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Toxic marine diatoms of Hong Kong: A paleoecological study

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Abstract——To determine whether toxic diatoms in Hong Kong's Victoria Harbour have become more common during the last four decades, a gravity core was taken from the anaerobic sediments of Kowloon Bay in the harbour.

Analysis of these sediments from a depth of 0—1 cm, 4—5 cm and 9—10 cm indicated that 6.4%, 2.4% and 1.5% of the diatoms observed at each of these three respective depths belonged to the genus *Pseudonitzschia*.

The abundance of *Pseudonitzschia* frustules in the surface sediments of Victoria Harbour was significantly higher ($p<0.05$) than it was in the harbour's deeper and older (ca. 1965) sediments. The increase in potentially toxic diatom species over the last 30 years was attributed to a variety of factors including the addition of large amounts of nutrients to Hong Kong's coastal waters as a result of man's activities.

Keywords: paleoecology; sediment cores; marine diatoms; toxic diatoms; *Pseudonitzschia*.

1 Introduction

Marine algal blooms (referred to hereafter as red tides) often begin in late February in Hong Kong and increase in frequency and intensity in March, April and May. In June and July they begin to decline and are relatively rare thereafter until the following March (Ho, 1991). In 1992, two deaths and several severe illnesses were associated with the consumption of shellfish from Hong Kong's Dapeng Bay (also referred to as Mirs Bay, Fig. 1, EPD, 1994).

The genus *Pseudonitzschia* contains nearly 20 species (Hasle, 1964). At present, domoic acid has been confirmed for only three of these species: *Pseudonitzschia multiseries* Hasle (Formerly *Pseudonitzschia pungens* (Grun.) Hasle f. *multiseries* (Hasle) Hasle), *Pseudonitzschia australis* Frenguelli and *Pseudonitzschia Pseudodelicatissima* (Hasle) Hasle (Martin, 1990).

Domoic acid was first isolated from the marine red alga *Chondria armata* (Kutz.) in Okamura, Japan (Takemoto, 1958). It is a glutamate analog that binds to glutamate receptors in the brain where it causes nerve cells to continuously transmit impulses. The excessive excitation of these cells ultimately leads to their death (Tasker, 1991). Domoic acid is a water so-

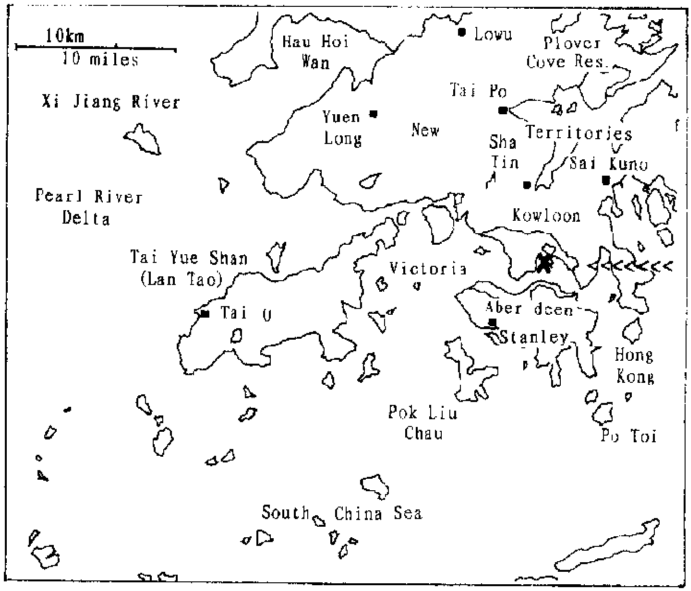

Fig. 1 The "*X*" at the end of the long arrow below the word Kowloon marks the sediment core sample site in Victoria Harbour. The point of land just above the "*X*" is the Kai Tak airport runway into Kowlcon Bay

luble amino acid, which is heat stable and therefore is not destroyed in the process of cooking (Villac, 1993).

Diatom produced domoic acid was also found in razor clams (*Siliqua patula* Dixin) on the pacific coast of Washington in the United States in late October, 1991 at levels up to 154 mg. g^{-1} wet weight and at lower levels in Dungeness crabs (*Cancer magister* Dana, Horner, 1993). From October 1991 to May 1992 (seven months) the level of domoic acid in the razor clam muscle tissue remained above 20 $\mu g \cdot g^{-1}$ wet weight and as a result the clams were not harvested from human consumption. This gave rise to speculation that domoic acid clearance rates in razor clams was quite low since the *Pseudonitzschia* diatom bloom lasted less than one month (Villac, 1993).

In the fall of 1987, more than 100 people became ill and three died after eating cultivated mussels from Prince Edward Island, eastern Canada (Todd, 1990). This was the first time that a diatom was implicated in shellfish poisoning. The U.S. Federal Drug Administration recommends closing a shellfish industry if domoic acid levels exceed 20 $\mu g \cdot g^{-1}$ wet weight. Closure criteria are based, in part, on Canadian studies of toxicity from mussels taken from Prince Edward Island.

According to J. C. Smith, "phytoplankton monitoring has proven to be an effective and highly economical strategy in detecting domoic acid events" (Smith, 1993). An example of the efficacy of a diatom monitoring program occurred in London Bay, Prince Edward Island, Canada, where a toxic bloom of diatoms was reported and shellfish producers and consumers were warned of this fact, thereby averting a possible re-occurrence of the tragic events of

1987 in Prince Edward Island that resulted in the *Pseudonitzschia* related deaths of 3 people.

2 Methods

A single sediment core was taken on 17 October, 1995 from Kowloon Bay at a site approximately 20 m west of the mooring buoy #38 which is located adjacent to the Kai Tak airport runway("*X*" on Fig. 1). A Kajak-Brinkhurst gravity coring device with a core liner having an internal diameter of 7.5 cm was used to take the core. The water depth at this site was 12m. Sediments were anaerobic as indicated by the presence of iron sulphide and there was a smell of hydrogen sulphide over the entire length of the 33 cm long core. No invertebrate life was observed in the core sections.

2.1 Core sectioning and slide preparation

In the lab, the sea water above the core's mud-water interface was removed with a U-shaped siphon. In this manner, almost all of the sea water was siphoned away without disturbing the flocculant surface sediments. The sediment core was held in place on a stand in such a way that the top of the perspex core liner fitted snugly into a metal sectioning plate. The sediment inside the core was then pushed up until the sediment's surface layer reached the same height as the top of the metal sectioning plate. A 1 cm high circular perspex collar, which had the same diameter as the corer, was placed on top of the metal sectioning plate so that the sediment could be extruded into it. A flat stainless steel blade was used to cut and transfer each 1 cm thick section to a labeled sterile Whirl-Park^{R} bag. The metal sectioning plate was then cleaned with tissue paper and the perspex collar washed to avoid cross-contamination of the sediment sections. This procedure was repeated at 1 cm intervals until all the sediment within the perspex core liner had been extruded.

The organic matter in the sediment was digested by boiling the sediment in concentrated nitric and sulphuric acid(Battarbee, 1987). Suspensions of the acid cleaned diatoms were then dispersed onto standard coverslip using special sedimentation plates(Battarbee, 1987). The coverslips were then air dried and mounted in Canada Balsam mounting medium. The resulting permanent slide preparations(slide mounts) were scanned under an Olympus B250 research microscope equipped with Nomarski interference optics. Diatoms were counted along line transacts until a minimum of 200 individuals had been counted for each of the four replicate slides at each of the three depths($n=2791$ frustules counted, Table 1).

Samples containing *Pseudonitzschia* were dried on coverslips mounted on stubs and coated with Au/Pd. The stubs were examined in a Cambridge S360 Analytical Scanning Electron Microscope(SEM) at the Electron Microscope Unit of the University of Hong Kong.

2.2 lead-210 and cesium-137 analyses

^{210}Pb activity was determined through the extraction and counting of a daughter isotope, ^{210}Po. Extraction of ^{210}Po was based on methods developed by Flynn(Flynn, 1968). The ^{210}Po and a ^{208}Po internal trace was plated onto silver planchets and counted on an alpha spec-

Table 1 A comparison of diatom frustule abundance in surface and subsurface sediments from Kowloon Bay, in Victoria Harbour(Only the 26 most common diatoms were chosen for enumeration sixteen transects per slide were counted in each of s4 replicates)

Species name	Abundance		
	0—1 cm	4—5 cm	9—10 cm
Actinocyclus spp.	3, 0, 3, 4	2,1,1,0	11,9,12,10
Biddulphia spp.	2, 2, 3, 1	3,4,5,4	3,2,2,1
Chaetoceros sp.	18, 13,14,16	31,30,30,33	21,18,22,25
Cocconeis sp.	3, 4, 2, 1	4,3,2,4	6, 5, 3,4
Coscinodiscus minor	2, 2, 3, 1	0, 0, 2,1	3, 5, 2,1
Coscinodiscus spp	17, 9, 11,16	13,15,12,13	17,17,19,20
Cyclotella comta	4, 5, 3, 6	2, 3, 5, 1	5, 7,9, 6
Cyclotella spp.	5, 3, 4, 4	5, 8, 5, 6	4, 8, 5, 3
Cyclotella striata	13,14,15,13	34,35, 33,31	24,28,27,29
Cyclotella striata var. baltica	0, 0, 0, 0	4, 3, 6, 5	5, 8, 6, 6,
Cyclotella stylorum	18,19, 17, 14,	21,17, 20, 19,	18,19,11,13
Diploneis bombus	3, 5, 3, 2	4, 6, 4, 5	7, 9, 8, 8
Diploneis spp.	2, 1, 4, 3	4, 6, 4, 7	7, 7, 9, 10
Hantzschia spp.	2, 3, 1, 2	3, 1, 1, 1	4, 7, 3, 3
Navicula spp	4, 6, 7, 6	5, 3, 2, 2	3, 6, 5, 4
Nitzschia cocconeifromis	3, 3, 5, 1	4, 6, 7, 4	4, 3, 3, 5
Nitzschia spp.	26,24,25, 25	16, 17,24,18	15,14,13,15
Paralia sulcata	15,17,18,15	17,18,16,19	5,8, 6, 6
Pseudonitzschia spp.	12,14,13,15	4,5,12,7	2,5,3, 3
Raphoneis spp.	1, 1, 0, 1	2, 0,0, 3	3, 2, 4, 3
Skeletonema costata	14,12,12, 13	8, 9, 5, 7	4, 7, 8, 5
Surirella spp.	4, 5, 3, 4	2, 0, 1,0	1, 3, 2, 2
Synedra spp.	3, 4, 2, 2	0, 1, 2, 0	2, 1, 0, 0
Thalassionema nitzschioides	31,34,33,31	57,62,68,62	24,25,26,21
Thalassiosira sp.	8, 6, 9, 4	7, 9, 11, 8	8, 9, 11, 3
Trachyneis spp.	3, 5, 3, 2	4, 5, 3, 2	4, 7, 4, 5
No. of frustules counted	216,211,213,202	256,267,281,262	210,239,223,211
Total no. of frustules counted	842	1066	883

trometer. Supported ^{210}Po was calculated from ^{226}Ra measured by a gamma spectrometer. The sediment age was calculated using the c. r. s model of (Appleby, 1978) according to the pattern of ^{210}Pb activity-depth curve(Han, 1986; Xiang, 1992).

2.3 ^{137}Cs dating method

^{137}Cs activity was determined by a gamma spectrometer using a dried sediment sample of

1 g. The maximum ^{137}Cs concentration was dated at 1963 based on the historical pattern of atmospheric fallout (Health and Safety Laboratory, 1977).

The accumulation rate of diatoms was calculated by multiplying the sedimentation rate, presented as sediment flux rate ($gcm^{2-} a^{-1}$) by the organic matter content. The result was then multiplied by the concentration of the diatoms.

3 Results

The valves of the *Pseudonitzschia* are long and narrow (Fig. 2 — Fig. 5). Valves of *Pseudonitzschia pungens* and *Pseudonitzschia multiseries* are of similar size (about 4m wide and 75m long), and could only be distinguished from one another with SEM analysis. The relative abundance of the 26 most abundant diatom taxa was determined for four replicate samples taken at sediment core depths of 0—1 cm, 4—5 cm and 9—10 cm (Table 1).

Based on the ^{137}Cs results (Table 2) the ^{137}Cs estimated sedimentation rate in Kowloon Bay, Victoria Harbour, Hong Kong is 3.20 mm. a^{-1} while the ^{210}Pb estimated sedimentation rate is 3.11 mm. a^{-1}. The average sedimentation rate of Kowloon Bay is therefore likely to be about 3.15 mm. a^{-1}.

Table 2 Lead-210 and cesium-137 stratigraphy in a Kowloon Bay core

Depth interval, cm	^{210}Pb, dpm/g	^{210}Pb exc, dpm. g	^{137}Cs, Bk/kg
0—1	5.28	2.69	1.5±0.8
1—2	4.90	2.31	1.4±0.7
2—3	4.53	1.94	1.7±0.9
3—4	4.66	2.07	<1
4—5	4.32	1.73	3.9±0.8
5—6	4.70	2.11	<1
6—7	4.32	1.73	<1
7—8	3.35	0.76	<1
8—9	4.45	1.86	<1
9—10	3.12	0.53	<1
10—11	3.70	1.11	
11—12	3.41	0.82	
12—13	3.59	1.00	<1
13—14	3.48	0.89	
14—15	3.48	0.89	
15—16	3.43	0.84	<1
16—17	3.31	0.72	
17—18	3.25	0.66	
18—19	2.91	0.32	

Table 2 (continued)

19—20	3.10	0.51
20—21	3.03	0.44
21—22	3.01	0.42
22—23	2.92	0.33
23—24	2.88	0.29
24—25	2.81	0.22
25—26	2.98	0.39
26—27	2.90	0.31
27—28	2.48	
28—29	2.62	
29—30	2.87	
30—31	2.81	
31—32	2.61	
32—33	2.61	

4 Discussion

Chinese research on red tides growth dramatically in recent years, in parallel with public concern about consuming shellfish following red tide events (Ho, 1991). The frequency of red tide events in southern China and Hong Kong has also increased over the last two decades (Ho, 1991). It would appear that as nutrient loading to the coastal waters of Hong Kong has increased over the last forty years so has the frequency of red tide events (EPD, 1991).

Studies of red tide events in Hong Kong waters carried out by EPD (Lam, 1987) indicate that Tolo Harbour displays the highest frequency of red tides with a peak in red tide events occurring in April and May. Sites located between Lantau Island and Hong kong Island were the second most frequent red tide locations (EPD, 1991).

4.1 Diatom taxonomy of *Pseudonitzschia*

Pseudonitzschia are marine planktonic diatoms with eccentric canal raphe systems, and elongate valves with clonal chains formed by connecting overlapping cell tips (Hasle, 1993).

Pseudonitzschia pungens (formerly *Pseudonitzschia pungens f. pungens*) is differentiated from *Pseudonitzschia multiseries* (formerly *Pseudonitzschia pungens f. multiseries*) by the number of rows of intercoastal periods. *Pseudonitzschia pungens* has two rows and *Pseudonitzschia multiseries* has three to four rows (Hasle, 1993; Fig. 2—Fig. 5). Because these rows of intercoastal punctae are very difficult to distinguish under the light microscope, scanning electron microscope examination is required in order to differentiate between these two species of *Pseudonitzschia*. As a result, we are unable to say what proportion of the *Pseudonitzschia* that we observed under the light microscope belonged to each species. It is possible to conclude, however, that the *Pseudonitzschia pungens* was far more common than the *Pseudonitzschia multiseries*.

Ninety five of the nearly 3000 common diatoms that we counted (3. 4%) belonged to the genus *Pseudonitzschia* (Table 1). According to a recent review by Villac *et al.* (Villac, 1993), *Pseudonitzschia* species are widely distributed around the world. Under the correct combination of light and nutrients (details as yet unknown), the *Pseudonitzschia* diatoms congregate to form long "ribbons" which often get swept up into the overlying water column

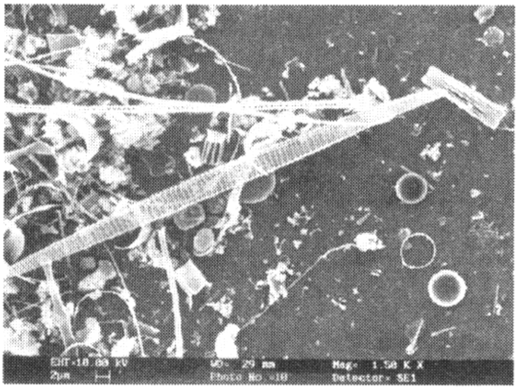

Fig. 2 SEM photomicrograph of *Pseudonitzschia pungens* magnified 1500 times (82 μ long × 4 μ wide)

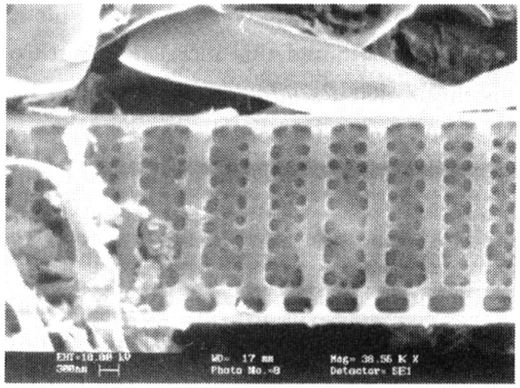

Fig. 3 SEM photomicrograph of *Pseudonitzschia pungens* magnified 38560 times. Note the two (and sometimes a partial third row) of intercoastal poroids. There are about 8—10 poroids across the width of the valve

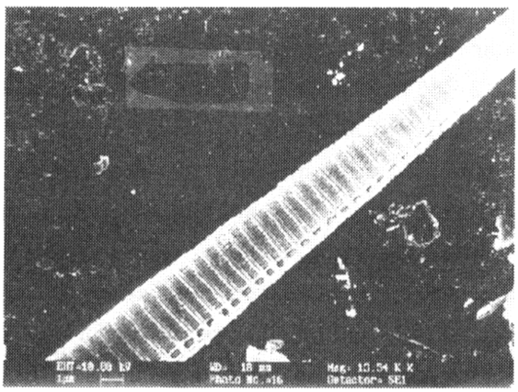

Fig. 4 SEM photomicrograph of *Pseudonitzschia multiseries* magnified 13540 times. The valve is 80 μ long and 4 μ wide

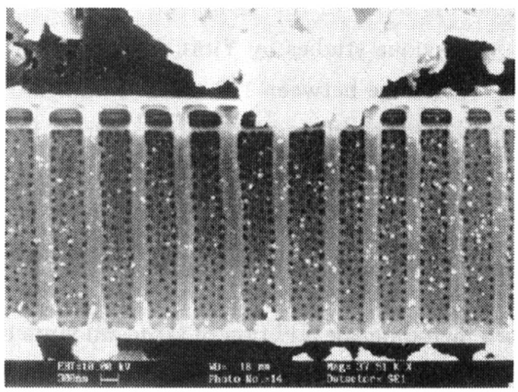

Fig. 5 SEM photomicrograph of *Pseudonitzschia multiseries* magnified 37510 times. Note the three to four rows of intercostal periods. There are about 20 poroids across the width of the valve

where they contribute to " red tide" development. The diatoms in these colonies often overlap ends but about one third or one fourth of their total cell length (Hasle, 1964). When we examined the surface sediment samples which we sampled in February, we expected to see the *Pseudonitzschia* diatoms congregating to form the long "ribbons" which we anticipated would get swept up into the overlying water column in March or April when the first algal blooms begin appearing but we failed to find any long "ribbons" of *Pseudonitzschia* in the unacidified surface sediment samples. In addition to the core sample reported here, we sampled the source sediments of Victoria Harbour and connecting channels but again failed to find only long "ribbons" of *pseudonitzschia* (Glenwright, 1996).

Qi observed an increase in the frequency of red tide events along the coast of southern China and attributed this increase to anthropogenic nutrient inputs (Qi, 1993). To test the hypothesis that *Pseudonitzschia* was also becoming increasingly more frequent we estimated their abundance at a core depth of 0—1 cm, 4—5 m and 9—10 cm. At a core depth of 0—1 cm 6.4% of the 842 diatoms belonged to the genus *Pseudonitzschia*. At a core depth of 4—5 cm only 3.4% of the 1066 diatoms belonged to this genus. At 9—10 cm only 1.5% of the diatoms counted ($n=883$) belonged to the genus *Pseudonitzschia*. Thus it was concluded that a significant increase in the relative abundance of *Pseudonitzschia* frustules ($p<0.05$) had occurred in the sediments of Kowloon Bay in Hong Kong Harbour.

4.2 Estimates of sedimentation rates in Kowloon Bay

According to Chalmers (Chalmers, 1984) inputs of sediments into Victoria Harbour are primarily derived from sewage solids, suspended loads from the Pearl River and losses from land reclamation and dredging projects. It was estimated that these sources contributed approximately 1000000m^3. a^{-1} of sediment to Victoria Harbour. Chalmers estimated sediment supply would result in a mean sedimentation rate of 40 mm a^{-1} if all the sediment entering Victoria Harbour remained there. However, wave and current action have been effective in removing much of this sediments.

Previous studies by Yim (Yim, 1983) have indicated that sedimentation rates in Victoria Harbour were between 1.2 and 2.5 mm. a^{-1} (i.e. 12—25 cm. a^{-1}) throughout the Holocene period. Our estimated sedimentation rate for Kowloon Bay of 3.15 mm. a^{-1} falls between estimates made by Yim and Chalmers. Our isotope estimated mean age of the 0—1 cm section was 1990. The 4—5 section would have an approximate age of 14 years and the 9—10 cm section would have an isotope estimated age of about 30 years (ca. 1965).

The recent increase in potentially toxic diatom species was attributed to a variety of factors including the addition of large amounts of nutrients to coastal waters as a result of man's activities. It would appear that as nutrient loading to the coastal waters of Hong Kong has increased over the lst thirty years so has the frequency of red tide events (EPD, 1994) and along with this, the frequency of *Pseudonitzschia* blooms.

A toxic algal blooms is both a public health hazard and an economic threat. It damages the shellfish industry, disrupts trade and discourages the expansion of the aquaculture industry (Shumway, 1989). The presence of potentially lethal diatoms in Hong Kong means that there is a real need for regular phytoplankton monitoring and additional shellfish monitoring to ensure that Hong Kong grown seafood is safe for human consumption.

5 Conclusions

Diatoms belonging to the genus *Pseudonitzschia* have increased in abundance during the last three decades ($p<0.05$) in Hong Kong's Victoria Harbour. Analysis of Kowloon Bay sediments from 0—1 cm indicated that 6.4% of the diatoms observed at this depth belonged to the genus *Pseudonitzschia*. At 9—10 cm (ca. 1965) only 1.5% of the diatoms belonged to the genus *Pseudonitzschia*.

Using only the light microscope, it was not possible to determine which *Pseudonitzschia* individuals produced the toxic domoic acid and which did not.

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