Dinoflagellate Cyst Distribution in Recent Sediments of Gwater Bay in the Northeast of Oman Sea in the Pre-and Post-Monsoon Season

Ayda Raisi[1], Gilan Attaran-Fariman[1*], Rouhollah Zare[1], Mohammad Ali Asefi[1]

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Abstract

Cysts produced by dinoflagellates act as the seeds involved in the initiation of algal bloom through blooming and at the end of bloom through the reformation of cysts. Therefore, they are of great importance in environmental studies. In this study, sampling of sediments in the Gwater Bay, located on Iran's southeast coast and the northeast of the Oman Sea, was done in the spring (pre-monsoon) and summer (post-monsoon) of 2013 to examine the distribution and abundance of dinoflagellate cysts. Twenty-four species belonging to 12 genera of the dinoflagellate cysts were identified for the first time in this area. The results of ANOVA indicated no significant difference between the abundance of cysts in different seasons. A study of the Shannon index in this area revealed a high diversity and abundance in the study area. In this study, 8 species of dinoflagellate were found as potentially toxic species.

Keywords: Cyst, Sediment, Gwater Bay, Monsoon, phytoplankton, Oman Sea

Introduction

Nearly 2000 species of marine dinoflagellates have been reported to date, of which nearly 10% can produce cysts resulting from hypnozygote produced by sexual reproduction (Dale, 2001). Cysts are extremely important ecologically (Dale, 1983). Indeed, since cysts act as seeds involved in the initiation of algal bloom through blooming and at the end of bloom through the reformation of cysts, they have great significance in environmental studies. Therefore, cysts in the sediments should be well studied from the point of view of algal bloom production. Furthermore, the study of cysts in certain areas can lead to reporting new species or those rarely seen as plankton in water columns. Since heterotrophic and autotrophic cysts indicate two important nutritional levels in the food web, each of which requires special food requirements, therefore they should be examined carefully. As a large number of plankton and heterotrophic dinoflagellates feed on small diatoms, their sudden growth can indicate eutrophication or upwelling (Harland et al., 2004).

1-Department of Marine Biology, Faculty of Marine Sciences, Chabahar Maritime University, Chabahar, Iran
*Corresponding author email: gilan.attaran@gmail.com

The study area, Gwater Bay, is one of the major bays with an area of 415.94 square kilometers on the eastern coast of Sistan and Baluchestan province on Iran's border with Pakistan (Alves-De-Souza et al., 2008). The Bay is considered a part of the Gando international wetland and a protected area, having unique ecological values. Southwest Monsoon winds (October-November) are one of the prominent features of the area, which have a major influence on the environmental and ecological characteristics of the Oman Sea. Monsoon currents and winds from the Indian Ocean, especially those occurring in the northwest of India, affect the Oman Sea such that they have special importance in regional ecological changes (Sedigh Marvasti et al., 2016; Aliabad et al., 2019; Ershadifar et al., 2020). A few studies on the dinoflagellate cysts have been performed on the southern coasts of Iran (Attaran-Fariman, 2010; Attaran-Fariman et al., 2012), however, so far no study has been done in Gwater Bay. The study was performed to identify the diversity, composition, and distribution of dinoflagellate cysts in recent sediments of the Gwater Bay, northeast of the Oman Sea in pre- and post-monsoon seasons.

Materials and Methods

The sediment sample was collected by Ekman grab with an area of 0.225 m3, from a depth of 1-2 m in early June (pre-monsoon) and early October (post-monsoon) in 2013 from 5 stations, each with three replications, in the Iranian waters of the Oman Sea, located at the Gwater Bay in the far south easoutheastahar port. The surface sediments of each grab were collected and stored in a container away from light and kept at the temperature. The geographical position of each station was determined using a GPS device on the floating vessel (Figure 1 and Table 1).

In the laboratory, 2-3 grams of surface sediment was stirred by Filtered Sea Water (FSW). The obtained water slurry was then sonicated (model RS-232C) for 2 min. After sonication, it passed through 125 and 20 μm sieves. The remaining sediment on the sieve was transferred with FSW to a Petri dish, using the panning technique after which the cyst was identified and counted under an inverted microscope (Nikon-TS100). Two-way ANOVA was employed to examine the abundance of cysts among the sampling stations and between two sampling seasons (pre- and post-monsoon) (10 stations, 2 seasons, and 24 species). Tukey's test was utilized to determine significant differences between stations. Finally, Excel statistical software was applied to draw diagrams, through which diversity indices were calculated by Ecological Methodology software version 6.

Results

In this study, a total of 24 types of cysts were recorded representing 12 genera in recent sediments of the Gwater Bay (Table 2 and Figs. 6, 7). The presence or absence of detected cysts is listed in Table 2 for different stations across two seasons of sampling. The relative abundance of cysts is also reported in Table 2 for different sampling stations

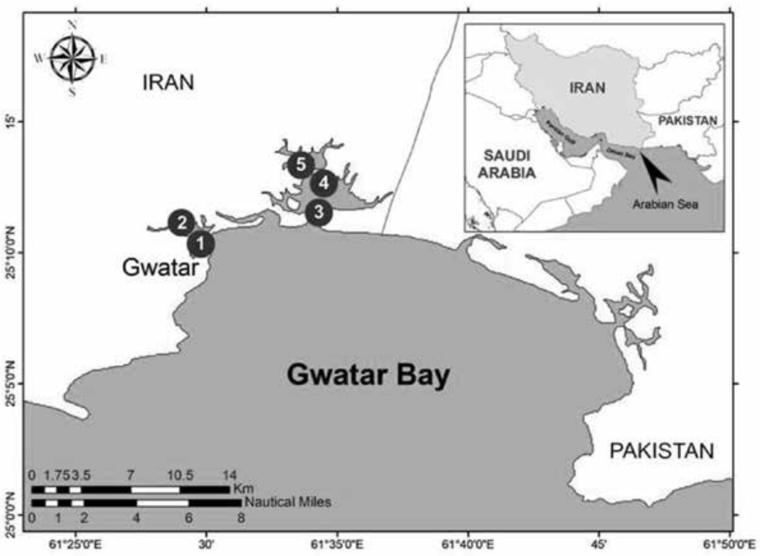

Fig. 1. Location of sampling sites in sediments of the Gwater Bay in the northeast of Oman Sea

Table 1. Geographical positions s of sampling sites with water depth in the study area

Site	Area	Latitude	Longitude	Depth (m)
1	End of estuary	25°10′15.2"	61°29′29.7"	1.8
2	Middle Estuary	25°10′31.9"	61°29′31.9"	1.5
3	Middle Estuary	25°11′58.9"	61°34′16.4"	1.7
4	Entrance of the estuary	25°13′08.0"	61°33′40.5"	1.4
5	Entrance of the estuary	25°11′03.1"	61°34′20.9"	1.1

in the two pre- and post-monsoon seasons. In the pre-monsoon, the highest and lowest abundance of cysts were recorded at station 1 (with 127 cysts no./10 g wet sediment) and station 2 (with 44 cysts no./10 g wet sediment), respectively. On the other hand, in the post-monsoon, they were recorded at station 4 (with 274 cysts no./10 g wet sediment) and station 2 (with 139 cysts no./10 g wet sediment), respectively. The abundance of autotrophic and heterotrophic cysts is also provided in Table 2 for each station in two sampling seasons. The maximum abundance of autotrophic cysts in the pre-monsoon and post-monsoon was found from station 1 and station 4, respectively, while the lowest abundance of these cysts in the pre-monsoon and post-monsoon occurred at station 2 (Table 2). The planktonic form of some of these cysts (e.g. *Alexandrium tamarense*, *Gonyaulax spinifera*, *Lingulodinium polyedra*, *Protoceratium reticulatum*, *Pyrodinium bahamense* and *Margalefidinium polykrikoides*) are toxic and, if they bloom, can cause the mortality of marine aquatic or human poisoning (Attaran-Fariman and Asefi, 2022).

The Shannon-Wiener index indicated that the maximum and minimum diversity in the pre-monsoon belonged to stations 1 and 5, while in the post-monsoon, the highest value of cysts diversity was found for station 1, and the minimum value of this index was assigned to stations 2 and 5 (Table 2). The abundance of

Table 2. Cyst concentrations (cysts no./10g wet sediment), percentages (%) of cysts in each season and autotrophic and heterotrophic dinoflagellates, and values of the Shannon-Wiener diversity index (H) of cysts in the Gwater Bay in the northeast of Oman Sea in Pre- and Post-monsoon seasons of 2013

Species/stations	Season									
	Pre-monsoon					Post monsoon				
	1	2	3	4	5	1	2	3	4	5
Order Gonyaulacales										
Alexandrium cf. *tamarense* (Lebour) Balech [a]	12	3	4	2	1	12	10	22	30	10
Alexandrium sp. [a]	5	0	0	3	0	0	0	0	0	0
Gonyaulax spinifera (Claparède & Lachmann) Diesing [a]	2	0	3	0	0	0	0	0	0	0
Gonyaulax cf. *scrippsae* Kofoid [a]	10	3	4	3	2	0	11	15	0	0
Gonyaulax digitalis (C.H.G.Pouchet) Kofoid [a]	9	3	3	12	0	0	0	0	0	0
Lingulodinium polyedra (F.Stein) J.D.Dodge [a]	3	0	0	2	0	21	8	9	25	11
Protoceratium reticulatum (Claparède & Lachmann) Bütschli [a]	7	0	3	2	0	0	0	0	0	0
Pyrodinium bahamense Plate [a]	4	1	5	26	6	0	0	0	0	0
percentages of Gonyaulacales cysts	**33**	**8**	**16**	**34**	**5**	**17**	**15**	**25**	**30**	**13**
Order Gymnodiniales										
Margalefidinium polykrikoides (Margalef) F.Gómez, Richlen & D.M.Anderson [a]	0	10	0	0	8	0	0	0	0	0
percentages of Gymnodiniales cysts	**0**	**56**	**0**	**0**	**44**	**0**	**0**	**0**	**0**	**0**
Order Peridiniales										
Diplopsalis lenticula Bergh [b]	14	10	18	20	12	14	10	18	20	12
Pentapharsodinium dalei Indelicato & Loeblich III [a]	2	3	6	3	10	19	0	12	11	0
Protoperidinium claudicans (Paulsen) Balech [b]	3	3	0	3	0	33	22	24	25	20
Protoperidinium conicum (Gran) Balech [b]	4	0	3	10	7	0	0	0	0	0
Protoperidinium obtusum (Karsten) Parke & J.D.Dodge [b]	0	0	0	0	0	25	0	24	45	18
Protoperidinium conicoides (Paulsen) Balech [b]	0	0	0	0	0	9	12	11	15	11
Protoperidinium leonis (Pavillard) Balech [b]	0	0	0	0	0	28	10	9	15	18
Protoperidinium oblongum (Aurivillius) Parke & Dodge [b]	0	0	0	0	0	0	0	0	0	0
Protoperidinium pentagonum (Gran, 1902) Balech [b]	0	0	0	0	0	18	13	16	20	14
Protoperidinium spp. [b]	0	0	0	0	0	34	20	17	20	30
Pyrophacus steinii (Schiller) Wall & Dale [b]	3	3	0	3	9	3	0	23	14	0
Scrippsiella acuminata (Ehrenberg) Kretschmann et al. [a]	8	5	9	4	5	4	5	9	4	5
Zygabikodinium lenticulatum Loeblich Jr. & Loeblich III [b]	0	0	0	0	0	14	18	20	30	18
percentages of Peridiniales cysts	**21**	**13**	**20**	**24**	**22**	**23**	**13**	**21**	**26**	**17**
overall cyst concentration	127	44	81	116	70	234	139	229	274	167
percentages of autotrophic cysts	32	9	16	29	14	22	13	27	28	10
percentages of heterotrophic cysts	25	11	22	24	18	23	13	20	26	18
Shannon-Wiener diversity index	2.667	2.165	2.2	2.25	2	2.4	2.3	2.6	2.5	2.3

a=autotrophic, b=heterotrophic

cysts in the pre-and post-monsoon seasons is provided in Figure 2.

As is shown in Figure 2, the abundance of cysts in the post-monsoon is higher than the pre-monsoon. The abundance of heterotrophic and autotrophic cysts is presented in Figure 3 for the two sampling seasons (pre- and post-monsoon). In the post-monsoon, the abundance of heterotrophic cysts increased while autotrophic cysts decreased, compared to the pre-monsoon.

The abundance of cysts is presented in Figure 4 for various stations across the two pre- and post-monsoon seasons. In general, the abundance of cysts in the post-monsoon was higher than that in the pre-monsoon for all stations.

Cluster analysis is presented in Figure 5 (A and B) for the two pre- and post-monsoon seasons. In the post-monsoon, cluster analysis consists of two main clusters: the first cluster (which is larger than the second) contains stations 4-3-1, while the second cluster covers stations 2-5. In the pre-monsoon, cluster analysis also consists of two main clusters - the first and the second clusters consisting of stations 2-5-3 and stations 1-4, respectively.

A two-way ANOVA was used to evaluate the abundance of cysts among stations, as sampling points, between the two seasons of sampling (pre- and post-monsoon). The results indicated that the mean number of cysts had no significant difference between the two

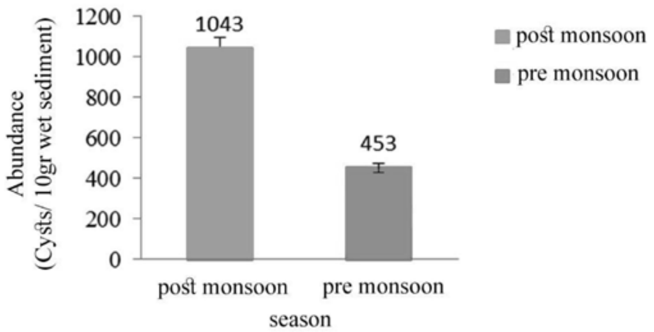

Fig. 2. Total abundance of dinoflagellate cysts (cysts/10 gr wet sediment) of the Gwater Bay in the northeast of Oman Sea in Pre- and Post-monsoon seasons (2013)

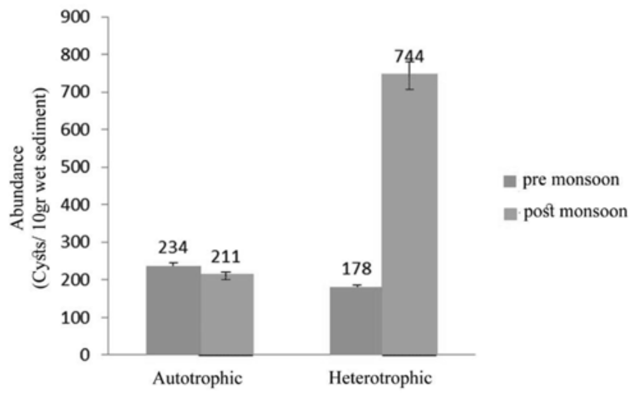

Fig. 3. Abundance of heterotrophic and autotrophic cysts (cysts 10 gr wet sediment) of the Gwater Bay in the northeast of Oman Sea in Pre- and Post-monsoon (2013)

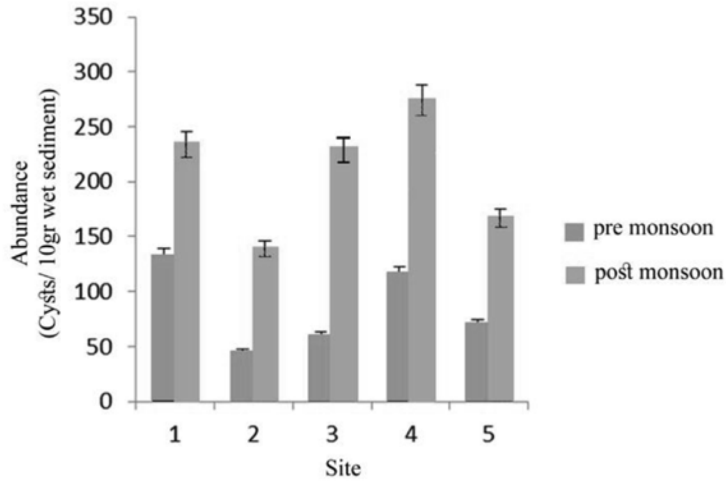

Fig. 4. Cysts abundance (cysts/ 10gr wet sediment) in sites of the Gwater Bay in the northeast of Oman Sea in Pre- and Post-monsoon seasons (2013)

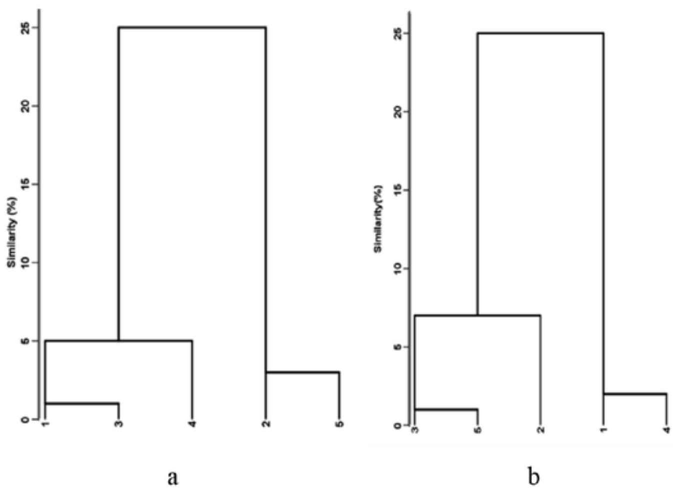

Fig. 5. a. Cluster analysis is presented in the Gwater Bay in the northeast of Oman Sea in Post-monsoon; b. Pre-monsoon

Table 3. A two-way ANOVA analysis Tests between subjects' effects

Source	Type III Sum of Squares	df	Mean Square	F	P value
Corrected Model	3144.786a	14	224.628	3.49	0
Intercept	5118.369	1	5118.369	79.6	0
Season	196.852	4	49.213	0.77	0.55
Site	2442.881	2	1221.44	19	0
Season site*	229.405	8	28.676	0.45	0.89
Error	13182.65	205	64.306		
Total	24673	220			
Corrected Total	16327.43	219			

a. R Squared = .193 (Adjusted R Squared = 137)

Dependent Variable: total

Table 4. Results of Tukey's multiple comparison tests

Tukey's multiple comparison test	Mean diff.	sig.	p-value	95% CI of the diff
St. 1 vs St. 2	-6.1709*	.000	p<0.05	-8.7867 to -3.5551
St. 1 vs St. 5	-9.7909*	.001	p<0.05	-16.0439 to-3.5379
St. 2 vs St. 1	6.1709*	.000	p<0.05	3.5551 to 8.7867
St. 2 vs St. 5	-3.62	.363	p>0.05	-9.899 to 2.659
St. 5 vs St. 1	9.7909*	.001	p<0.05	3.5379 to 16.0439
St. 5 vs St. 2	3.62	.363	p>0.05	-2.659 to 9.899

Observed means, The error term is Mean Square (Error) = 64.306, * The mean difference is significant at the 0.05 level.

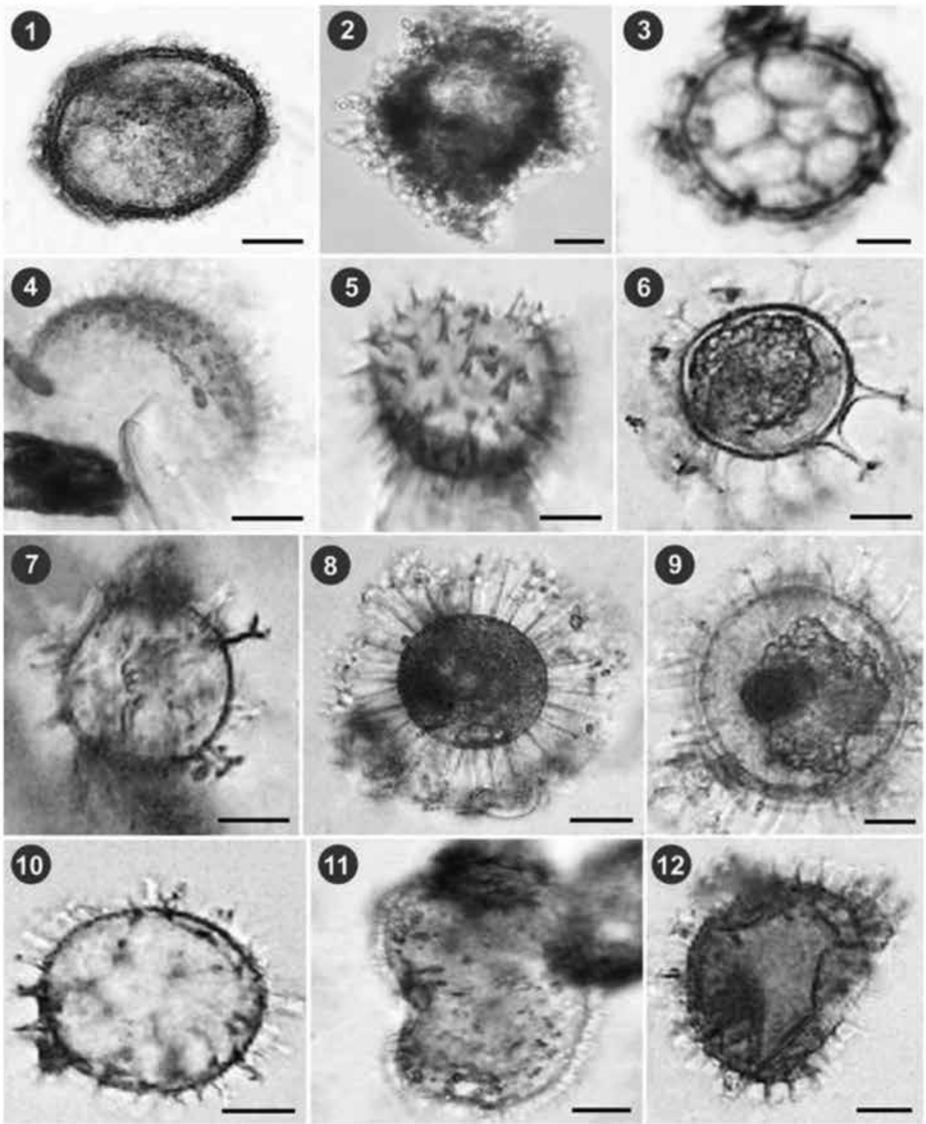

Fig. 6. 1) Light micrographs of *Alexandrium* cf. *tamarense*, 2) *Alexandrium* sp., 3) *Margalefidinium polykrikoides*, 4) *Diplopelta lenticular*, 5) *Pyrodinium cf. bahamense* 6) *Gonyaulax digitalis*, 7) *Gonyalax* cf. *scrippsae*, 8) *Lingulodinium polyedra*, 9) *Pentapharsodinium da lei*, 10) *Protoceratium reticulatum*, 11) *Protoperidinium claudicans*, 12) *Protoperidinium conicum*, Scale bar=10 µm.

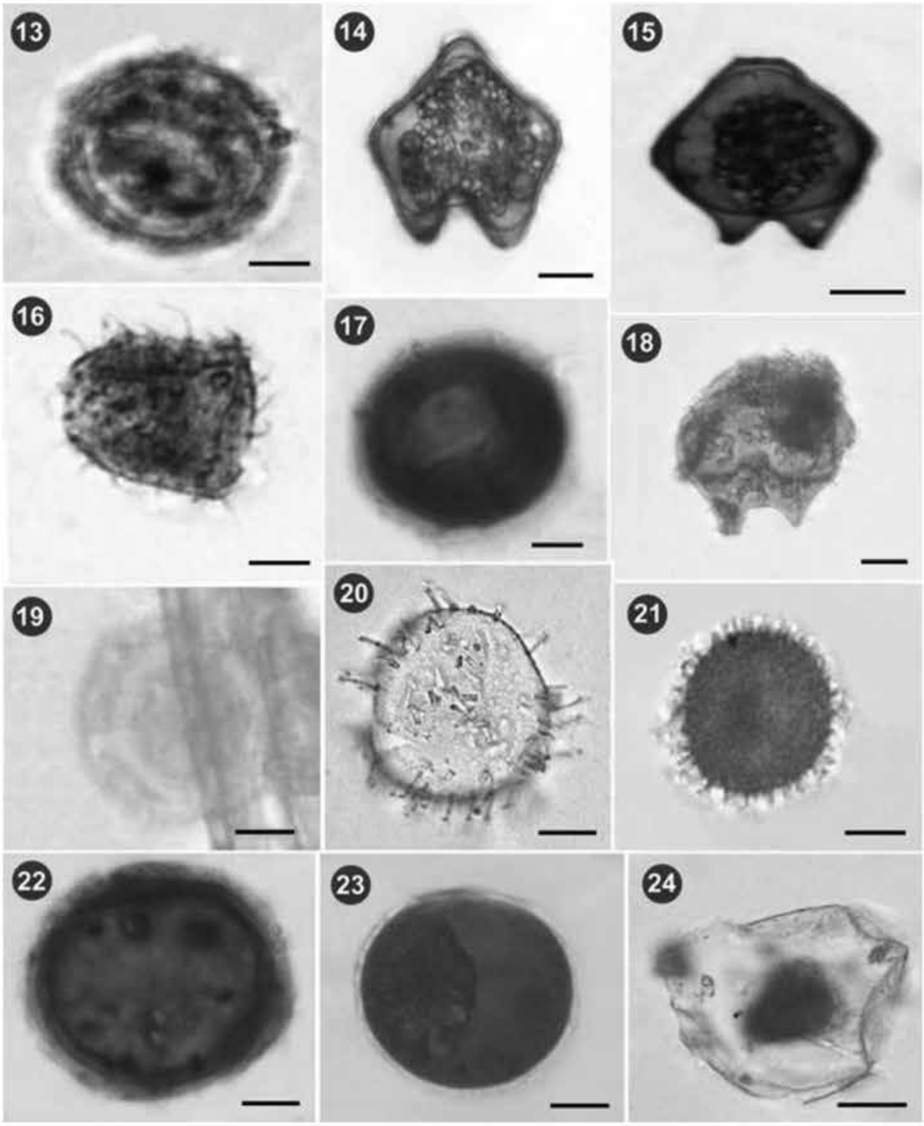

Fig. 7. 13) Light micrographs of *Protoperidinium conicoides*, 14) *Protoperidinium oblongum*, 15) *Protoperidinium* sp. 1, 16) *Protoperidinium* sp. 2, 17) *Protoperidinium obtusum*, 18) *Protoperidinium leonis*, 19) *Pyrophacus steinii*, 20) *Pyrodinium bahamense*, 21) *Scrippsiella trochoidea*, 22) *Zygabikodinium lenticulatum*, 23) Unknown cyst 1, 24) Unknown cyst 2, Scale bar=10 μm.

sampling seasons (P> 0.05) (Table 3).

Finally, the Tukey test was used to determine significant differences between stations, the results of which are listed in Table 4.

Discussion

Previous reports have provided basic information on cysts in the Oman Sea (Attaran-Fariman et al., 2011 and 2012). This study is the first record of the identification of dinoflagellate cysts in the sediments of the Gwater Bay in the northeast of the Oman Sea and provides useful information on the distribution of dinoflagellate species in a tropical region. In this study, 24 types of cysts were identified (Figs. 7, 8). In sediments from this Bay, 22 cysts species were identified and counted at the species level (Table 2). According to Figure 2, the abundance of cysts increased considerably in the post-monsoon. Note that the climate of the Oman Sea is completely different from other southern regions of the coasts (the Persian Gulf). Summer monsoon winds along the coast cause a rise in the water and the occurrence of the phenomenon of upwelling along the coastline (Barratt et al., 1984). The raised water contains high amounts of nutrients. Therefore, it considerably increases the primary production of phytoplankton, thereby strengthening the food web after the summer monsoon. In such circumstances, the number of autotrophic phytoplankton, especially small diatoms like Skeletonema costatum, increases, where abundant small autotrophs (phytoplanktons) provide the ideal food for heterotrophic dinoflagellates Fazeli and Zare, (2011), which is reflected by the high abundance of heterotrophic cysts in the summer)Table 2 and Figure 3).

The study by Wang et al. (2004) showed that generally, eutrophication by loading heavy food (especially nitrates) increases autotrophic phytoplanktons (e.g. diatoms) fed by heterotrophic phytoplanktons. This will be followed by an increase in heterotrophic cysts in these areas (Table 2 and Figure 3). A significant increase was also reported in response to rising food cysts (e.g. Alves-De-Souza et al., 2008; Liu et al., 2012). According to Figure 4, the abundance of cysts varies at different stations. Indeed, the spatial and temporal variations in species composition of phytoplankton cysts can be affected by several factors including environmental factors, primary production, competitiveness, and environmental adaptation (Simpson and Hunter, 1974). In general, compared to the four other stations, the abundance of cysts (especially for autotrophic cysts) was very low at station 2, which is located in the route of movement of boats introducing too much oil into the sea (Table 2 and Figure 3). Because of high friction and long-term wear of engine parts, these oils contain large amounts of metal particles. At this station, the abundance of autotrophic cysts has decreased compared to that of heterotrophic cysts. They can be changed and compressed by stress and oxidative changes in autotrophic cells due to metals in chloroplasts; also, in some *Goyaulax* genera such as *Alexandrium*, the cell is compressed and chloroplasts become star-shaped (Sætre et al., 1997). The light capacity diminishes due to the reduced level of peridinin, especially in *P. reticulatum*, causing the inhibition of the photosynthesis functional system in autotrophic cysts. Therefore, one can generally say that it is an important strategy to survive in polluted conditions (Bravo and Figueroa, 2014). Considering the toxicity of heavy metals, the abundance of autotrophic dinoflagellates can be reduced faster than that of heterotrophic dinoflagellates due to changes in the size of the population of phytoplanktons, where pollutants can reduce the diversity, complexity, and stability of algal communities (Liu et al., 2012). Middle estuary (stations 3, 4) showed a higher density compared to the two stations of estuary entrance and exit, for various reasons (e.g., avoiding the impact of sea waves occurring in the region of the estuary entrance) (station 5), as well as reducing stress conditions, such as increased salinity and drought during the tides.

Several species from this area were reported for the first time in this study. It became clear that the cysts have both heterogeneous distribution and abundance. Perhaps, the changes in species composition at each station will be due to the difference in the amount of food at another station. For example, a high percentage of heterotrophs can be attributed to high levels of food and low levels of predators. However, considering the cyst density and distribution in the sediments, further studies are required that can show the patterns of distribution, abundance, and diversity of dinoflagellate cysts. Figures 5 a and b demonstrate the cluster analysis of sampling stations across pre-and post-monsoon based on the abundance of cysts. The goal of cluster analysis is to achieve a criterion for a more appropriate classification of variables and samples based on intra-group similarity and differences between the groups as much as possible. In this way, the variables are divided into groups with the maximum similarity and the greatest difference between them. In the similarity matrix between variables, distance coefficients (or coefficients of variation) are used as a criterion of similarity and consistency between them. According to Figure a, which is composed of two main clusters, it can be concluded that the stations in each cluster have a maximum similarity. The minimum distance represents the maximum similarity between the two variables. This is also confirmed by the tree pattern obtained based on similarities in Figure B. Among the toxic species detected, genus Alexandrium is considered as the major dinoflagellate producing PSP (Taylor et al., 1995), which was present in our study area in both pre-and post-monsoon seasons. The high diversity and presence of this genus are due to its great adaptation to different environments (Satta et al., 2013). The negative effects of the species of this genus are well known worldwide such that their harmful bloom in the Mediterranean has caused huge economic losses (Cho and Matsuoka, 2001). About 16 species of Alexandrium have been reported by researchers in the Oman Sea and the Persian Gulf, of which 6 species have been introduced as species with the potential to produce toxins (Attaran-Fariman and Asefi, 2022). The abundance and diversity of cysts were different among the sampling locations. In the pre-monsoon, the diversity was higher in station 3, which is located on the estuary and where phytoplankton are more abundant due to reduced stress conditions.

In this study, six potentially toxic species of phytoplankton cysts were identified representing 5 genera. Lingulodinium polyedra and Protoceratium reticulatum can cause a toxic bloom. The harmful bloom of these species has been reported to mostly occur in the northern Adriatic Sea (Pistocchi et al., 2012). However, the active presence of these species has been reported in the Oman

Sea (Attaran-Fariman and Asefi, 2022). In Mexico, the abundant presence of cysts of the species *L. polyedra* led to the predicted bloom in the area (Peña-Manjarrez et al., 2005). *L. polyedra* produces homoyessotoxin in the Santos Bay, a subtropical region of Mexico, cysts were introduced as a source responsible for the annual bloom of the species (Peña-Manjarrez et al., 2005). Although the species *P. reticulatum* has been reported in Chabahar Bay (Attaran-Fariman, 2010), there is no report on the harmful bloom. The toxicity of this species needs more studies, and the abundance of cysts of these species is poorly known. *Pyrodinium bahamense*, which is native to warm waters with high salinity and areas with mangroves, produces Paralytic Shellfish Poisoning (PSP) which can cause infection in oysters and scalp as well as in some fishes, such as sardines and probably shrimp. The cysts of this species were also observed in this study. The species is present in the Gwater Bay which is covered with mangroves. It has not been reported in previous studies (Attaran-Fariman, 2010; Attaran-Fariman et al., 2012) from Iran's southeastern coasts. *Margalefidinium polykrikoide*s (formerly known as *Cochlodinium polykrikoides*) cyst wall is grainy and brownish (Aydin et al., 2011), and was detected in the sediments of the Gwater Bay in this study. This species created a great deal of blooming and economic damage and loss on the coasts of the Oman Sea and the Persian Gulf in 2009 (Attaran-Fariman et al., 2011). In general several species from this area were reported for the first time in this study. It became clear that the cysts have both heterogeneous distribution and abundance across different stations and seasons.

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