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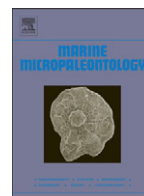
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## Research paper

## The impact of different pollution sources on modern dinoflagellate cysts in the Sishili Bay, Yellow Sea, China

Dongyan Liu<sup>a,\*</sup>, Yajun Shi<sup>a,b</sup>, Baoping Di<sup>a</sup>, Qianli Sun<sup>c</sup>, Yujue Wang<sup>a</sup>, Zhijun Dong<sup>a</sup>, Hongbing Shao<sup>a</sup><sup>a</sup> CAS and Shandong Provincial Key Laboratory of Coastal Environmental Processes, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, 264003, Yantai, Shandong, PR China<sup>b</sup> Graduate University of the Chinese Academy of Sciences, 100049, Beijing, PR China<sup>c</sup> State Key Laboratory for Estuarine and Coastal Research, East China Normal University, 200062, Shanghai, PR China

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## ABSTRACT

The spatial distribution of dinoflagellate cysts in the surface sediment of Sishili Bay, Yellow Sea, China, was studied, with the purpose of understanding the impact from nutrient enrichment and industrial pollution. Thirty-five dinoflagellate cyst taxa belonging to 15 genera and 3 unknown cysts were identified and quantified at 22 sampling sites. Autotrophic cysts (e.g., *Spiniferites bentori* var. *truncata*) and heterotrophic cysts (*Brigantedinium* sp.1 and *Quinquecupis concreta*) dominated the sediment samples. The spatial distribution of cyst abundance showed a significant positive correlation with increased nutrients, but was negative to heavy metal pollution. The highest cyst abundance (with an average of 539 cysts g<sup>-1</sup> DW) occurred in Zone A, corresponding to nutrient enrichment caused by domestic sewage discharge. In contrast, the lowest cyst abundance (with an average of 131 cysts g<sup>-1</sup> DW) occurred in Zone E, impacted heavily by the industrial pollution. The abundance of autotrophic cysts decreased dramatically in Zone E compared with heterotrophic cysts and showed a sensitivity to industrial pollution. How heavy metals affect physiological mechanisms in autotrophic and heterotrophic cysts differentially is in need of in-depth study.

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## 1. Introduction

Sishili Bay (SB) is located in the northern Yellow Sea, China, and is surrounded by the city Yantai (Fig. 1). Over the last two decades, SB has been developed as an important harbor and coastal aquaculture base by the local government. The aquaculture area covers 70% of the bay and has provided more than 1.5 million tons of seafood for the market over the last 5 years (Yantai Statistics Bureau, 1985–2008). However, the increased population, marine aquaculture production, freight ships and sewage discharge along the Yantai coastline have significantly impacted the SB marine ecosystem (Yantai Statistics Bureau, 1985–2008) (Fig. 2). Domestic sewage and industrial waste water increased almost three-fold during 1995–2007 from 8.5 × 10<sup>7</sup> tons to 22.1 × 10<sup>7</sup> tons. As a result, increased red tides and jellyfish blooms indicated the deterioration of the SB ecosystem over the last 10 years (Wu et al., 2001; Chi, 2008; Dong et al., 2010). Thus, it is important to understand the impact of different sources of pollution on the SB ecosystem ahead of development of the policy on environmental protection and restoration.

Phytoplankton, as the most important primary producers in marine ecosystems, are sensitive to environmental changes, as indicated

by the fluctuation of species composition and abundance. The fossil phytoplankton assemblages in the sediment have been used as proxies for past environmental changes (e.g., temperature, salinity and eutrophication) (McMinn and Wells, 1997; Matsuoka, 1999, 2001; Shin et al., 2010; Tuovinen et al., 2010; Wang et al., 2011). About 200 marine dinoflagellate taxa can produce cysts that sink to the seabed to serve as benthic resting stages, and their abundance and composition have been used to predict red tides (Anderson et al., 1982; Siringan et al., 2008), reflect pollution loads and indicate temperature change and hydrodynamic signals (Matsuoka, 1999, 2001; Dale, 2001, 2009; Pospelova et al., 2005, 2008). Thus, dinoflagellate cysts have been applied widely in the study of modern and past environments as an effective biological indicator.

Dale (2001, 2009) pointed out that dinoflagellate cysts showed different responses to nutrient enrichment and industrial pollution, respectively. The nutrient enrichment can increase the abundance of cysts in the sediment. For example, Pospelova et al. (2005) compared the spatial distribution of cysts from several polluted estuaries in the northeast coast of USA, and found that cyst abundance increased progressively with distance from the major sources of nutrient enrichment. However, the industrial pollution might decrease the cyst abundance or change the ratio between heterotrophic and autotrophic cysts (Sætre et al., 1997; Matsuoka, 2001; Dale, 2009). For example, Sætre et al. (1997) found that cyst abundance in sediment cores declined with increased pollution and suggested that industrial

\* Corresponding author. Tel.: +86 535 2109116; fax: +86 535 2109116.

E-mail address: [dylu@yic.ac.cn](mailto:dylu@yic.ac.cn) (D. Liu).

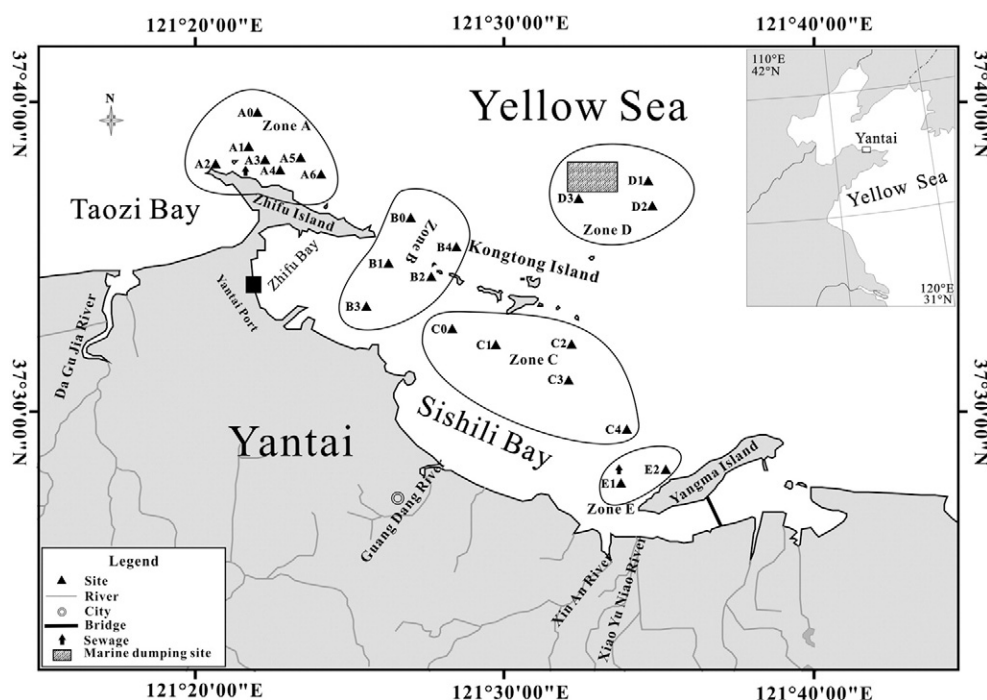


Fig. 1. Map showing the sampling sites in Sishili Bay, Yellow Sea, China.

pollution could cause a shift toward more heterotrophic species. However, due to the limited number of case studies to date, it is still hard to define the changes to dinoflagellate cysts under industrial pollution conditions. In addition, nutrient enrichment and industrial pollution often occurred together in the coastal waters. For example, Matsuoka (1999, 2001) found that the relative proportion of heterotrophic dinoflagellate cysts in Yokohama Port, Tokyo Bay, Japan was mostly consistent with the trend of eutrophication and industrialization

levels since the 1970s. Thus, it is necessary to do more case studies to understand the response of dinoflagellate cysts to industrial pollution.

In this study, we chose SB as a survey region, with the aim of finding out the relationship between the spatial distribution of dinoflagellate cysts and different pollutants. The cyst composition and abundance in the surface sediment of SB were surveyed at 22 sites, and these sites covered the sea areas impacted by nutrient enrichment and industrial waste water. The trophic types and abundance

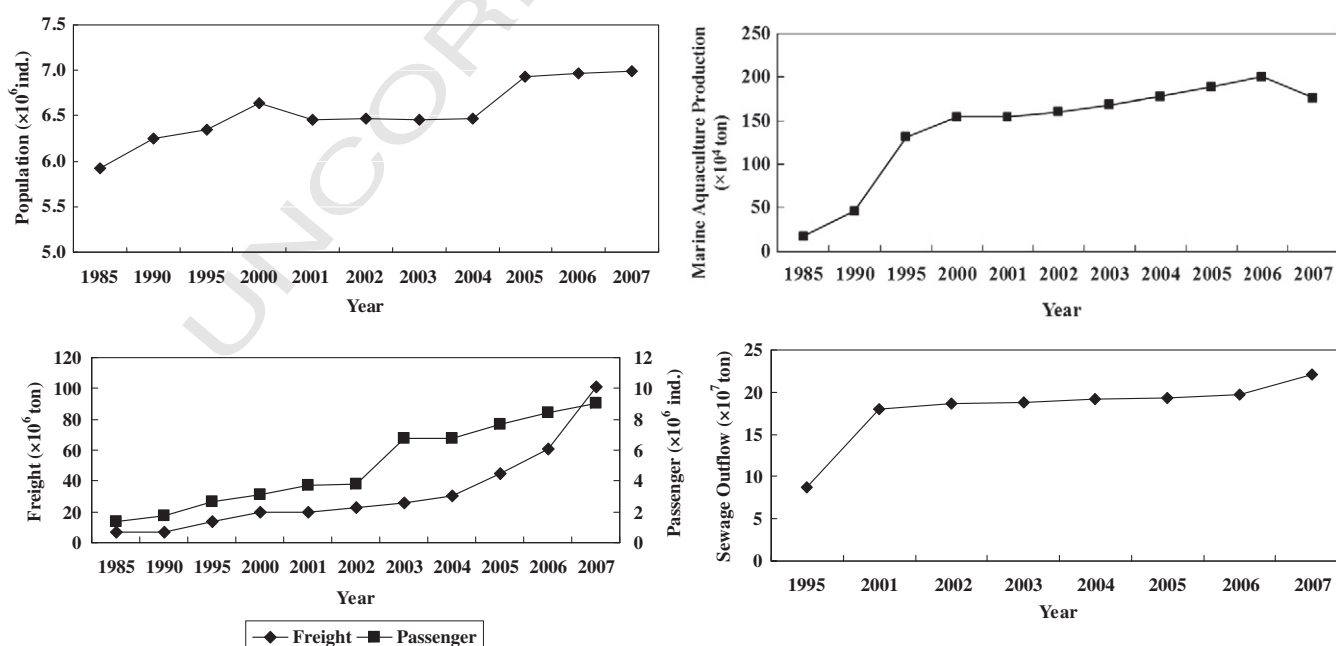


Fig. 2. The increased population, marine aquaculture production, freight ships and sewage outflow in Yantai (Yantai Statistics Bureau, 1985–2008).

of dinoflagellate cysts are discussed in relation to the environmental parameters, in order to further understand the spatial variation of cyst distribution.

## 2. Methods

### 2.1. Sampling sites

Sishili Bay (SB) is an ear-shaped semi-closed bay with a total area of about 130 km<sup>2</sup> (Fig. 1). It is a typical temperate coastal bay with a temperature of 23.3–27.4 °C in summer and 2.5–3.5 °C in winter. Only four small ephemeral rivers (Guangdang River, Majia River, Xin An River and Xiao Yuniao River) flow into SB (Wu et al., 2001), where the salinity ranges from 29.6 to 33.0 during the wet and dry seasons. The water depth in SB is generally less than 15 m, and the hydrodynamic process is mainly controlled by the movement of tide and wind-induced currents (Zhang and Dong, 1990). Along the periphery of the bay, the tidal currents move mainly back and forth in a north-west–southeast direction, which causes the pollutants to move parallel to the shore, while in the inner bay, due to the weak tidal current and obstructing small islands, water exchanges are limited with the outer bay. Moreover, the prevailing southeasterly wind can carry and accumulate pollutants into SB, consequently deteriorating the water quality.

Based on the types of pollution, twenty-two sampling sites were chosen and divided into five zones (Fig. 1). Zone A (sites A0–A6) is significantly affected by the nutrients discharged from an urban domestic sewage plant (Sang and Sun, 2010); Zone B (sites B0–B4) is along the channel of Yantai port (Li et al., 2006), which can be impacted by the pollutants from Zone A under the action of tidal currents; Zone C (sites C0–C4) is near to the marine aquaculture area (Zhao et al., 2001), and the waste and excretion from the aquaculture have significantly increased the nutrient level in this area; Zone D (sites D1–D3) is near to the marine dumping area (Ji et al., 2003), impacted by the dumped waste and the pollutants that are carried by the coastal current from Zone A; Zone E (sites E1 and E2) is near to Yangma Island and the Xin An River sewage plant, which is heavily impacted by industrial pollutants, particularly heavy metals from an electroplating plant near to the river (Jia et al., 2007).

### 2.2. Analysis of dinoflagellate cysts and grain size

The surface sediment at each site was collected using a box corer (0.05 m<sup>2</sup>) in November 2008. A 0–5 cm sediment sample representing “modern” environmental conditions was carefully taken using a transparent plastic tube with a diameter of 5 cm from the undisturbed part of each core. The samples were preserved in an icebox, and then transported back to the laboratory and kept in the freezer in the darkness to avoid germination. Five grams of the top 5 cm of sediment of each sample were oven-dried at 60 °C for 48 h to calculate the water content, and 5 g of sediment of each sample were used for the analysis of dinoflagellate cysts. The samples were processed following the methods of Matsuoka and Fukuyo (2000). Sediment samples were treated by palynological and sieving procedures. For palynology, sediment samples were treated with HCl (10%) to remove carbonates, and HF (40%) solutions were used to remove silicates. After the chemical treatment, samples were sonicated for 30 s and sieved through 125- and 15-μm-pore-sized meshes to remove sundries (e.g., the sand and the mud). The residue on the 15-μm mesh was suspended in 3 mL of distilled water.

Dinoflagellate cysts were identified based on published descriptions (Matsuoka and Fukuyo, 2000; Fujii and Matsuoka, 2006; Wang, 2007; Matsuoka et al., 2009; Rochon et al., 2009). Specimens were identified to the generic level, if identification to the species level was not possible. For each treated sample, a minimum of 100 dinoflagellate cysts were counted in a tubular plankton chamber

(HYDRO-BIOS, Germany) under an inverted microscope (Olympus IX81) at ×400 magnification. Cyst abundance was calculated as cysts per gram of dry weight sediment (cysts g<sup>−1</sup> DW).

Grain sizes of 22 sediment samples were measured using a Mastersize 2000 Laser Particle Sizer. Before measurements, samples were oxidized by 10% H<sub>2</sub>O<sub>2</sub> to remove organic matter and dispersed in 0.05% (NaPO<sub>3</sub>)<sub>6</sub> to isolate discrete particles. Grain sizes were divided into 3 groups (<4 μm, 4–63 μm and >63 μm) (Folk et al., 1970). The sediment description was based on Folk's triangle classification and its nomenclature (Folk et al., 1970).

### 2.3. Data analysis

Shannon–Wiener index (*H'*) (Shannon and Weaver, 1949) was calculated based on the cyst taxon composition and their abundances.

$$H' = - \sum_{i=1}^S P_i \times \log_2 P_i$$

<i>H'</i>	Shannon–Wiener index	179
<i>S</i>	total number of cyst taxa	180
<i>P<sub>i</sub></i>	the proportion of each cyst taxon in the sample	181

The abundances of three unknown cysts were excluded when calculating the ratio of heterotrophic–autotrophic dinoflagellate cysts (*H:A*), heterotrophic cyst abundance and autotrophic cyst abundance. Based on the data of total cyst abundances including 38 taxa and 22 samples, the similarity was analyzed by software PRIMER 6.0 packages (Clarke and Warwick, 1994), and then a resemblance matrix was created (Clarke and Warwick, 1994). Based on the resemblance matrix, the hierarchical cluster and multidimensional scaling (MDS) were analyzed. The correlation analysis between cyst abundances and environmental factors was determined by SPSS 11.5 (Rosner, 2000).

## 3. Results

### 3.1. Taxonomic composition of dinoflagellate cysts in the surface sediment of SB

A total of 35 dinoflagellate cyst taxa belonging to 15 genera, as well as 3 unknown cyst taxa were identified from the 22 sediment samples (Table 1, Fig. 3 and Appendix A). The thirty-five taxa in this study were classified into four taxonomic groups: gonyaulacoid (14 taxa), calciodinellid (one taxon), gymnodinioid (two taxa) and protoperidinioid (18 taxa). The cyst richness was distributed unevenly at different sites, with a range of 19–28 taxa (Fig. 4a). In contrast, higher cyst richness occurred at the domestic sewage outlet (Zone A) and aquaculture areas (Zone C) (Fig. 4a). The Shannon–Wiener index showed a tendency of Zone C (4.00) > Zone A (3.86) > Zone E (3.82) > Zone D (3.75) and Zone B (3.74), based on the average values (Fig. 4b). There were some higher values (>4.0) that occurred at sites A4, A5, A6, B1, C1 and C2.

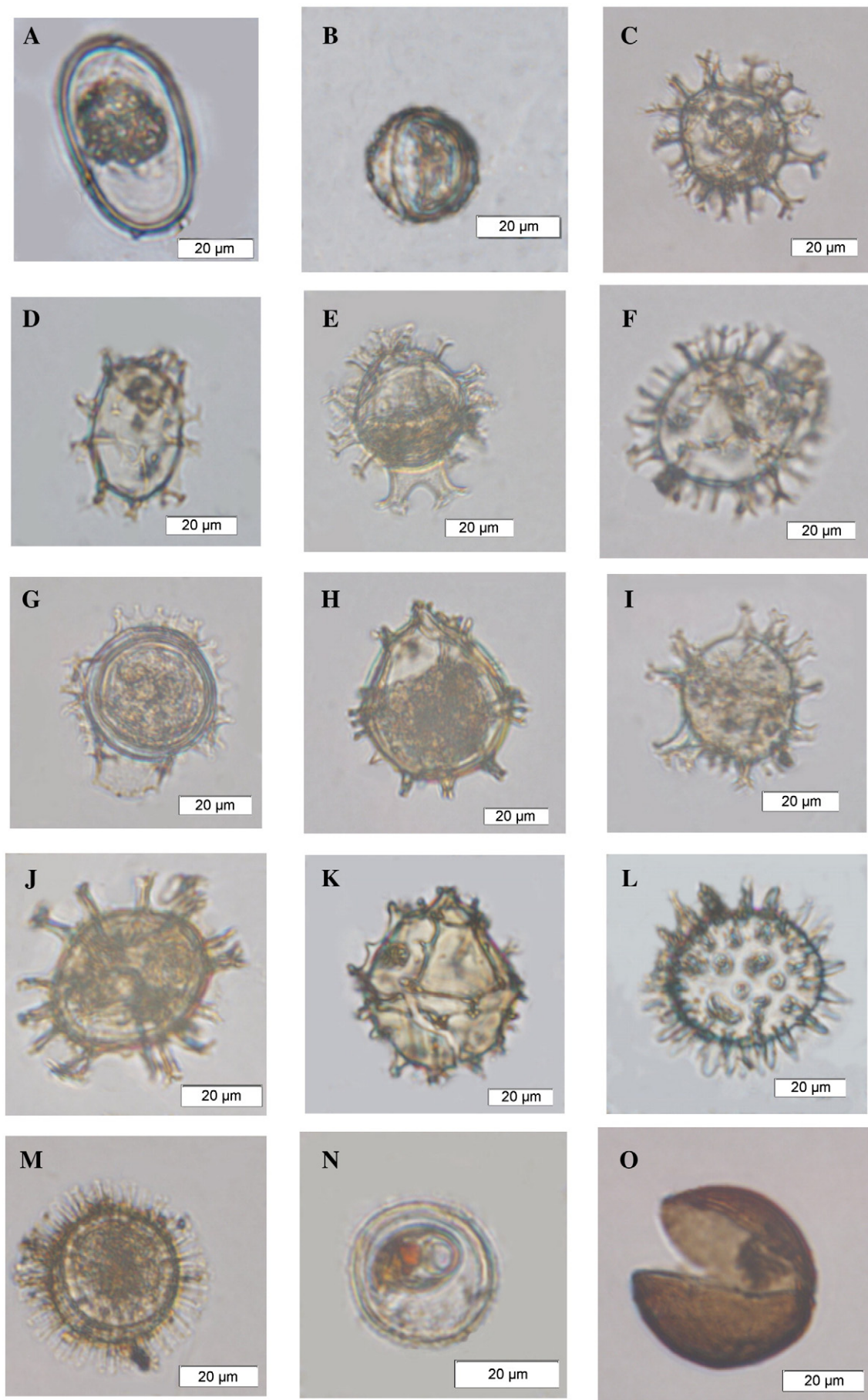
The identified dinoflagellate cysts were divided into sixteen autotrophic and nineteen heterotrophic cysts in accordance with Matsuoka (2001) and Wang (2007) (Table 1). The results showed that autotrophic cysts dominate at most sites (Table 1). *Spiniferites bentori* var. *truncata* was the dominant autotrophic cyst, and *Brigantedinium* sp.1 and *Quinquecupis concreta* were dominant heterotrophic cysts in the sediment samples. Cysts of *Alexandrium minutum*/affine, *Spiniferites ramosus*, *S. bentori* var. *truncata*, *Spiniferites* spp., *Spiniferites* sp. cf. *delicates*, *Operculodinium centrocarpum*, *Protoperidinium* sp.2, unknown cyst 1 and unknown cyst 2, were observed widely in SB (Table 1).

**Table 1**  
Cyst abundance (cysts g<sup>-1</sup> DW), richness, proportion of living cysts (%) and Shannon–Wiener index in the surface sediment of Sishili Bay.

Taxa/site		A0	A1	A2	A3	A4	A5	A6	B0	B1	B2	B3	B4	C0	C1	C2	C3	C4	D1	D2	D3	E1	E2
Biological name	Paleontological name																						
<i>Autotrophs</i>																							
<i>Gonyaulacoid</i>																							
<i>Alexandrium</i>	Cyst of <i>Alexandrium</i>	14	0	10	4	0	13	0	2	3	4	0	6	0	3	10	7	0	9	10	1	1	1
<i>Alexandrium tamarense/catenella</i>	<i>tamarense/catenella</i>																						
<i>Alexandrium minutum/affine</i>	Cyst of <i>Alexandrium</i>	16	3	20	4	2	26	34	26	7	39	4	21	4	29	3	25	31	30	15	0	12	5
<i>Gonyaulax elongata</i>	<i>minutum/affine</i>																						
	<i>minutum/affine</i>	2	3	10	0	2	0	22	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0
<i>Gonyaulax scrippsae</i>	<i>Spiniferites elongatus</i>	4	0	20	7	2	4	17	0	0	0	4	3	6	0	3	4	0	0	5	1	0	0
	<i>bulloideus</i>																						
<i>Gonyaulax spinifera</i>	<i>Spiniferites</i>	51	45	89	66	29	34	22	21	27	25	68	24	48	6	26	47	31	36	88	13	1	0
	<i>hyperacanthus</i>																						
<i>Gonyaulax spinifera</i>	<i>Spiniferites mirabilis</i>	7	0	30	11	6	4	11	1	7	0	4	9	4	3	6	4	9	6	15	10	1	5
<i>Gonyaulax spinifera</i>	<i>Spiniferites ramosus</i>	2	3	49	7	4	22	6	8	13	7	16	15	6	15	10	7	9	3	5	11	3	4
<i>Gonyaulax</i> sp.	<i>Spiniferites bentori</i>	88	122	187	95	46	73	101	69	60	128	116	47	38	94	58	54	18	77	137	25	1	3
	var. <i>truncata</i>																						
<i>Gonyaulax</i> sp.	<i>Spiniferites</i> sp. cf. <i>bentori</i>	12	0	0	11	0	0	11	3	3	0	24	6	4	0	6	0	0	15	0	0	0	0
<i>Gonyaulax</i> sp.	<i>Spiniferites delicatus</i>	2	13	10	15	6	9	6	1	3	0	0	21	0	0	0	7	4	3	10	0	1	0
<i>Gonyaulax</i> sp.	<i>Spiniferites</i> sp. cf. <i>delicatus</i>	23	6	30	26	11	13	0	2	17	11	28	18	13	9	26	18	8	15	15	14	1	1
<i>Gonyaulax</i> spp.	<i>Spiniferites</i> spp.	30	19	148	80	27	43	34	4	23	18	36	6	4	32	19	54	53	15	44	17	4	2
<i>Lingulodinium polyedrum</i>	<i>Lingulodinium</i>	0	0	0	0	0	0	0	2	0	0	0	27	2	0	0	4	0	3	0	0	1	1
<i>Protoceratium reticulatum</i>	<i>machaerophorum</i>																						
	<i>Operculodinium centrocarpum</i>	19	13	187	26	8	34	112	4	23	11	40		21	23	13	69	53	36	113	19	8	11
<i>Calceidiniellid</i>																							
<i>Scripsiella trochoidea</i>		5	0	20	4	0	0	11	8	7	18	12	0	2	3	13	0	9	0	10	0	0	8
<i>Gymnodinioid</i>																							
<i>Gymnodinium catenatum</i>		0	6	0	4	2	0	11	0	0	0	0	0	0	0	3	4	0	0	0	0	0	1



Heterotrophs																							
Gymnodinioid																							
Polykrikos schwartzii		2	3	0	0	0	0	11	0	0	0	0	0	0	3	0	7	0	0	0	0	1	0
Protoperidinioid																							
Protoperidinium	Selenopemphix	4	0	0	4	0	0	6	1	0	0	0	0	0	3	4	0	0	0	4	1	0	
	conicum																						
Protoperidinium	Xandaradinium	0	3	0	0	4	4	0	0	3	0	0	0	2	0	0	0	18	0	0	1	0	0
	divaricatum																						
Protoperidinium		0	0	0	0	0	0	6	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0
	latissimum																						
Protoperidinium	Quinquecuspis	2	0	20	0	2	4	0	0	7	4	4	0	6	0	16	7	18	3	0	0	3	22
	leonis																						
Protoperidinium	concreta	2	0	59	4	17	13	17	1	17	0	4	0	4	6	6	4	0	3	15	0	1	2
	minutum																						
Protoperidinium	Votadinium calvum	5	3	20	7	6	9	6	3	7	0	8	3	2	6	13	4	13	3	10	0	7	1
	oblongum																						
Protoperidinium	Trinovantedinium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	3	0
	capitatum																						
Protoperidinium	Selenopemphix	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0
	nephroides																						
Protoperidinium sp.	Brigantedinium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
	aymmetricum																						
Protoperidinium sp.	Brigantedinium sp.1	7	19	148	18	38	39	28	17	20	21	12	12	6	9	35	7	26	21	25	0	15	24
Protoperidinium sp.	Brigantedinium sp.2	0	6	39	7	6	0	28	10	7	4	4	12	2	3	13	14	0	3	15	4	5	0
Protoperidinium sp.	Lejeunecysta sp.1	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	1
Protoperidinium sp.	Votadinium sp.1	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
Protoperidinium sp.1		7	10	0	4	0	4	17	1	0	0	0	0	0	12	0	18	4	0	5	7	0	0
Protoperidinium sp.2		0	10	69	7	11	30	6	10	17	7	20	9	8	20	16	11	40	6	20	8	7	13
Protoperidinium sp.3		0	0	0	0	2	0	6	0	0	0	4	0	0	3	0	4	0	3	5	3	1	0
Protoperidinium spp.	Brigantedinium spp.	5	6	0	4	2	0	6	7	17	11	4	0	4	6	3	4	4	3	5	0	0	2
Protoperidinium spp.		2	22	39	11	8	22	17	4	7	36	8	0	4	18	3	11	9	3	20	3	3	2
Unknown cysts																							
Unknown cyst 1		16	6	39	15	8	30	17	4	20	21	20	9	8	15	13	33	48	33	29	1	31	11
Unknown cyst 2		9	22	79	15	15	17	6	15	27	32	8	30	15	15	23	22	62	50	25	3	9	12
Unknown cyst 3		0	3	0	4	13	9	0	1	3	0	4	6	0	6	0	18	0	3	0	0	0	7
Sum																							
Total		336	350	1322	457	280	457	573	227	342	395	457	281	223	342	341	470	475	382	637	147	122	141
Autotrophs		276	234	809	358	147	276	399	152	193	260	353	201	156	216	196	304	224	246	466	113	36	44
Heterotrophs		51	90	434	80	105	155	169	59	120	103	92	44	53	105	122	127	189	83	147	32	77	79
Unknown cyst		9	26	79	18	27	26	6	16	30	32	12	35	15	20	23	40	62	53	25	3	9	19
Autotrophic taxon number		14	10	13	14	12	11	14	13	12	10	11	12	13	10	13	13	10	12	12	10	11	11
Heterotrophic taxon number		9	9	7	9	10	8	12	9	9	6	10	4	10	11	9	12	10	10	9	7	11	8
Unidentified species number		2	3	2	3	3	3	2	3	3	2	3	3	2	3	2	3	2	3	2	2	2	3
Total species number		25	22	22	26	25	22	28	25	24	18	24	19	25	24	24	28	22	25	23	19	24	22
Proportion of living cysts		35.26	36.70	52.24	29.60	56.39	57.55	42.16	62.02	61.17	52.25	50.88	52.63	49.06	47.01	60.38	50.00	63.89	50.39	44.62	21.70	86.96	82.17
Shannon–Wiener index		3.71	3.48	3.92	3.76	4.02	4.03	4.07	3.59	4.14	3.39	3.73	3.83	3.91	3.84	4.10	4.15	3.98	3.82	3.72	3.71	3.81	3.82



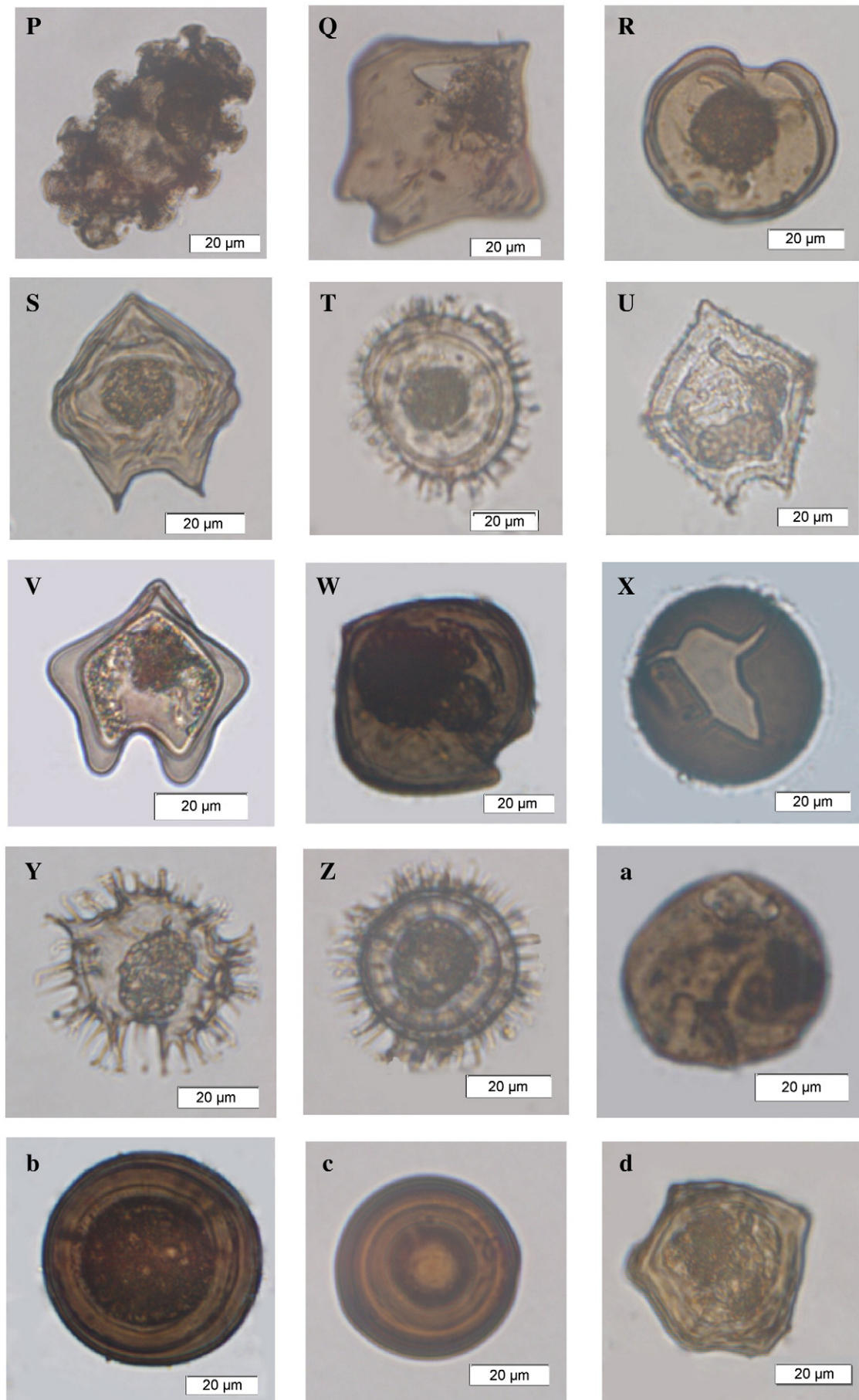
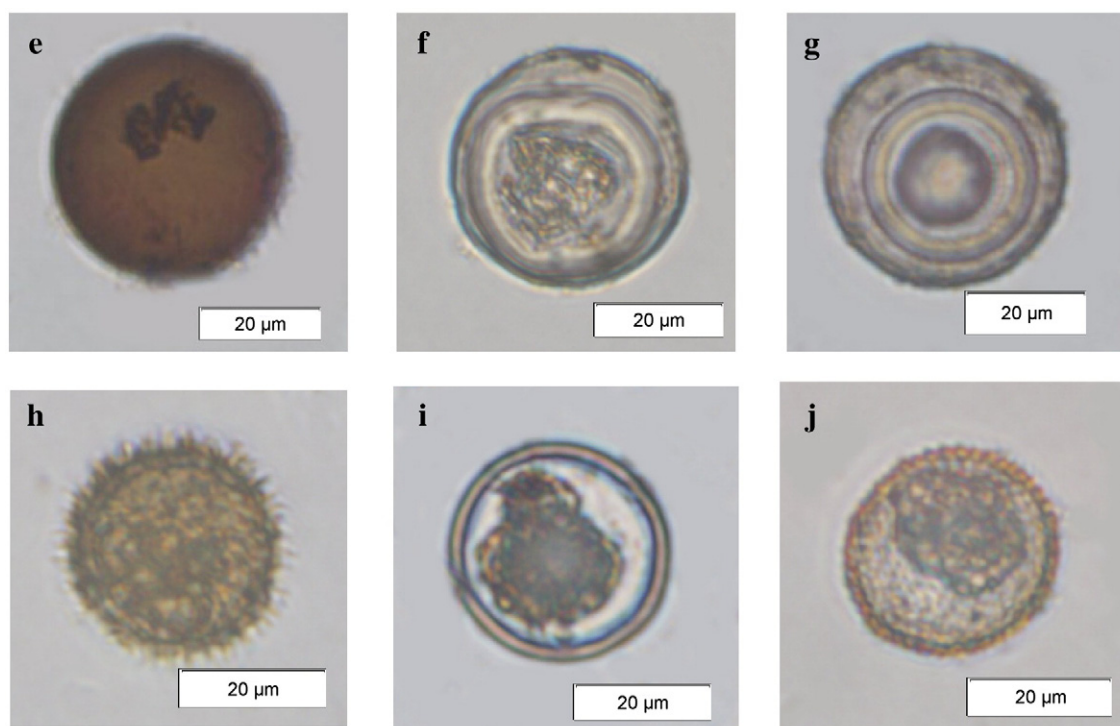


Fig. 3. (continued).





**Fig. 3.** Light micrographs of dinoflagellate cysts identified from the surface sediments of Sishili Bay (A: Cyst of *Alexandrium tamarense/catenella*, B: Cyst of *Alexandrium minutum/affine*, C: *Spiniferites bulloideus*, D: *Spiniferites elongatus*, E: *Spiniferites* sp. cf. *delicatus*, F: *Spiniferites hyperacanthus*, G: *Spiniferites mirabilis*, H: *Spiniferites bentori* var. *truncata*, I: *Spiniferites delicatus*, J: *Spiniferites ramosus*, K: *Spiniferites* sp. cf. *bentori*, L: *Lingulodinium machaerophorum*, M: *Operculodinium centrocarpum*, N: *Scrippsiella trochoidea*, O: *Gymnodinium catenatum*, P: *Polykrikos schwartzii*, Q: *Protoperidinium latissimum*, R: *Selenopemphix nephroides*, S: *Quinquecuspsis concreta*, T: *Selenopemphix quanta*, V: *Trinovantedinium capitatum*, U and W: *Votadinium calvum*, X: *Brigantedinium asymmetricum*, Y: *Xandaradinium xanthum*, Z: *Protoperidinium minutum*, a: *Votadinium* sp.1, b: *Brigantedinium* sp.1, c: *Brigantedinium* sp.2, d: *Lejeunecysta* sp.1, e: *Protoperidinium* sp.1, f: *Protoperidinium* sp.2, g: *Protoperidinium* sp.3, h: Unknown cyst 1, i: Unknown cyst 2, j: Unknown cyst 3).

### 3.2. The abundance of dinoflagellate cysts in the surface sediment of SB

The abundance of dinoflagellate cysts was distributed unevenly in SB and ranged from 122 to 1322 cysts  $g^{-1}$  DW at different sites, with an average of 396 cysts  $g^{-1}$  DW (Fig. 5a); the highest abundance occurred at A2 site with a value of 1322 cysts  $g^{-1}$  DW, and the lowest abundance occurred at E1 site (122 cysts  $g^{-1}$  DW) (Fig. 5a). The average values in five zones displayed a tendency of Zone A (539 cysts  $g^{-1}$  DW) > Zone D (389 cysts  $g^{-1}$  DW) > Zone C (370 cysts  $g^{-1}$  DW) > Zone B (340 cysts  $g^{-1}$  DW) > Zone E (131 cysts  $g^{-1}$  DW) (Fig. 5a). Cyst abundance in Zone A was significantly higher than in the other zones, particularly compared to Zone E that was impacted by industrial pollution. However, the proportion of living cysts displayed an opposite tendency of Zone E (84.6%) > Zone B (55.8%) and Zone C (54.1%) > Zone A (44.3%) > Zone D (38.9%) (Table 1). The characteristics of autotrophic and heterotrophic cyst distribution were analyzed, respectively (Fig. 5b,c). The tendency of autotrophic cysts in five zones displayed Zone A (357 cysts  $g^{-1}$  DW) > Zone D (275 cysts  $g^{-1}$  DW) > Zone B (232 cysts  $g^{-1}$  DW) > Zone C (219 cysts  $g^{-1}$  DW) > Zone E (40 cysts  $g^{-1}$  DW) (Fig. 5b). Obviously, the autotrophic cyst in Zone E was much lower than in the other zones. In contrast, heterotrophic cysts in Zone E did not decrease so dramatically as autotrophic cysts, based on the tendency of Zone A (155 cysts  $g^{-1}$  DW) > Zone C (119 cysts  $g^{-1}$  DW) > Zone D (87 cysts  $g^{-1}$  DW) > Zone B (84 cysts  $g^{-1}$  DW) > Zone E (78 cysts  $g^{-1}$  DW) (Fig. 5c). As a result, the ratio of heterotrophic and autotrophic dinoflagellate cysts (H:A) displayed a tendency of Zone E (1.42) > Zone C (0.44) > Zone A (0.38) > Zone B (0.31) > Zone D (0.24) (Fig. 5d).

### 3.3. The characteristics of dinoflagellate cysts distributed in the surface sediment of SB

Based on the cyst composition and abundance at these sites, a similarity analysis by PRIMER 6.0 was conducted. The result showed that the cysts at twenty-two sites can be classified into four groups (Fig. 6). The cyst assemblages at sites E1 and E2 were significantly different from those of the other twenty sites. The reason was the dominant species shift, with heterotrophic cysts dominating instead of autotrophic cysts at sites E1 and E2 (Fig. 5b). Moreover, the coarse silt sediment at E1 site could have negative effect on the preservation of cysts (Fig. 7). The cyst assemblage at A2 site was separated from other groups due to the highest abundance and diversity. In addition, D3 site was also separated from the main group due to its low abundance and species richness.

### 3.4. Grain size of surface sediment in SB

The grain size was analyzed in order to understand the sediment property and the results were sorted into three groups (<4  $\mu m$ , 4–63  $\mu m$  and >63  $\mu m$ ) based on Folk et al. (1970). According to Udden (1914), Wentworth (1922) and the scope of sediment grain size, the sediment can be regarded as clay (<4  $\mu m$ ), fine silt (4–16  $\mu m$ ), coarse silt (16–63  $\mu m$ ) and sand (>63  $\mu m$ ). Our result showed that the silt dominated in the sediment and the proportion of clay and silt represent more than 60% at most sites except for E1 (Fig. 7). The abundance of total cysts, autotrophic and heterotrophic cysts and H:A ratios were used for correlation analysis with grain size (Table 2). The result showed that total cysts and heterotrophic

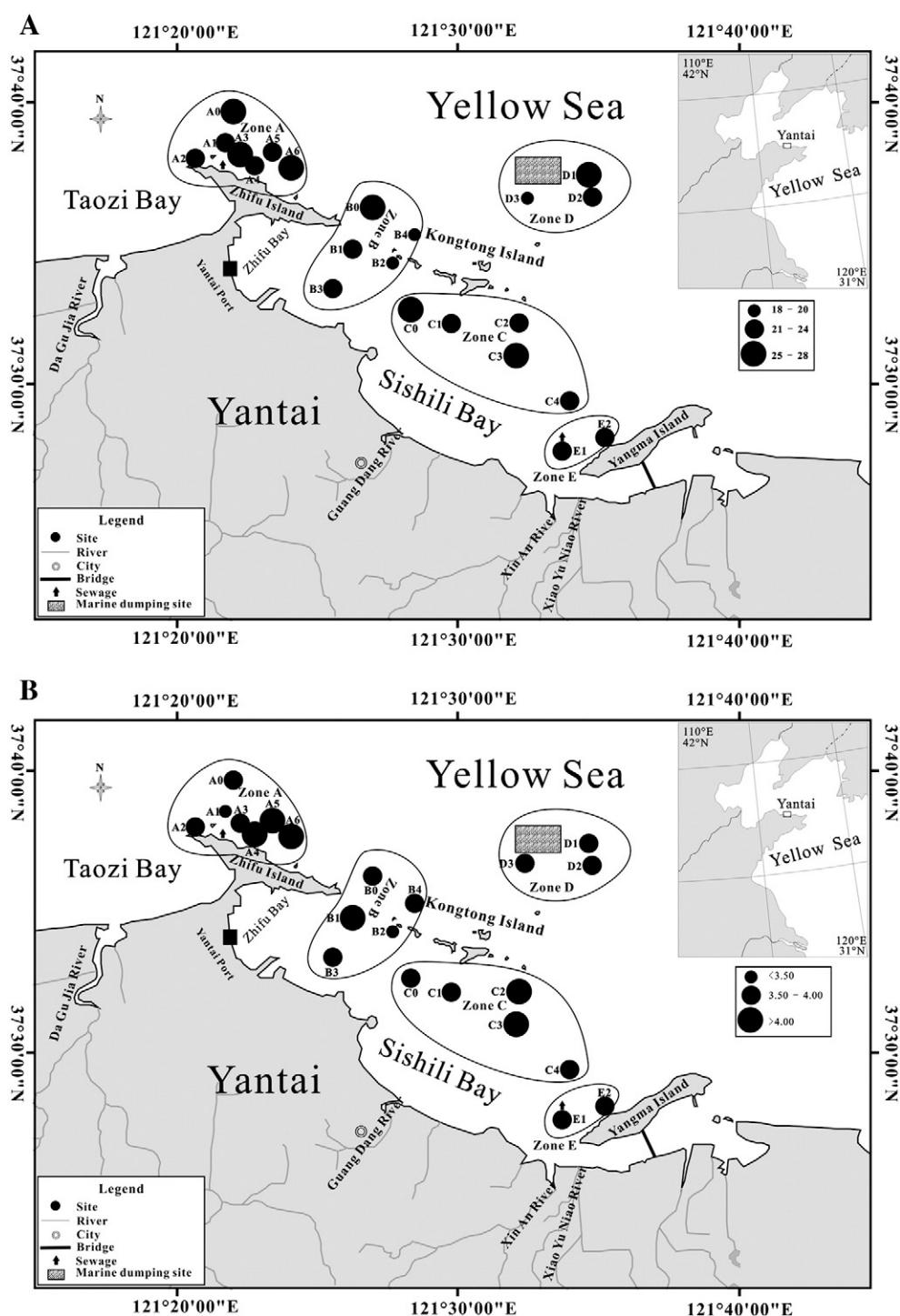


Fig. 4. Spatial distributions of cyst richness (a) and Shannon-Wiener index (b).

cyst abundances were not significantly correlated to the sediment grain size ( $P > 0.05$ ) (Table 2), but autotrophic cyst abundance presented a significant positive relativity to the silt concentration (4–63  $\mu\text{m}$ ) and negative relativity to the sand concentration (>63  $\mu\text{m}$ ) ( $P < 0.05$ ) (Table 2). H:A ratios presented significant negative relativity to the silt concentration (4–63  $\mu\text{m}$ ) and positive relativity to the sand concentration (>63  $\mu\text{m}$ ) ( $P < 0.05$ ) (Table 2).

#### 4. Discussion

Previous studies have found that a clear eutrophic signal can be identified from the cyst assemblages in the sediment, with cyst

abundances gradually increasing with increased nutrients (Persson et al., 2000; Matsuoka, 2001; Pospelova et al., 2005). In this study, the spatial distribution of total cyst abundance showed a significant correlation with the levels of dissolved inorganic nitrogen (DIN) in SB, particularly with autotrophic cysts (Table 3). High nutrient levels were found in Zone A, based on the surveys in SB during 2008–2009 (Y. Wang, personal communication; Bai et al., 2010). The spatial distribution of DIN in seawater was characterized by a tendency of Zone A (13.0  $\mu\text{M}$ ) > Zone B (10.8  $\mu\text{M}$ ) > Zone D (9.7  $\mu\text{M}$ ) > Zone C (9.2  $\mu\text{M}$ ) > Zone E (7.1  $\mu\text{M}$ ), showing the significant impact from the sewage plant near to Zone A. This is quite consistent with the distribution of total cyst abundance in SB (Zone A (539 cysts  $\text{g}^{-1}$

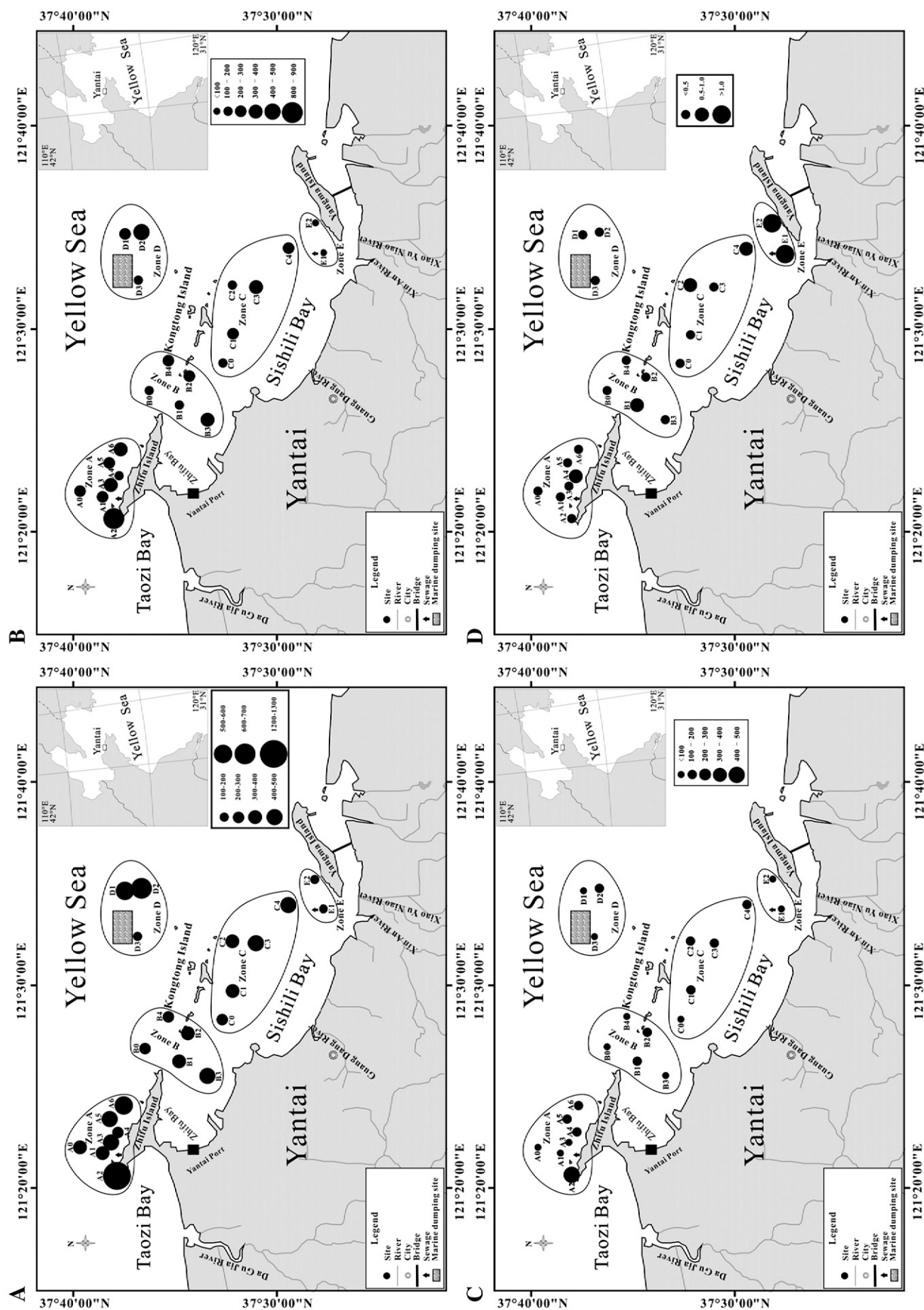


Fig. 5. Spatial distribution of dinoflagellate cyst abundance (a: total dinoflagellate cyst, cysts g<sup>-1</sup> DW; b: heterotrophic dinoflagellate cyst, cysts g<sup>-1</sup> DW; c: autotrophic dinoflagellate cyst, cysts g<sup>-1</sup> DW; d: heterotrophic dinoflagellate cyst; heterotrophic dinoflagellate cyst (H:A)).



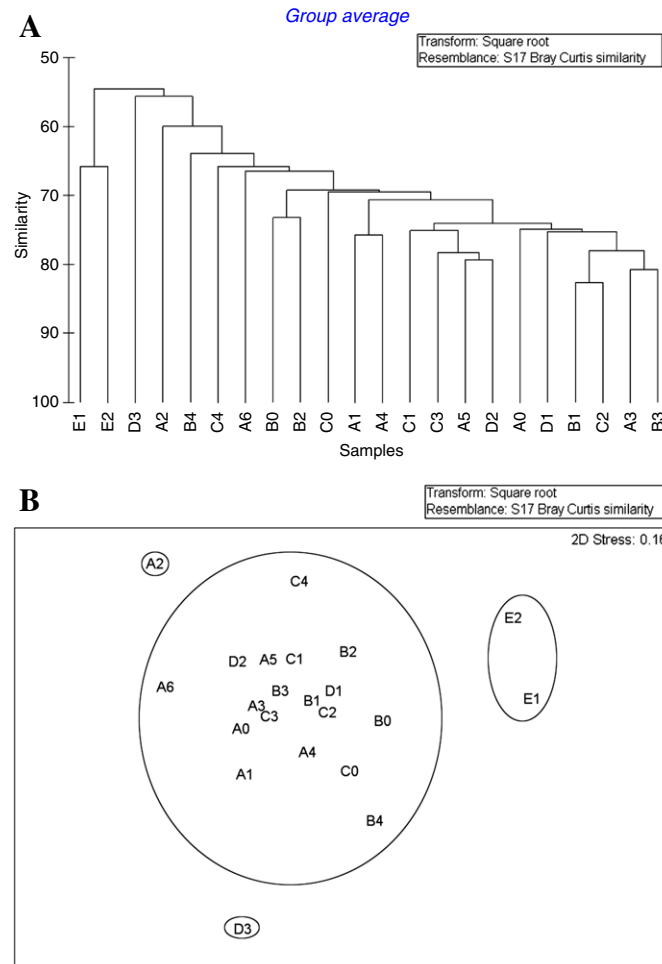


Fig. 6. The similarity analysis for dinoflagellate cysts of twenty-two surface sediment samples in Sishili Bay (a: hierarchical cluster, b: MDS).

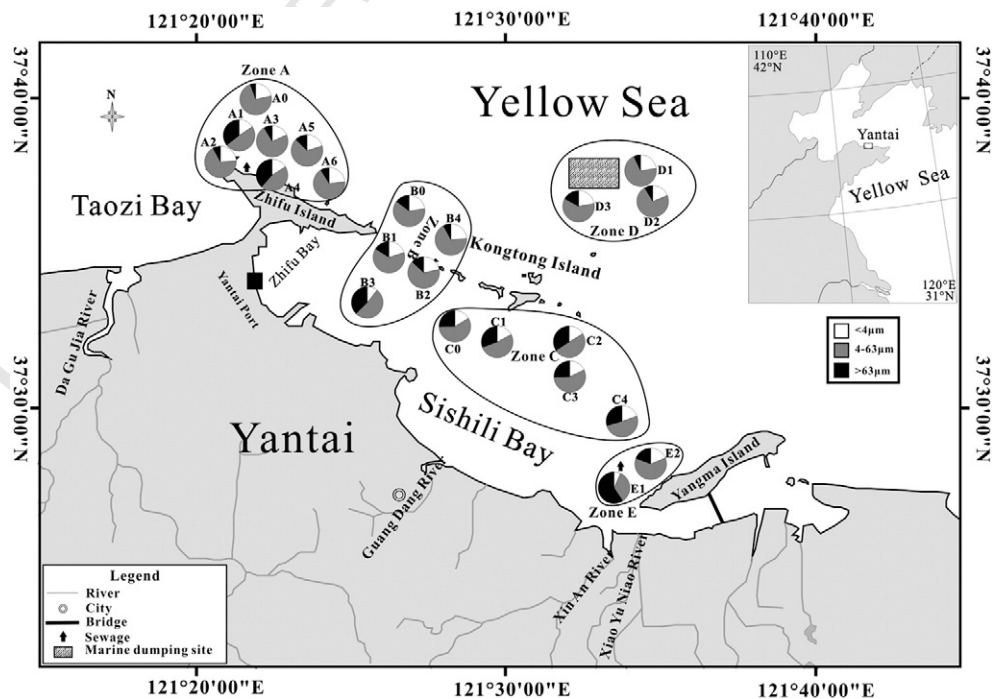


Fig. 7. Spatial distribution of grain size proportion in surface sediment (%).



**Table 2**  
The correlation between the cyst abundance and grain size of sediment.

Items		Grain size		
		<4 $\mu\text{m}$	4–63 $\mu\text{m}$	> 63 $\mu\text{m}$
Total cyst abundance	Pearson correlation	0.296	0.389	−0.378
	N	22	22	22
Autotrophic cyst abundance	Pearson correlation	0.336	0.467*	−0.448*
	N	22	22	22
Heterotrophic cyst abundance	Pearson correlation	0.168	0.187	−0.191**
	N	22	22	22
H:A	Pearson correlation	−0.422	−0.482*	0.487*
	N	22	22	22

\* Correlation is significant at the 0.05 level (2-tailed).

\*\* Correlation is significant at the 0.01 level (2-tailed).

DW) > Zone D (389 cysts  $\text{g}^{-1}$  DW) > Zone C (370 cysts  $\text{g}^{-1}$  DW) > Zone B (340 cysts  $\text{g}^{-1}$  DW) > Zone E (131 cysts  $\text{g}^{-1}$  DW)) (Fig. 5a), and corroborated the significant response of cysts to the increased nutrients as mentioned in previous studies. Moreover, an extremely high cyst abundance was found at A2, which distinguished it from other sites in Zone A using the similarity analyses (Fig. 6). Except for nutrient enrichment, hydrodynamics could also play an important role in determining the level of abundance. Zhang and Dong (1990) found a small anticyclonic eddy around A2, which was caused by tide action and the shallow sea floor. The anticyclonic eddy can confine dinoflagellates to the eddy center (Rodríguez et al., 2003) and more cysts can be collected into the sediment during deposition.

Compared with the other four zones, cyst abundance in Zone E was much lower, particularly for autotrophic cysts (Fig. 5a,b). Zone E is near to the outflow of the Xin An River sewage plant, which was established in 2003 (Jia et al., 2007). This plant was used to treat small amounts of domestic sewage and large industrial wastewater from the neighboring industrial plants. Heavy metals such as mercury, lead and cadmium were reported in Zone E by the local EPA (Wu et al., 2006; Bai et al., 2010), and showed significant pollution from industrial wastewater discharge. Consequently, high concentrations of heavy metals were also found in the sediment (Liu et al., 2011) (Table 3). Thus, Zone E is characterized as an environment with low nutrients and high heavy metals, compared with the other zones. Based on limited case studies to date, industrial pollution is associated with a decrease in cyst abundance or change in the ratio between heterotrophic and autotrophic cysts (Sætre et al., 1997; Matsuoka, 2001; Dale, 2009). Our result supported this hypothesis, with low cyst abundance and high H:A ratios observed in Zone E. Autotrophic cyst abundance decreased more rapidly than heterotrophic cysts in Zone E, indicating a kind of sensitivity to industrial pollution.

Not many studies explored the physiological mechanism of how heavy metals act on cysts, but the existing studies still provided useful information and have contributed to our understanding. The previous

studies can be summarized as follows: 1) by changing the oxidative stress of the cell: Chloroplasts are cell compartments that are highly susceptible to oxidative stress, and pollutant metals accelerate the generation processes of reactive oxygen species in chloroplasts, which exacerbate the oxidative stress in photosynthetic organisms (Pinto et al., 2003). Okamoto and Colepicolo (1998) also pointed out that oxidative stress is an important mediator of metal toxicity in *Protoceratium reticulatum* (formerly *Gonyaulax grindleyi*). 2) Reducing the light-harvesting capacity: Heavy metals can reduce the light-harvesting capacity by decreasing the peridinin levels in *P. reticulatum* (Okamoto et al., 2001). Miao and Wang (2006) also found that Cd toxicity can inhibit the growth and maximal photosynthetic system II quantum yield of the autotrophic dinoflagellate *Prorocentrum minimum*. The above studies pointed to chloroplasts of autotrophic dinoflagellates playing an important role in the regulation of metal toxicity. However, heterotrophic dinoflagellates could be impacted by heavy metals in a different way as they lack chloroplasts and pigments (Gaines and Elbrachter, 1987). This could cause the autotrophic dinoflagellates to decrease much faster than heterotrophic dinoflagellates under the stress of heavy metals and would lead to high H:A ratios. In Zone E, the concentrations of heavy metals in the industrial sewage were higher than those of the other four zones. Due to the toxicity of heavy metals, the abundance of autotrophic dinoflagellates could decrease faster than that of heterotrophic dinoflagellates. Therefore, H:A ratios were higher in Zone E than that of the other four zones. 3) Accelerating cyst formation: Our result showed a higher proportion of living cysts found in Zone E in contrast to other zones, indicating that cyst germination could be difficult under the stress of heavy metals. Lage et al. (1994) and Okamoto et al. (1999) found that dinoflagellate cells (*P. reticulatum*, *Amphidinium carterae* and *Prorocentrum micans*) exposed to metals can promptly undergo encystment, which is an important strategy for surviving metal exposure.

Anderson et al. (2003) found that dinoflagellate cyst abundance was correlated to the grain size of the sediment, and it was easier for silt and clay sediment to collect dinoflagellate cysts in contrast to sandy sediment. Moreover, the fine sediment also indirectly indicates a stable and favorable sedimentation process. The sediment in SB is dominated by silt and clay except for site E1 and showed little impact on the spatial distribution of total cyst abundance, but autotrophic cysts displayed a preference for fine silt (Table 2). Kawamura (2004) also found that autotrophic dinoflagellate cyst abundance, for example of *Spiniferites* species, favored silt (13–18  $\mu\text{m}$ ), but heterotrophic dinoflagellate cysts, for example *Brigantedinium* species, were strongly related to sandy sediment (70  $\mu\text{m}$ ). The property of sediment at site E1 could contribute to the lower levels of autotrophic cysts.

In a conclusion, our case study in SB supported the hypothesis that dinoflagellate cysts could show different responses to nutrient enrichment and industrial pollution (Dale, 2001, 2009). Nutrient enrichment significantly increased the abundance of dinoflagellate cysts in SB, whereas industrial pollution decreased their abundance. Moreover, autotrophic cysts showed more sensitive characteristics to the industrial pollution than heterotrophic cysts. Eddy and sediment grain

**Table 3**  
The environmental factors and their correlation with the cyst abundance.

Items	Cr	Cu	Zn	As	Pb	DIN	DIP
Concentration							
A zone (average)	72.91	31.82	91.61	13.41	32.56	12.97	0.28
B zone (average)	72.4	25.93	81.72	11.61	29.27	10.8	0.25
C zone (average)	68.84	22.4	72.26	9.94	27.87	9.21	0.28
D zone (average)	70.71	22.75	77.32	11.4	26.47	9.7	0.27
E zone (average)	76.57	28.4	91.01	9.64	42.95	7.13	–
Total cyst abundance	–0.565	0.16	–0.11	0.835	–0.661	0.920*	–0.843
Autotrophic cyst abundance	–0.573	0.099	–0.146	0.854	–0.721	0.919*	–0.881*
Heterotrophic cyst abundance	–0.286	0.46	0.176	0.628	–0.197	0.733	–0.443

Data of heavy metals in the sediment from Liu et al. (2011); unit is  $\text{mg kg}^{-1}$ .

Data of nutrients in water column from annual survey of SB (Dr. Y. Wang, personal communication; Bai et al., 2010); unit is  $\mu\text{M}$ .

\* Correlation is significant at the 0.05 level (2-tailed).

size can enhance or weaken the processes of cyst accumulation in the sediment and these can cause problems in clarifying ecological information. The different physiological mechanisms determining how heavy metals affect autotrophic and heterotrophic cysts need to be studied in detail.

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## Appendix A

- Unknown Cyst 1** The round brown cyst has a single layer with many spines (3–8  $\mu\text{m}$  in diameter), and is 25–40  $\mu\text{m}$  in diameter excluding spines. These spines are acuminate in shape, solid and rigid (Fujii and Matsuoka, 2006).
- Unknown Cyst 2** The spherical or ovoid and colorless cysts with one or double membranes are 20–60  $\mu\text{m}$  in diameter with a smooth surface and without ornamentation and chromatophore (Fujii and Matsuoka, 2006).
- Unknown Cyst 3** The ovoid and colorless cysts (30–40  $\mu\text{m}$  in diameter) with a granulated surface are probably *Scrippsiella* cysts, although they do not have any ornamentation due to palynological treatment (Fujii and Matsuoka, 2006).

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