the Aliağa and Nemrut Bay (Table 5). *G. nolleri* had significantly positively correlated with level of Cu, little positive correlations with level of Pb, Cr, Zn and showed negative poor correlation with level of Hg. There were some positive correlations between *Operculodinium centrocarpum* concentrations with TOC percentage. Concentration of *Trinovantedinium pallidifurvum* and *Votadinium calvum* had high correlation with some metals (Pb, Cu and Zn), whereas concentration of *Protoperidinium minutum* showed significant negative correlation with Cd, Pb, Cu and Zn level. Liu *et al.* (2012) pointed that phytoplankton has some physiological mechanisms to deal with the high level of toxic metal. *Lingulodinium polyedrum* (vegetative stage of *L. machaerophorum*) changes to resting stage under high toxic metal level and the high level of Hg, Cd and Pb were reported as

Table 5. Pearson's correlation values between some cyst taxa, heavy metal level, total organic carbon content (TOC) and sediment structure (coefficients, r, for present data and bold values correspond to P<0.05 significance)

	Hg	Cd	Pb	Cr	Cu	Zn	TOC	Sand	Mud	A.cat[a]	O.cen[b]	O.isr[c]	G.nol[d]	P.min[e]	T.pal[f]	V.cal[g]	D.sp.[h]	Auto[i]	Hete[j]	TCC[k]	TCN[l]
Hg	0																				
Cd	0.38	0																			
Pb	-0.23	0.66	0																		
Cr	0.35	0.42	0.39	0																	
Cu	-0.32	0.52	0.97	0.31	0																
Zn	0.03	0.83	0.95	0.54	0.88	0															
TOC	0.36	0.70	0.54	0.74	0.44	0.68	0														
Sand	0.20	0.16	-0.31	-0.49	-0.45	-0.23	-0.32	0													
Mud	-0.20	-0.16	0.31	0.49	0.45	0.23	0.32	-1.00	0												
A. cat	-0.61	-0.30	0.11	0.29	0.18	-0.01	0.20	-0.65	0.65	0											
O. cen	0.22	0.09	0.17	0.64	0.09	0.21	0.59	-0.21	0.21	0.26	0										
O. isr	-0.66	-0.21	0.23	0.11	0.34	0.08	-0.07	-0.58	0.58	0.79	-0.08	0									
G. nol	-0.48	0.16	0.65	0.41	0.74	0.55	0.22	-0.74	0.74	0.60	-0.07	0.80	0								
P. min	-0.16	-0.68	-0.57	0.07	-0.45	-0.61	-0.17	-0.54	0.54	0.63	-0.02	0.39	0.13	0							
T. pal	-0.34	0.50	0.96	0.23	1.00	0.86	0.38	-0.42	0.42	0.14	0.01	0.35	0.73	-0.47	0						
V. cal	-0.10	0.73	0.68	0.17	0.65	0.72	0.36	-0.08	0.08	0.04	-0.23	0.42	0.58	-0.47	0.67	0					
D. sp.	-0.25	-0.13	0.05	0.64	-0.01	0.06	0.25	-0.31	0.31	0.66	0.55	0.37	0.33	0.36	-0.09	-0.18	0				
Auto	-0.46	0.13	0.62	0.48	0.71	0.53	0.26	-0.78	0.78	0.64	0.02	0.81	0.99	0.18	0.69	0.54	0.40	0			
Hetero	-0.17	-0.23	-0.07	0.64	-0.09	-0.07	0.35	-0.49	0.49	0.79	0.63	0.44	0.30	0.59	-0.17	-0.23	0.91	0.38	0		
TCC	-0.46	0.11	0.60	0.50	0.69	0.51	0.27	-0.79	0.79	0.67	0.06	0.82	0.99	0.21	0.66	0.51	0.44	1.00	0.44	0	
TCN	0.25	-0.08	-0.22	0.69	-0.23	-0.11	0.47	-0.51	0.51	0.59	0.52	0.33	0.20	0.65	-0.30	-0.11	0.63	0.28	0.84	0.33	0

[a]*Alexandrium catenella/tamarense* complex, [b]*Operculodinium centrocarpum*, [c]*Operculodinium israelianum*, [d]Cyst of *Gymnodinium nolleri,* [e]Cyst of *Protoperidinium minutum*,[f]*Trinovantedinium pallidifurvum*, [g]*Votadinium calvum*, [h]*Dubridinium* sp., [i]Total autotrophic cyst concentration, [j]Total heterotrophic cyst concentration, [k]Total cyst concentration, [l]Total cyst number.

Table 6. Comparison of metal (µg g^{-1}dws) concentrations in the sediments from different areas

Area	Zn	Cu	Cr	Pb	Cd	Hg	Reference
Izmir Bay, Turkey	14-412	2.2-109	19-316	3.1-119	0.005-0.82	0.05-1.3	Kucuksezgin *et al.* (2011)
Nemrut Bay, Turkey	75.0-271	9.6-43.7	35.7-98.8	22.3-89.4	0.005-0.25	1.70-9.60	Esen *et al.* (2010)
Coast of Aliağa, Turkey	86.4-970	19.6-703	65.5-264	26.5-440	0.06-3.94	0.32-7.02	Neşer *et al.* (2012)
Candarli Bay, Turkey	63.2-104.5	2.1-28.7	-	2-35.5	-	-	Taş *et al.* (2007)
Thermaikos Gulf, Greece	74-358	19-165	7-172	10-218	-	-	Christophoridis *et al.* (2009)
Thau Lagoon, France	23.8-51.5	12.2-27.2	13.3-38.2	8.2-20.9	0.21-0.47	-	Rigollet *et al.* (2004)
Abu Qir Bay, Egypt	25.23-104.08	10.24-22.85	-	1.90-16.79	0.31-4.89	-	Ghani *et al.* (2013)
Visakhapatnam Harbour, India	160-328	30-89	81-245	86-288	6.0-42.0	-	Sarma *et al.* (1996)
Buzzards Bay, USA	37.3-564.9	4.2-706.8	23.4-296.2	5.8-222.1	0.06-1.39	-	Pospelova *et al.* (2005)
Sishili Bay, China	72.26-91.61	22.4-31.82	68.84-76.57	26.47-42.95	-	-	Liu *et al.* (2012)
Nemrut and Aliaga Bay, Turkey	67.1-660.0	25.7-337.0	20.0-66.8	48.2-1277.0	0.061-1.048	0.09-12.72	Present study

extremely toxic metal for vegetative cells (Okamato *et al.*, 1999). The lower levels of Cu and Cd do not have any significant effect on vegetative cell growth of phytoplankton species (Herzi *et al.*, 2013). Some metal elements are important component in phytoplankton physiology and effects of metal toxicity on dinoflagellate life cycle is not well understood. Liu *et al.* (2012) reported that autotrophic dinoflagellate and heterotrophic dinoflagellate can be affected via different way by metal toxicity and under the stress condition decreasing of autotrophic dinoflagellate is faster than heterotrophic dinoflagellate. In the study area, total concentration of autotrophic cyst taxa were observed higher than the total concentration of heterotrophiccyst taxa. According to Pearson's correlation, total autotrophic cyst concentration positively and significantly linked to level of Pb and Cu in the sediment. Moreover, level of Cr and Zn showed positive little correlations with total autotrophic cyst concentration while Hg had a negative poor correlation with the total autotrophic cyst concentration. There is no correlation found between total heterotrophic cyst concentration and toxic metal levels except level of Cr in the sediment. The abundance of total and autotrophic cyst concentrations seems to be good indicator for the metal toxicity in the Aliağa and Nemrut Bay.

Total number of cyst was reported as a general indicator of industrial pollution from the Buzzards Bay, USA and toxic metal and organic carbon content were negatively correlated to species richness (Pospelova *et al.*, 2005). Different from Pospelova *et al.* (2005), more dinoflagellate cyst diversity were observed at stations which are detected higher level of metals. Total number of cyst ranged between 2-27 and high cyst diversity were found at mostly the Nemrut Bay stations. Lower cyst diversity were observed at stations in the Aliağa Bay, especially station 1 where is close to river outfall and station 4 where is the level of Pb, Cr and Zn the highest in the sediment. Shannon Weiner and Fisher's diversity index also were determined higher at stations in the Nemrut Bay. Pearson's correlation showed no correlation between number of cysts and metal levels except level of Cr. The influence of anthropogenic pollutants can be detected in the Nemrut Bay and high organic matter was detected owning to the untreated domestic wastes through to the Nemrut Bay (Esen, 2010; Esen *et al.*, 2010). Furthermore, total organic content were determined with higher percentage (3.55-6.37%) in the Nemrut Bay in the present study.According to our knowledge, there is limited data about physicochemical parameters of Aliağa and Nemrut Bays. In this study, sea surface parameter (such as temperature and salinity) were also not determine as the main purpose of this study was to investigate industrial impact on dinoflagellate cysts in study areas. Liu *et al.* (2012) pointed out industrial pollution and eutrophication often occurred together in some coastal waters. Due to difficulties of separating these pollution sources in coastal waters, cyst abundance and distribution should be studied in detail with nutrients and other environmental factors in the study area.

Conclusion

Dinoflagellate cysts are commonly used as a proxy of environmental changes especially monitoring anthropogenic impacts such as eutrophication and pollution. In the Aliağa and Nemrut Bay, industrial pollution has been appeared due to the industrial usage of the area and the ship recycling zone. In the present study, toxic metal levels were high. Total cysts concentration and autotrophic cyst concentration indicated the metal toxicity for the area, whereas total cyst number showed no correlation with level of metals for the study area. The distribution and abundance of dinoflagellate cysts assemblages also showed variation corresponding to sediment structure in the area.

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