Occurrence and germination of dinoflagellate cysts in surface sediments from the Red Sea off the coasts of Saudi Arabia

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KEYWORDS Cyst Dinoflagellates Red Sea Saudi Arabia Toxic species

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## Abstract

The distribution and abundance of dinoflagellate cyst assemblages were investigated in surface sediments from south-western Red sea coasts of Saudi Arabia at six sites during March 2010. A total of 19 taxa of dinoflagellate cysts were identified from all sites. The sampling sites showed a similar cyst assemblage, but they differed in total cyst abundance (3 to 4083 cysts  $g^{-1}$  dry weight). Cyst abundance was strongly correlated with sediment characteristics, the highest numbers being recorded in sediments with large contents of organic carbon, silt and clay. Cyst assemblages were dominated by cysts of potentially toxic species, including *Cochlodinium polykrikos, Prorocentrum minimum, Dinophysis acuminata, Alexandrium catenella* and *Scrippsiella trochoidea*. Most cysts germinated successfully at different rates at 15 and 25°C. This study suggests that surface sediments from all Saudi Red Sea coasts should be monitored for the presence of dinoflagellate cysts to give ample warning of the presence and abundance of toxic species in a given area.

The complete text of the paper is available at http://www.iopan.gda.pl/oceanologia/

## 1. Introduction

Dinoflagellates constitute the major phytoplankton group in marine environments with harmful species, causing red tides and shellfish poisonings in coastal areas (de Vernal & Marret 2007). The life cycle of many dinoflagellates consists of an asexual vegetative phase, with production by binary fission, and a sexual phase, involving reproduction by gamete fusion (Pfiester & Anderson 1987). Sexual reproduction yields a motile cell, the zygote, which can either return to the vegetative stage or become a hypnozygote, or resting cyst, which is unable to swim and sinks to the bottom sediments (Figueroa et al. 2007). Cysts can remain viable in sediments for 5–10 years or longer (Anderson et al. 1995). Cysts constitute a seed bank for the region where they are present and supply the initial inoculum to form plankton blooms once favourable conditions (mainly temperature) are re-established (Lewis et al. 1999). In addition, cysts of toxic species such as Alexandrium spp. and Gymnodinium catenatum may be more toxic than their motile vegetative cells (Dale et al. 1978, Oshima et al. 1992) and may therefore represent a source of paralytic shellfish poisoning (PSP) toxins (Schwinghamer et al. 1994).

Although studies of dinocyst distributions in marine surface sediments are increasing worldwide, there is no published literature on dinoflagellate cyst assemblages in Saudi coastal areas of the Red Sea. However, incidents of algal blooms and dinoflagellate red tides did occur along Saudi coasts of the Red Sea during the period 2004–2006 (Mohamed & Messad 2007). Although these blooms have since disappeared from this area, there is a possibility of their recurrence in the original bloom area and elsewhere. Therefore, the collection and counting of resting cysts during non-bloom periods offer a potential tool for the prediction of future toxic blooms (Hallegraeff & Bolch 1992, Anderson 1997, Persson et al. 2000). Hence, the objective of this study was to investigate the occurrence of dinoflagellate cysts in surface sediments collected from previously infected areas with algal blooms on south-western Saudi coasts of the Red Sea. The germination ability of these cysts was also evaluated.

## 2. Material and methods

## 2.1. Study area and sample collection

The study area was located in the Red Sea off the south-western coast of Saudi Arabia, extending from 19.65° to 19.80°N (Figure 1). The coastal region of this area is subject to drainage from surrounding rainwater pools and is affected by aquaculture wastewater discharges from a nearby shrimp farm. The surface sediments collected from the study area were



**Figure 1.** Map showing the study area and locations of sediment samples along south-western Saudi coasts of the Red Sea

characterized as fine sand and mud (Table 1). Surface sediments were collected from 6 sites throughout the study area during March 2010. The sites are ca 20 km distant from each other. Three sediment samples were collected from different spots (located about 10 m away from one another) at each site with a flat spade and the subsamples put into plastic jars. Three

Sites	Depth [m]	Organic matter [%]	Silt [%]	Clay [%]	$\begin{array}{c} \text{Sand} \\ [\%] \end{array}$
site 1	4	1.1	3.5	10.1	85.3
site 2	3.5	2.3	1.3	33.8	62.4
site 3	0.5	0.3	8.2	53.6	37.9
site 4	8	0.5	1.2	5.5	93.8
site 5	6	9.7	9.3	3.8	77.8
site 6	1	9	7.3	63.4	20.3

**Table 1.** Contents of organic matter, silt, clay and sand in surface sedimentscollected from Saudi coasts during the present study

replicate subsamples were taken from the top 5 cm using a 1.5-cm-diameter syringe with a cut-off top. The three replicates were pooled and placed into containers that were then tightly sealed to prevent germination. All the samples were stored in the dark at 4°C until processing. Aliquots of the samples were oven-dried at 105°C for 6 h to determine sediment dry weight. The sediments were analysed for grain size following Folk & Ward (1957), and their organic carbon content was determined according to el Wakeel & Riley (1957).

## 2.2. Sediment preparation, microscopy and cyst germination

The sediment samples from each site were homogenized with a glass rod, and subsamples of the sediment were extracted with a spoon and sieved through 100  $\mu$ m and 25  $\mu$ m Retsch stainless steel sieves using filtered seawater. The sediments remaining on the 25  $\mu$ m sieve were collected in a 50 ml glass container. The collected material was transferred to a test tube and made up to a total volume of 10 ml with filtered seawater. The slurry was homogenized by shaking the tube, and a 200  $\mu$ l aliquot was transferred to a new test tube. This new aliquot was made up with filtered sea water to a total volume of 10 ml and poured into an Utermöhl-type sedimentation chamber (Utermöhl 1958). To prevent their germination, the cysts were counted and identified within 8 hours at magnifications of 200x, 400x and 1000x under a Zeiss inverted microscope. A minimum of 100 cysts were identified and counted in each sample. The cyst concentration in each sample was given as cysts per gram (cysts/g) of dry weight sediment.

Cysts were identified to species level whenever possible based on the literature listed in the References section; images were obtained from Dino-Atlas at http://www.pangaea.de/Projects/Dino-Atlas/dinoflagellates.html (Marret & Zonneveld 2003). Additional taxonomic references for dinoflagellate cysts, including those of Rochon et al. (1999), Matsuoka & Fukuyo (2003) and Fensome & Williams (2004), were also used. The biological taxonomy system was used throughout this study. Photographs were taken with an Olympus OM4 camera connected to the relevant microscope. Changes in cyst assemblages were described by the total cyst concentration, species richness (number of taxa), the proportion of cysts of heterotrophic and autotrophic dinoflagellates, as well as the value of the Shannon-Weaver diversity index (H) (Shannon & Weaver 1949).

## 2.3. Cyst germination

To study the viability of the cysts from the sediments collected and to confirm their original species (identification), germination experiments were conducted. Single cysts (20) were isolated with a glass micropipette and transferred to 96-well tissue culture plates containing 100  $\mu$ l f/2 (Guillard 1975) or filtered seawater (Millipore, 0.22  $\mu$ m). Plates were incubated at 15 and 25°C using a 12:12 h light:dark cycle provided by cool white illumination tubes at 80  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. The germination experiment was carried out in triplicate for each cyst species. Cysts were monitored every 2 days for germination and growth for a maximum of one month, and the percentage germination was calculated for each cyst type.

### 2.4. Statistical analysis

Differences in cyst abundance among the study sites were determined by one-way ANOVA (P < 0.05). Spearman rank correlation coefficients were used to measure the degree of association between the cyst abundance, and the contents of organic matter, silt, clay and sand in the sediments collected.

### 3. Results

## 3.1. Composition and distribution of dinoflagellate cysts

A total of 19 taxa of dinoflagellate cysts representing 9 genera and 19 species were identified from all sites during the present study (Table 2, Figures 2 and 3). These dinoflagellate assemblages comprised 2 species of Gonyaulacales (19% of the total number of dinoflagellate cysts in all samples), 6 species of Gymnodinales (33%), 9 species of Peridiniales (16%), 1 species of Prorocentrales (18%) and 1 species of Dinophysiales (13%). Cyst abundance at the study sites ranged from 3 to 4083 cysts  $g^{-1}$  dry weight sediment. In general, the proportion of autotrophic cysts (70-83%) in the cyst abundance was larger than that of heterotrophic ones (17-30%). Of the individual cyst types, cysts of potentially toxic dinoflagellate species were more abundant than those of non-toxic species. *Cochlodinium polykrikos* was the most abundant at all sites (31%), followed by *Prorocentrum minimum* (18%), Dinophysis acuminata (13%), Alexandrium catenella (11%) and Scrippsiella trochoidea (10%). Although Protoperidinium cysts were found in very small numbers at all sampling sites (0.03-1.6%) of the total cyst abundance), this genus was represented by more species (six) than any other dinoflagellate genera during the present study (Table 2): P. claudicans, P. conicum, P. curtipes, P. leonis, P. minutum and P. subinerme.

Species richness (number of species) of dinoflagellate cysts varied significantly among the sites studied (F = 3.93, df = 5, P = 0.024). The highest number of species was recorded at sites 2, 3 and 5, while the number of species was the lowest at site 4. Species richness was weakly correlated with the total cyst abundance (r = 0.2) and the percentage of silt in the

**Table 2.** Species compositions, abundances (cysts  $g^{-1}$  dry weight) and dominance of dinoflagellate cysts in surface sediments of the Red Sea off the south-western coasts of Saudi Arabia

Resting cyst species		$\begin{array}{c} \text{Abundance} & \text{D} \\ (\text{cysts } \text{g}^{-1} \text{ dry weight}) \end{array}$				Dominance [%]	
		Sampling sites					
	1	2	3	4	5	6	
Gonyaulacales							
$A lexandrium \ catenella^{a*}$	413	784	381	342	941	1063	10.65
$A lexandrium \ minutum^{a*}$		823	582		831	943	8.63
Gymnodinales							
$Cochlodinium \ polykrikoides^{a*}$	653	1325	949	632	4083	3678	30.7
$Cochlodinium \text{ sp.}^{a}$	34	81	19		97		0.7
$Gymnodinium \ instriatum^{a}$	24	65	54	48	69	31	0.8
$Gymnodinium \text{ sp.}^a$	13	11	3		8		0.1
$Gyrodinium \text{ sp.}^a$	28	43	16	5	6	11	0.3
$Polykrikos\ schwartzii$	63	98	47	44			0.7
Peridiniales							
Diplosalis sp.	14	11	5		103	39	0.47
Protoperidinium claudicans	39	51	46	62	111	63	1
Protoperidinium conicum	13	26	39	88	145	89	1.1
Protoperidinium curtipes	24	18		67	123	97	0.9
Protoperidinium leonis	89	87	69	91	151	86	1.6
Protoperidinium minutum	11		5	13	38	31	0.03
Protoperidinium subinerme	68	54	22	10	26		0.48
Scrippsiella trochoidea <sup>a*</sup>	676	823	524		956	863	10.42
Scrippsiella ramonii <sup>a</sup>		13	6	3	7	2	0.1
Prorocentrales							
$Prorocentrum \ minimum^{a*}$	1312	1545	1562	376	319	1519	18
Dinophysiales							
Dinophysis acuminata <sup>a*</sup>	843	977	813	466	192	1608	13.32
Total no. of cysts	4317	6835	5142	2247	8206	10123	100
Percentage of autotrophic cysts [%]	83	71.5	76.7	70	70	76.2	
Percentage of heterotrophic cysts [%]	17	28.5	23.3	30	30	23.8	
Species richness (no. of species)	17	18	18	14	18	15	
Diversity (H)	1.93	2.1	1.92	2.25	1.77	1.84	

 $^{a}$ Autotrophic species

\*Harmful



**Figure 2.** Dinoflagellate cysts isolated from surface sediments off the southwestern coasts of Saudi Arabia: a) Alexandrium catenella, b) Alexandrium minutum, c) Cochlodinium polykrikoides, d) Cochlodinium sp., e) Gymnodinium instriatum, f) Gymnodinium sp., g) Gyrodinium sp., h) Polykrikos schwartzii, i) Prorocentrum minimum. Scale bars: 20 μm

sediments (r = 0.3). The Shannon-Weaver diversity index (H) calculated for the study sites did not vary significantly among them (F = 1.11, df = 5, P = 0.4), but the species diversity at sites 2 and 4 was higher (H = 2.1, 2.25, respectively) than at other sites. The diversity index was negatively correlated with species richness (r = -0.45, P = 0.18, n = 6) and total cyst abundance (r = -0.72, P = 0.0, n = 6). Total cyst concentration varied from as many as 10123 cysts g<sup>-1</sup> in the sediments from site 6 to as few as 2247 cysts g<sup>-1</sup> in site 4 sediments (Table 2). Cyst abundance was strongly correlated with sediment characteristics. The highest cyst abundance was associated with sediments of high organic carbon (r = 0.86, P = 0.01, n = 6), silt (r = 0.6, P = 0.1, n = 6), and clay (r = 0.82, P = 0.02, n = 6) contents, but was negatively correlated with the sand content (r = -0.7, p = 0.05, n = 6) (Tables 1 and 2).

#### 3.2. Cyst germination

The results of the germination experiment showed that most cysts were successfully germinated at rates from 74 to 90% at  $15^{\circ}$ C and from 48 to 64% at  $25^{\circ}$ C (Table 3). However, the germination of *Alexandrium* cysts was not



**Figure 3.** Dinoflagellate cysts isolated from surface sediments off the southwestern coasts of Saudi Arabia: a) *Diplosalis* sp., b) *Protoperidinium claudicans*, c) *Protoperidinium conicum*, d) *Protoperidinium curtipes*, e) *Protoperidinium leonis*, f) *Protoperidinium minutum*, g) *Protoperidinium subinerme*, h) *Scrippsiella trochoidea*, i) *Scrippsiella ramonii*, j) *Dinophysis acuminata*. Scale bars: 20 μm

significantly affected by the change in temperature (P = 0.12), where the maximum germination rate was 94% at 15°C and 95.6% at 25°C (Table 3).

## 4. Discussion

This study provides the first data about the abundance, composition and distribution of dinoflagellate cysts, including toxic species, in the Red Sea sediments off the south-western coasts of Saudi Arabia. The results showed a considerable similarity in the cyst compositions at the different study sites, which may be explained by the transportation along with the flood and ebb tides of dinoflagellate cysts produced in one area to other areas, where they sink (Hwang et al. 2002, Shin et al. 2007). Earlier such a similarity in the species composition of dinoflagellate cysts was

Cyst species	Germination rate [%]				
	$15^{\circ}\mathrm{C}$	$25^{\circ}\mathrm{C}$			
Gonyaulacales					
Alexandrium catenella	$94\pm0.5$	$95.6\pm2.4$			
Alexandrium minutum	$94.3\pm0.8$	$95\pm2.2$			
Gymnodinales					
Cochlodinium polykrikos	$76 \pm 1$	$49.6\pm0.7$			
Cochlodinium sp.	$78.7 \pm 1.2$	$51\pm0.6$			
Gymnodinium instriatum	$74\pm0.9$	$48\pm0.8$			
<i>Gymnodinium</i> sp.	$80.5\pm1.3$	$56\pm0.9$			
Gyrodinium sp.	$76 \pm 1$	$49\pm1$			
Polykrikos schwartzii	$81\pm1.4$	$57 \pm 1$			
Peridiniales					
Diplosalis sp.	$83\pm0.9$	$61.8 \pm 1.55$			
Protoperidinium claudicans	$89 \pm 2$	$63\pm1.7$			
Protoperidinium conicum	$90 \pm 2.1$	$64 \pm 1$			
Protoperidinium curtipes	$88.7 \pm 1.8$	$59.4 \pm 1.1$			
Protoperidinium leonis	$90 \pm 2$	$62.6 \pm 1.6$			
Protoperidinium minutum	$90 \pm 1.6$	$61.8\pm0.8$			
Protoperidinium subinerme	$90 \pm 2.1$	$62\pm0.9$			
Scrippsiella trochoidea	$84 \pm 1.2$	$60 \pm 1$			
Scrippsiella ramonii	$86.4\pm1$	$61\pm1.1$			
Prorocentrales					
Prorocentrum minimum	$88.6 \pm 1.4$	$64\pm1.3$			
Dinophysiales					
Dinophysis acuminata	$79.2\pm1$	$58 \pm 1.2$			

**Table 3.** Germination rates [%] of cyst species isolated from Saudi surface sediments during the present study

demonstrated in recent sediments from the eastern coasts of Russia (Orlova et al. 2004). On the other hand, the species composition of dinoflagellate cysts from the sediments of Saudi coasts can be compared to that recorded in marine sediments off Japan, Korea, Russia, India, Sweden, Chile and China (Godhe et al. 2000, Persson et al. 2000, Matsuoka et al. 2003, Orlova et al. 2004, Wang et al. 2004, Shin et al. 2007, Alves-de-Souza et al. 2008). As there are no earlier records of recent dinoflagellate cysts from the Saudi coasts off the Red Sea, comparison with nearby Saudi localities is not possible. In addition, the assemblages comprised mainly cosmopolitan dinoflagellate cyst genera such as *Alexandrium, Cochlodinium, Gymnodinium, Polykrikos, Diplosalis, Protoperidinium, Prorocentrum* and *Scrippsiella* (Matsuoka & Fukuyo 2003).

In this study, cysts of heterotrophic dinoflagellates were present in low proportions (17-30%) compared to the huge numbers of cysts of autotrophic dinoflagellates (70-83%). These results are actually contrary to those of most studies, which report the dominance of cysts of heterotrophic species over those of autotrophic species (Godhe & McQuoid 2003, Matsuoka et al. 2003, Fujii & Matsuoka 2006, Harland et al. 2006, Radi et al. 2007). These authors correlated higher abundances of heterotrophic dinoflagellate cysts in nutrient-rich areas with high diatom abundances. The discrepancy in the results between our study and previous studies could be due to the sampling locations of the sediments: our study was carried out on surface sediments, whereas most studies were done using sediment traps. Therefore, the results of the present studies support the hypothesis that heterotrophic dinoflagellate cysts are dominant in upwelling areas, because diatoms, being prey organisms for these heterotrophic dinoflagellates, are abundant (Matsuoka et al. 2003), and that the concentration of heterotrophic cysts could be reduced up to half in surface sediments (Pitcher & Joyce 2009). The results of the present study also revealed a low richness of dinoflagellate cyst taxa (19 species) compared to other studies. The decrease in species richness of dinoflagellate cysts may indicate that the study region is polluted and highly eutrophic, as suggested by Pospelova et al. (2002). In addition, we recorded cysts of heterotrophic taxa, e.g. *Protoperidinium*, which has been reported as a high productivity indicator (Dale & Fjellså 1994, Sprangers et al. 2004, Uzar et al. 2010).

In our study, cyst abundance was closely correlated with sediment characteristics, where higher concentrations of dinoflagellate cysts were found in sediments with high contents of silt, clay and organic matter, and lower cyst concentrations in sandy sediments. These results are thus in agreement with those of previous studies, which reported that muddy sediments have higher concentrations of dinoflagellate cysts than sandy sediments (Nehring 1993, Anderson et al. 2005, Olli & Trunov 2010). This may be due to the fact that the depositional behaviour of dinoflagellate cysts is like that of fine particles, and that their abundance increases in sediments with higher mud contents (Dale 1983).

The present study also showed that most dinoflagellate cysts identified in Saudi sediments germinated successfully, with germination rates varying significantly among cyst types at different temperatures. This finding thus concurs with the conclusions drawn from previous studies that temperature is the major factor regulating the germination of marine phytoflagellate cysts (Dale 1983, Pfiester & Anderson 1987, Ishikawa & Taniguchi 1996, Ishikawa & Taniguchi 1997), and that cyst germination is stimulated in different organisms by different water temperatures (Meksumpun et al. 2005). Our results showed that an increase in temperature from 15 to  $25^{\circ}$ C lowered the germination rates of dinoflagellate (*Alexandrium*) cysts from Saudi sediments. These results are in agreement with those of Meksumpun et al. (2005), who reported that some dinoflagellate cysts (but not *Alexandrium* cysts) can germinate well at temperatures between 10 and  $28^{\circ}$ C. Also, Ishikawa & Taniguchi (1996) found that *Scrippsiella* cysts can germinate at temperatures between 5 and  $25^{\circ}$ C. Therefore, the increase in temperature may act to prevent seeding or the maintenance of blooms in the water column during summer periods (Genovesi et al. 2007).

Unlike other cyst types, the germination of *Alexandrium* cysts was not affected by the difference in temperatures, with maximum germination rates reaching as high as 95.6%. Perez et al. (1998) reported that temperature had no significant effect on the germination of Alexandrium cysts collected from the St. Lawrence Estuary, Canada. The germination rate of Alexandrium cysts from Saudi sediments is higher than that obtained (48-52%) by Bravo et al. (2006), but is comparable with that reported by Garcés et al. (2004) (up to 91%). Such a remarkable difference in the germination rates of *Alexandrium* cysts between the two studies may be explained by the presence of some distinctive internal features, such as globular content, or other, genetic or external, factors (Bravo et al. 2006). Germination success can also be affected by excystment medium conditions, where higher rates of germination were found for A. catenella cysts isolated in seawater than in L1 medium (Figueroa et al. 2005). Overall, such information on the germination of dinoflagellate cysts may be helpful for understanding the mechanism of the outbreak of dinoflagellate red tides along Saudi coasts, as cyst bank germinations contribute to the initial seeding of blooms (Genovesi et al. 2007).

Our study also highlighted the presence of harmful marine dinoflagellate cysts in Saudi marine sediments. The cysts belonged to the potentially toxin-producing species of *Alexandrium catenella*, *A. minutum*, *Cochlodinium polykrikoides*, *Dinophysis acuminata*, *Prorocentrum minimum* and *Scrippsiella trochoidea*, and were recorded at all sampling sites with high abundances compared to other dinoflagellate cyst species. The presence of harmful marine dinoflagellate cysts in marine sediments has been documented worldwide (Matsuoka & Fukuyo 2003, Anderson et al. 2005, Fahnenstiel et al. 2009, Pitcher et al. 2009) and has been suggested as being one of the dominant vectors responsible for the apparent global increase in harmful algal blooms (Hallegraeff 1998). In addition, the dinoflagellate cysts themselves can be very toxic, containing up to 10 times the toxin content of vegetative cells, thus constituting a possible source of poison to organisms long after the motile forms have disappeared from the water column (Oshima et al. 1982). Furthermore, the higher abundance of the cysts of these toxic species in Saudi surface sediments could be a reflection of the large bloom of these species that recently occurred in this area, as suggested by Matsuoka & Takeuchi (1995), Kremp & Heiskanen (1999) and Wang et al. (2004, 2007). These authors reported that large numbers of resting cysts are produced at the end of blooms and that cyst formation is regarded as one of the major factors in bloom termination.

To summarize, this is first study of dinoflagellate cysts in marine sediments off the Saudi Red Sea coast. It therefore contributes to our knowledge of dinoflagellate cysts and provides a basis for further studies. The dinoflagellate cysts did not show a significant difference in species composition or diversity, but both species richness (the number of species) and cyst abundance varied significantly among the study sites. The abundance of dinoflagellate cysts was markedly correlated with sediment characteristics: cyst concentrations were high at sites containing large amounts of organic matter, silt and clay, but lower on sandy sediments. All dinoflagellate cysts were successfully germinated, and the maximum germination rate for cyst species was temperature dependent. Our study also showed that cysts of six potentially toxic and harmful species were detected in almost all localities in high abundances. The presence of such high numbers of toxic dinoflagellate species not only reflects the recent occurrence of large-scale blooms of these species in the study area, but can also be a risk factor and constitute an early warning of future harmful algal blooms. It was stated earlier that a rich cyst bank is not only the witness of past blooms, but also portends further blooms (Pati et al. 1999). Therefore, the present study suggests that cyst surveys should be conducted in other areas of Saudi Red Sea coastlines not yet investigated, in order to monitor and manage the formation of harmful algal blooms in this country.

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