International Conference on Chemical, Environmental and Biological Sciences (CEBS-2015) March 18-19, 2015 Dubai (UAE)

Dinoflagellate Cyst Assemblage and Marine Sediment Characteristics of Colombo Port, Sri Lanka

Dulanjali Wijethilake*[1] and R R M K P Ranatunga[1]

***Abstract*— The present study was to investigate dinoflagellate cyst assemblage in relation to characteristics of sediments in Colombo Port. Sampling was conducted between June and October 2013. Sediment samples collected using small (2cm) and large (18cm) core samplers and analyzed for occurrence of dinoflagellate cysts, particle size and organic content of the sediment. Sea Surface Temperature (SST) and Sea Surface Salinity (SSS) were also measured at each sampling site. Eight dinoflagellate cysts were recorded from the sediment samples. They were *Protoperidinium leonis, Protoperidinium pentagonum, three other Protoperidinium spp., Scrippsiella spp., Spiniferites spp.* and *Polykrikos spp.* The *Scrippsiella spp.* is known to form harmful algal blooms which lead to fish mortality. The mean SST varied between 28–29.5^{0}C and SSS between 15.5–33.5 ppt. Organic content was between 6.63–14.02% and sediment texture ranged from very fine sand to coarse sand. The study revealed that the dinoflagellate cysts were associated with fine sediments and showed inverse relationships with organic content. Port environment and adjacent coastal environments should be closely monitored for non-indigenous species which would otherwise cause deleterious effects on native biota.

***Keywords*—Colombo Port, Dinoflagellate Cysts, Organic Content, Particle Size.

I. Introduction

DINOFLAGELLATES are one of the most influential biological entities in the marine and estuarine systems since they act as primary producers. Most of the planktonic groups such as dinoflagellates, diatoms, copepods, cladocerans and tintinnids include non motile resting cyst or egg in their life cycle. The dinoflagellates are categorized under division Pyrrhophyta and class Dinophyceae. Dinoflagellates are noticeable harmful microalgal group causing red tide and shellfish poisonings [16]. The autotrophic and heterotrophic dinoflagellate cyst distribution correlates well with the marine sediment characteristics [9], [11]. Sediment texture is one of the key feature of marine sediments. Marine sediments are complex mixtures of a number of solid phases. Sediment may be loose sand, clay and silt that deposit at the bottom of water. Sediment particles of 13-18µm size range dominated by *Spiniferites spp.* cysts and in contrast fine grained sediment mostly occupied by Protoperidiniods [8]. The use of sediments properties and oceanographic data can determine cyst distribution in surface sediments [2]. The other phenomenon is that there are inverse relationship between particle size and organic content. Fine grained sediments are richer in organic content than coarse grained sediments [3]. General objective of the study is to identify dinoflagellate cysts and to analysis sediment in Colombo port. Specific objective of the study is to identify distribution and possible areas where dinoflagellate cysts can aggregate and find any relationship between sediment characteristics and dinoflagellate species availability.

Dulanjali Wijethilake[1]. University of Sri Jayewardenepura, Gangodawila, Nugegoda, 10250, Sri Lanka. (0094711327051, dulanjali1014@gmail.com).
R R M K P Ranatunga[1]. Department of Zoology, University of Sri Jayewardenepura, Gangodawila, Nugegoda, 10250, Sri Lanka. (0094714463970, kamal.ranatunga@gmail.com).

II. Materials And Methods

Sampling was conducted between June 2013 and October 2013 at monthly intervals. Sediment samples were collected using small (d=2cm) and large (d=18cm) core samplers with the assistance from divers. Samples were collected at day time from 9.00am to 3.00pm. Ten sites were selected (Table 1)for collection of sediment samples representing less disturbed isolated areas since dinoflagellate cysts prefer to inhabit in undisturbed and slow water moving regions over a long time. Standard methods were used when collecting sediment samples for both biological and physico-chemical analysis. Small core samples were treated according to a standard protocol: 5.0g was weighed, treated with HF (40%) to dissolve silicates and HCL (10%) to remove carbonates and silicoflourides. The residues were rinsed with distilled water, sonicated for about 30s and sieve through two stainless steel screens (90µm and 20µm). Residues on the 20µm mesh were turned in to aliquots by adding pure water. Dinoflagellate cysts were identified to the nearest possible taxon using published literature and expert assistance was sought for confirmation.

SST and SSS were measured using calibrated standard meters. Soil pH, particle size and organic content analysis were carried out in the laboratory. For soil pH, 10.0g of collected sediment were mixed with 25ml of distilled water in 50ml beaker and stirred well at regularly intervals for 20 minutes and left to settle. Then soil pH was directly read using digital pH meter. To measure particle size determination, sediment samples were air dried, weight of empty sieve was measured prior to the sieving, 50.0g of air dried samples were sieved through the sieve set (4mm, 2mm, 1.7mm, 500µm, 125µm, 90µm, 63µm, 45µm). Then weight of each sieve was recorded. The proportion of each sieve fraction was calculated as percentage of the total dry sample. The classification system used to distinguish sediment texture accordance to the Wentworth scale.

Total dry weight = ∑ Weight of each sieve fraction

$$\text{Percentage of each fraction} = \frac{\text{Dry weight of each component}}{\text{Total dry weight}} \times 100\%$$

To measure organic content, approximately 25g of unseived sediment samples were air dried for 24 hours. Air dried samples were then dried in an oven at 80^{0}C for 48 to 96 hours. Then 10.0g of oven dried samples were transferred in to crucibles and then ashed in the muffle furnace at 480^{0}C. Then crucibles were transferred in to desiccating chamber and allowed to cool for 1 hour prior to being reweighted.

$$\text{Organic content (\%)} = \frac{\text{Net dry weight} - \text{Net ash dry weight}}{\text{Initial dry weight}} \times 100\%$$

TABLE I
SELECTED SAMPLING SITES

Site No.	Sampling site
1	Prince Vijaya Quay (PVQ)
2	South Jetty (SJ)
3	Dock No 1 (DN1)
4	New Dock Pier (NDP)
5	New Dock Pier Entrance (NDPE)
6	Jaya Container Terminals – Extension North (JCTEN)
7	China Pier Cross Berth (CPCB)
8	Canal Berth 1 (CB 1)
9	Canal Berth 2 (CB 2)
10	Passenger Jetty (PJ)

III. RESULTS

Eight species of dinoflagellate cysts were detected from sediment samples. They were *Protoperidinium leonis, Protoperidinium pentagonum, Protoperidinium spp.* 1, *Protoperidinium spp.* 2, *Protoperidinium spp.* 3, *Scrippsiella spp., Spiniferites spp.* and *Polykrikos spp.* According to table 2 the most of dinoflagellate cyst species are belong to genus Protoperidinium. This genus disperses in every location and usually common in port sediment where as not confined to one area. The *Polykrikos spp.* found only in the site 4 located in the Colombo dockyard. It is a very rare genus in port sediments and has identical characteristics for identification (Fig. 2). Most of the Lowest numbers of species were present in site 2. The *Scrippsiella spp.* is known to form harmful algal blooms which lead to fish mortality. The mean SST varied between 28–29.5^{0}C and SSS between 15.5–33.5 ppt. Organic content was between 6.63–14.02% and sediment texture ranged from very fine sand to coarse sand (Table 3).

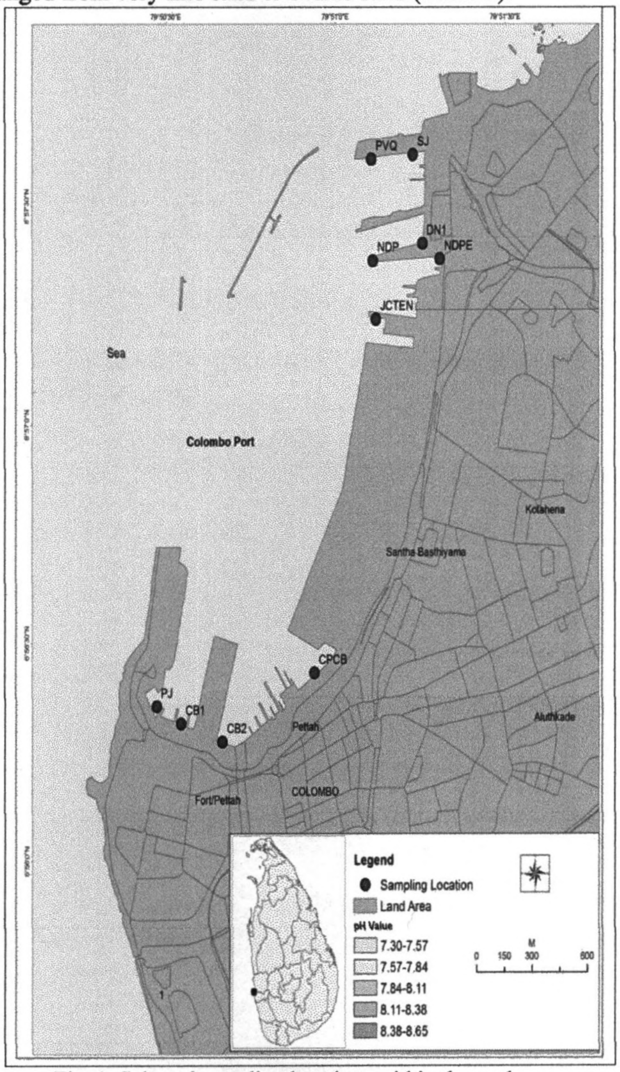

Fig. 1. Selected sampling locations within the study area

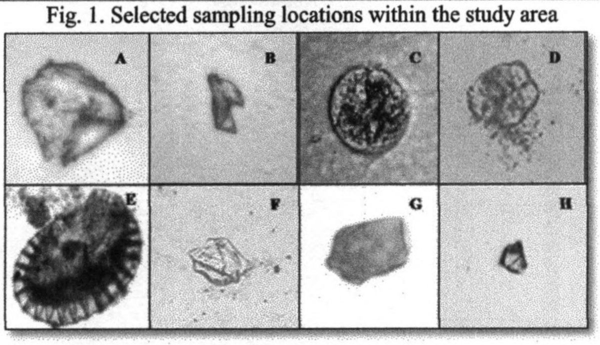

Fig. 2. Dinoflagellate cyst species recorded in Colombo port A - *Protoperidinium spp.* 1 (10×10) B - *Protoperidinium leonis* (10×10) C - *Scrippsiella spp.* (10×40) D - *Spiniferites spp.* (10×10) E - *Polykrikos sp.* (10×10) F - *Protoperidinium spp.2* (10×10) G - *Protoperidinium pentagonum* (10×10) H - *Protoperidinium spp.*3 (10×10).

IV. Discussion

A. Biological Parameters

In present study, eight species of dinoflagellate cysts were identified from the collected sediments samples from Colombo port. They belong to *Protoperidinium spp., Scrippsiella spp., Spiniferites spp.* and *Polykrikos spp.* The cyst assemblage also elicits some significant differences within selected sites. Identified cysts belong to both autotrophic and heterotrophic species. With reference of the autotrophs, *Spiniferites spp.* were prominent in few sites whilst heterotrophs composed of *Protoperidinium spp.* predominant in most of the study area. Thus heterotrophs comprised of 75% of the total identified dinoflagellate species. Rare species named as *Polykrikos spp.* were belong to heterotrophs [13] was found only in the site 4 (NDP) which is close to Colombo dockyard. The cysts of *Protoperidinium spp.* were largely distributed among all the sites of Colombo port. Five species of adult *Protoperidinium spp.* in Colombo port [4]. The *Protoperidinium leonis* recorded in this study and present study where as *Protoperidinium pentagonum* not reported in previous work. Other heterotrophs species was identified in the current study as *Polykrikos spp.* which was also recorded in the study [4]. No past literature was found in the field of dinoflagellate cyst identification in Sri Lanka. However results of present study tally with other foreign studies. The previous study had reported that *P. leonis, P. pentagonum and Polykrikos spp.* in sediments from the West coast of India [6]. These species were similar to the present study. This study was the first record of dinoflagellate cysts from the West coast of India while inimitable tropical environment govern by monsoon. This study adds up valid to present study by reason of both regions are in the same geographical area and obtained results from present study was more or less similar to study carried out by [6]. The *Polykrikos spp.* were found in very lower abundance in few sites nevertheless *Protoperidinium spp.* found in all sites at high cyst abundance was observed in this study with same to present study. The cysts of *Scrippsiella spp.* were also recorded [6], [11] and which responsible for commonly occurring harmful algal blooms which can lead to fish mortality. The cysts of *Scrippsiella spp.* were also found in present work and it is the best warning signal for harmful algal blooms monitoring in Colombo port. This finding indicates *Scrippsiella spp.* might be living in other areas other than sampling sites. The records of nuisance cysts provide a baseline data for future studies on potential harmful dinoflagellates. Present data are noteworthy for the management of bio invasion. Unidentified species were also recorded and it might be another *Protoperidinium spp.* due to shape it possesses. Although further studies are need to clarify this. Another most notable relationship had stated as dinoflagellate cysts are mostly found in areas with low organic matter [2]. This statement clearly proved by the current study due to lowest organic content was recorded in the site 8 at the same time highest number of total dinoflagellate species were recorded in there. Site 4 (NDP) had relatively low organic content and the total number of dinoflagellate species were recorded relatively high in there. Strikingly inverse relationship was also found in between both organic content and species richness [12]. Absolutely contrast finding was reported in the study [11], where the highest cyst abundance was associated with sediments with high organic content (r=0.86) from Red Sea. It might be due to cyst abundance depends on another factors rather than organic content. Several studies were stated that autotrophic dinoflagellates noticeably decreased in polluted areas. In fact Industrial pollution decrease dinoflagellate cyst abundance [9]. Site 7 connects with water source from fish markets in Pettah region while site 8 receiving water from latrine outlet. These sites might contain nutrient rich waters above the surface sediments. Freshwater inflow from inland areas partly responsible for nutrient loading, specially Nitrogen into ocean [14]. River discharge is a dominant influence on the nutrient budget of the sea [5]. The high abundance of heterotrophic dinoflagellate cysts are presence in nutrient rich conditions [6]. The *Protoperidinium spp.* were mostly presents in the site 7 and 8 and corroborate with the above statement. Increase in cysts of heterotrophic species in Yokohama port in Japan with the existing sewage and industrial pollution conditions [10]. This was might be due to increase diatoms under nutrient enriched conditions. Hence, the prey species were increased. Thus cysts of heterotrophic species become increase. Elevated levels of heterotrophic dinoflagellates (*Protoperidinium spp.*) are significant in trophic dynamics [6]. Nutrient enrichment notably increased the dinoflagellate cyst abundance in Yellow Sea, China [9]. The high abundance of cysts of heterotrophic dinoflagellate species were also recorded in Southern Chile [1]. Sites 1, 4, 7 and 8 contain very fine sand (63-125μm) texture and high total numbers of species were recorded in those sites. Reason for this observation might be due to the depositional behavior of dinoflagellate cysts prefer to the fine particles with high surface area. Total cyst and heterotrophic cyst abundance was not correlated ($p>0.05$) with sediment grain size [9]. Moreover, this study was reported autotrophic cyst abundance positively correlates with silt concentration meanwhile negatively correlate with sand concentration. In current study all are in >63μm size in diameter and identification of relationships are not possible statistically. Furthermore, the fine sediments also indirectly elaborate stable sedimentation process [9]. Highest cyst abundance associated with high silt and clay contents whereas inverse correlation with sand content was also observed in the study [11]. Cysts of *Spiniferites spp.* generally prefer sediments with fine to medium silt (13-18μm) [8] while observation contrast with current study. Environmental parameters such as SST, SSS and nutrient levels related to formation of dinoflagellate cysts and dinoflagellate productivity [14]. Several studies concern main environmental factors such as SST and SSS. Although highlighted the importance of nutrient enrichment for dinoflagellate cyst assemblage rather than SST and SSS [15]. In country like Sri Lanka, the SST varies within narrow range throughout the year. In faith dinoflagellate cyst assemblages controlled by other factors rather than SST. Current study proves this hypothesis due to mean ambient SST more or less similar among sites although numbers of total species vary highly within the sites. Nevertheless salinity is also another well known determinant factor in cyst distribution patterns [7]. Site 7 had the lowest value of SSS due to freshwater source reaching the site where as at the same time there were no considerable different in number of cyst species with other sites. Therefore there was no evidence for relationship between dinoflagellate cyst distribution and SSS in present study. Less saline and more turbid water favour the formation of *Spiniferites* species [13]. This observation tally with present study due to the fact that site 7 has low SSS and present *Spiniferites spp.* The soil pH did not correlate with dinoflagellate cyst distribution because no variation was observed within sampling sites. The major changes of dinoflagellate cysts are coincident with the other factors such as increase of organic carbon input, PAHs, PCBs and heavy metal concentration that may have cumulative effects [12]. On other hand, composition of dinoflagellate cysts from the marine sediments of Sri Lankan coasts can be compared to these records of Indian, Korean and Chinese studies [6], [14], [16] respectively. As there are no previous records of recent dinoflagellate cysts from the Sri Lankan coastal sediments, therefore comparison with nearby localities is not possible.

TABLE II
DINOFLAGELLATE SPECIES RECORDED IN COLOMBO PORT

Dinoflagellate species	1	2	3	4	5	6	7	8	9	10
Protoperidinium leonis	+	-	-	+	+	-	+	+	+	-
Protoperidinium pentagonum	-	-	+	-	+	+	+	+	+	-
Protoperidinium spp. 1	-	-	-	-	+	-	+	+	+	+
Protoperidinium spp. 2	-	+	-	-	-	+	-	+	-	-
Protoperidinium spp. 3	-	-	-	+	-	+	-	-	+	+
Scrippsiella spp.	+	-	+	+	-	-	-	+	-	+
Spiniferites spp.	+	-	-	+	-	-	+	+	-	-
Polykrikos sp.	-	-	-	+	-	-	-	-	-	-
Unidentified sp. 1	+	-	+	-	-	+	-	-	-	-
Total Number of Species	4	1	3	5	3	4	4	6	4	3

TABLE III
MEAN AMBIENT SST, SSS, SOIL PH, PARTCLE SIZE AND ORGANIC CONTENT OF SAMPLING SITES IN COLOMBO PORT

Parameter	1	2	3	4	5	6	7	8	9	10
SST (^{0}C)	28.5	29.0	28.0	29.0	28.5	29.0	29.5	29.0	29.0	28.5
SSS (ppt)	31.5	29.0	30.5	31.5	33.5	31.5	15.5	31.5	30.5	31.0
Soil pH	8.09	8.63	8.06	8.16	7.53	7.70	7.33	7.86	7.91	8.02
Partcle size	VFS	MS	CS	VFS	VFS	MS	VFS	VFS	FS	VFS
Organic contenet (%)	12.37	13.64	10.59	9.74	12.66	12.35	13.57	6.63	14.02	9.65

*VFS - Very Fine Sand, MS – Medium Sand, CS – Coarse Sand

B. Physical parameters

In addition organic content values undoubtedly relate to site specific activities. The highest value was recorded in site 9. This site receive latrine canal from other areas. The lowest value was obtained from the site 10 due to no local source found for elevated organic content. Organic content varies from 0.3 to 9.0% in sediments from Red Sea [11] and it indicates that Colombo Port sediments highly polluted with organic matter. Furthermore, soil pH is more or less same among all sites and showed alkaline conditions. Other published studies not aware about soil pH. It might be that soil pH does not an important factor for cyst distribution or other factors.

V. CONCLUSION

The dinoflagellate cysts show a relationship with fine sediments and an inverse relationship with organic content. Nevertheless heterotrophic dinoflagellates are most favorable for conditions like nutrient enriched environment. No high influence on dinoflagellate cyst distribution by conditions such as SST, SSS and soil pH although nutrient enrichment plays a major role.

VI. Recommendations

Additional dinoflagellate cyst surveys should be implemented in other areas of Sri Lankan coasts where it has not yet investigated. Sri Lankan baseline data are in lower level when compared with India. The monitoring and management programs should be carried out to detect formation of HABs in island wide coastal belt. Management of ocean and coastal system is not easy matter because pollutants enter from multiple sources. It is necessary to have through waste management programmes as a strategy to minimize marine pollution by reason of prevention is better than cure. The government agencies have a moral, legal and economic responsibility to give permissions and to prepare standard levels for wastes discharged into the ocean. There are strict regulations for discharge waste water in Australian and in New Zealand coastal waters. The results of this study emphasize the importance of proactive measurements to manage and control pollution in coastal waters.

References

[1] Alves-de-Souza, C., Varela, D., Navarrete, F., Fernandez, P., Leal, P.: Distribution, abundance and diversity of modern dinoflagellate cyst assemblages from Southern Chile. J. Botanica Marina. 51, 399-410 (2008) http://dx.doi.org/10.1515/BOT.2008.052

[2] Azanza, R. V., Siringan, F. P., Diego-Mcglone, M. L. S., Yniguez, A. T., Macalalad, N. H., Zamora, P. B., Agustin, M. B., Matsuoka, K. : Horizontal dinoflagellate cyst distribution, sediment characteristics and benthic flux in Manila Bay, Philippines. J. Phycological Research, 52, 376-386 (2004) http://dx.doi.org/10.1111/j.1440-1835.2004.tb00346.x

[3] Cammen, L. M.: Effect of particle size on organic content and microbial abundance within four marine sediments. J. Marine Ecology Progress Series, 9, 273-280 (1982) http://dx.doi.org/10.3354/meps009273

[4] Chandrasekara, W. U., Fernando, M. A. S. T.: Accidental introduction of alien plankton into the Sri Lankan coastal zone through ballast water of cargo ships. J. Aquatic Sciencev, 14, 84-103 (2009)

[5] Chen, L., Zonneveld, K. A. F., Versteegh, G. J. M.: Paleoclimate of the Southern Adriatic Sea region during the 'Medieval Climate Anomaly' reflected by organic walled dinoflagellate cysts. J. The Holocene, 23(5), 645-655 (2013) http://dx.doi.org/10.1177/0959683612467482

[6] D'Silva, M. S., Anil, A. C., Borole, D. V., Nath, B. N., Singhal, R. K.: Tracking the history of dinoflagellate cyst assemblages in sediments from the west coast of India. J. Sea Research, 73, 86-100 (2012) http://dx.doi.org/10.1016/j.seares.2012.06.013

[7] Harland, R., Nordberg, K., Filipsson, H. L.: A major change in the dinoflagellate cyst flora of Gullmar Fjord, Sweden, at around 1969/1970 and its possible (2010)

[8] Kawamura, H. (2004). Dinoflagellate cyst distribution along a shelf to slope transect of an Oligotrophic tropical sea, Sunda shelf, South China Sea. J. Phycological Research, 52, 355-375 (2004) http://dx.doi.org/10.1111/j.1440-1835.2004.tb00345.x

[9] Liu, D., Shi, Y., Di, B., Sun, Q., Wang, Y., Dong, Z., Shao, H.: The impact of different pollution sources on modern dinoflagellate cysts in Sishili Bay, Yellow Sea, China. J. Marine Micropaleontology, 84-85, 1-13 (2012) http://dx.doi.org/10.1016/j.marmicro.2011.11.001

[10] Matsuoka, K.: Eutrophication process recorded in dinoflagellate cyst assemblages - a case of Yokohama Port, Tokyo Bay, Japan. J. Science total Environment, 231(1), 17-35 (1999) http://dx.doi.org/10.1016/S0048-9697(99)00087-X

[11] Mohamed, Z. A., Al-Shehri, A. M.: Occurrence and germination of dinoflagellate cysts in surface sediments from the Red Sea off the coasts of Saudi Arabia. J. Oceanologia, 53(1), 121-136 (2011) http://dx.doi.org/10.5697/oc.53-1.121

[12] Pospelova, V., Chmura, G. L., Boothman, W. S., Latimer, J. S.: Dinoflagellate cyst records and human disturbance in two neighboring estuaries, New Bedford Harbor and Apponagansett Bay, Massachusetts, USA. J. Science of the Total Environment, 298, 81–102 (2002) http://dx.doi.org/10.1016/S0048-9697(02)00195-X

[13] Price, A. M., Pospelova, V.: High-resolution sediment trap study of organic-walled dinoflagellate cyst production and biogenic silica flux in Saanich Inlet, BC, Canada. J. Marine Micropaleontology, 80, 18-43 (2011) http://dx.doi.org/10.1016/j.marmicro.2011.03.003

[14] Shin, H. H., Matsuoka, K., Yoon, Y. H., Kim, Y. O.: Response of dinoflagellate cyst assemblages to salinity changes in Yeoja Bay, Korea. J. Marine Micropaleontology, 77, 15-24 (2010) http://dx.doi.org/10.1016/j.marmicro.2010.07.001

[15] Verleye, T. J., Louwye, S.: Recent geographical distribution of organic-walled dinoflagellate cysts in the southeast Pacific and their relation to the prevailing hydrographical conditions. J. Palaeogeography, Palaeoclimatology, Palaeoecology, 298, 319-340 (2010) http://dx.doi.org/10.1016/j.palaeo.2010.10.006

[16] Wang, Z., Matsuoka, K., Qi, Y., Chen, J., Lu, S.: Dinoflagellate cyst records in recent sediments from Daya Bay, South China Sea. J. Phycological Research, 52, 396-407 (2004) http://dx.doi.org/10.1111/j.1440-1835.2004.tb00348.x.