Journal of Environmental Sciences Vol. 10, No. 1, pp. 13—24, 1998 ISSN 1001—0742

Deformity frequencies of benthic marine diatoms associated with contaminated sediments in Hong Kong

Mick Dickman

Ecology and Biodiversity Department, The University of Hong Kong, Pokfulam Road, Hong Kong

Abstract—The relationship between contaminated sediments and deformity frequencies for benthic diatoms was the subject of this study. There were two basic types of diatom deformities observed in this study of Tolo Harbour diatoms: 1. Diatoms with deformed cell wall morphology and 2. diatoms with deformed ornamentation.

Deformed individuals of *Fragilaria capucina* and *Achananthes hauckiana Grun*. displayed external cell wall deformities while deformed individuals of *Diatoma* displayed deformities in valve ornamentation.

Abnormal cell morphology was used as an indicator of a degraded ecosystem. There was a significant correlation($P < 0.05$, $r_s = 0.652$) between sediment toxicity as reflected by the microtox test and benthic diatom diversity.

Species diversity and species richness levels at the twelve sites were closely correlated with one another and inversely correlated with sediment toxicity as reflected by our microtox results($r_s = 0.809$, $P < 0.01$). The microtox test for acute toxicity was correlated ($P < 0.05$) with diatom species richness. The deformity frequencies and microtox tests were not significantly correlated. This lack of correlation was expected as in numerous laboratory studies, toxic substances were not necessarily mutagenic.

The use of diatom deformity frequencies as a means of assessing the mutagenic/teratogenic impacts of anthropogenic substances on a marine benthic diatom community necessitates the enumeration of a large number of diatoms making this method extremely labor intensive.

Keywords: deformity frequencies; benthic marine diatoms; contaminated sediments.

1 Introduction

The toxic substances are not always mutagenic. Thus standard tests for mutagenicity such as the SOS Chromotest (Quilardet, 1985) and the Mutatest (Ames, 1975) have resulted in the identification of numerous substances which were not toxic but were mutagenic.

The sediments of coastal areas around Hong Kong such as Victoria Harbour and Tolo Harbour are contaminated with a variety of heavy metals and persistent organic compounds (Enviro-Chem Engineering and Laboratory Co. Ltd, 1994, Hong Kong Environmental Protection Department, 1994). A number of studies have been conducted to assess sediment toxicity in Hong Kong but none have been carried out to assess sediment mutagenicity.

Ecological conditions in coastal waters near Hong Kong are a concern to its citizens and scientists alike because a number of toxic chemicals are present that have been linked to reproductive, metabolic, neurologic and behavioural abnormalities (Hong, 1993). The elevated levels of heavy metals in Tolo Harbour (EPD, 1994) was the main reason that this study was undertaken in this part of Hong Kong. Lam *et al*. (Lam, 1987) concluded that the assimilative capacity of Tolo Harbour was very limited due to its enclosed characteristics, weak currents and vertical salinity and thermal stratification. Copper, chromium and zinc in Tolo Harbour, are all elevated above the U. S. EPA guidelines (Lam, 1987).

In the present study, 12 sites were examined to determine their: 1. diatom diversity; 2. sediment toxicity as indicated by the microtox test; 3. heavy metal concentrations of the sediments and 4. the frequency of diatom deformities.

Diatoms(as indicator organisms) are algae which are classified by the shape and ornamentation of their silica cell walls. These cell walls preserve extremely well and , as a result, diatoms can be efficiently and permanently identified and archived. Cell shape and ornamentation in natural diatom populations vary only slightly within a species and deformities in diatom morphology from unpolluted waters are rare (Schmid, 1990).

2 Methods

2.1 Procedures for the removal of sediment samples

Sediments are usually sampled with a gravity corer or grab sampler or sediment dredge. An Ekman grab sampler was used to sample the sediments for heavy metals and microtox determinations but such an approach proved unsuitable for quantitative samples of sediment diatoms because the sand grains in the grab samples obscured the diatom preventing their accurate enumeration. As a result, an epipelic diatom suction sampler was used to sample the diatoms at all sites that were less than 1 m deep. The epipelic suction sampler had the advantage that it removed most of the diatoms and organic debris leaving sand and clay behind. Because it did not sample the diatoms clinging to sand grains it was referred to as an epipelic rather than an epipsammic sampler. The lack of sand grains and other inorganic silicous material in the epipelic samples made diatom counting far easier for the reason described above. Diatoms are difficult to separate from sand grains even when differential desimentation or filtration are employed.

The epipelic diatom sampler could not be used at all sites because sites where the water depth exceeded the 1m length of the suction tube necessitated the used of an Ekman dredge. The Ekman dredge penetrated the sediment to a depth of about 4cm ± 1cm depending on the amount of sediment consolidation.

Samples were taken from January, 1994 to February, 1995 at six sites in Tolo Harbour (Fig.1) and three sites at Ping Chau and three sites at Cape d'Aguilar on Hong Kong Island. Three replicate samples were taken at each site and 1000 diatom frustules were counted for each replicate. The six sites in Tolo Harbour were chosen because their sediments had high levels of heavy metal and organic persistent contaminants while the three sites on Ping Chau and the three sites at Cape d'Aguilar had much lower concentrations of these contaminants. The degree of metal contamination in the sediment was assessed by sending the samples to the AWT Science and Environment Consulting Co. (51 Hermitage Road, West Ryde, New South Wales, 2114 Australia) for analysing. They measured the total content of seven metals(Cd, Cr, Cu, Fe, Ni, Pb, Zn) using atomic absorption spectrometry on nitric acid digested samples.

The biological assessment of the contaminants present in the sediment was carried out by screening for both acute toxicity (Hong Kong Environmental Protection Dept.) and the frequency of diatom deformities (Ecotoxicology Laboratory, The University of Hong Kong).

The samples from each of the 12 sites were coded to avoid unconscious bias. After 36000 diatoms had been counted and the number of individuals of each species recorded, the number of deformed diatoms on each slide was noted and the slides were then decoded.

2.2 Toxicity testing

The method chosen for sediment toxicity screening was the non-extractive microtox solid phase test(Microbics Corp, 1992). This is a bacterial inhibition bioassay, using a 50% reduction in the bioluminescence of a colony of *photobacterium phosphoreum* as the criterion for calculating the effective concentration. The wet sediments collected from each site were centrifuged and the resulting pellet was made into an aqueous suspension before being serially diluted. The effective concentration (EC_{50}), which is the concentration(% W/V) of the sediment sample causing a 50% decrease in bioluminescence measured after 25 minutes total contact time, was then determined (Microbics Corp, 1992).

The microtox test is a rapid and simple method for comparing and ranking the relative toxicity of sediment samples taken from different locations. A detailed discussion of the physiology of *photobacterium phosphoreum* the influence of test conditions on the final toxicity values, and the experimental procedure of this bioassay have been provided by Krebs(Krebs, 1983). Unlike methods which require solvent extracts or elutriates to be prepared from the sediments, the microtox solid phase test allows the photobacterium phosphoreum bacteria to come into direct contact with the sediment. The bacteria are then separated using a filter column and analyzed using the microtox toxicity analyzer Model M 500(Beckman, 1982).

2.3 Diatom deformity testing

A Pasteur Pipette was used to transfer 5 ml of the homogenized sediment-water mixture to an Utermohl sedimentation tube which was observed using an Plympus research compound microscope (Model BX 50 equipped with a photoautomat). A total of one thousand diatoms were counted for each of three replicates samples at 400 times magnification. Diatoms were identified at 1000 times magnification using Cheng *et al*. (Cheng, 1993), Germain(Germain, 1981), Jin *et al*. (Jin, 1986) and Patrick (Patrick, 1966) and Reimer (Reimer, 1975) procedures. Deformities were photographed using the Olympus photoautomat.

2.4 Sample sites

Among the 6 sites sampled in Tolo Harbour(Fig.1), the first 4 were in the Tai Mei Tuk Typhoon Shelter(Fig.1). Site 5 was located near Tai Po Industrial Estate and site 6 was located at the mouth of a small stream that enters Tolo Harbour near the northern end of the Plover Cove Reservoir(Fig.1). Six "control" or reference sites were sampled from areas in Hong Kong which displayed low levels of heavy metals in the sediments. Three of these reference sites (site 7, 8 and 9) were located on Ping Chau Island(Fig.1). The three other reference sites (sites 10, 11 and 12) were located at Cape d'Aguilar at the southeastern corner of Hong Kong Island(Fig.1).

2.5 Site descriptions

Site 1: The sediments at this site had a brownish-yellow colour and were collected using an

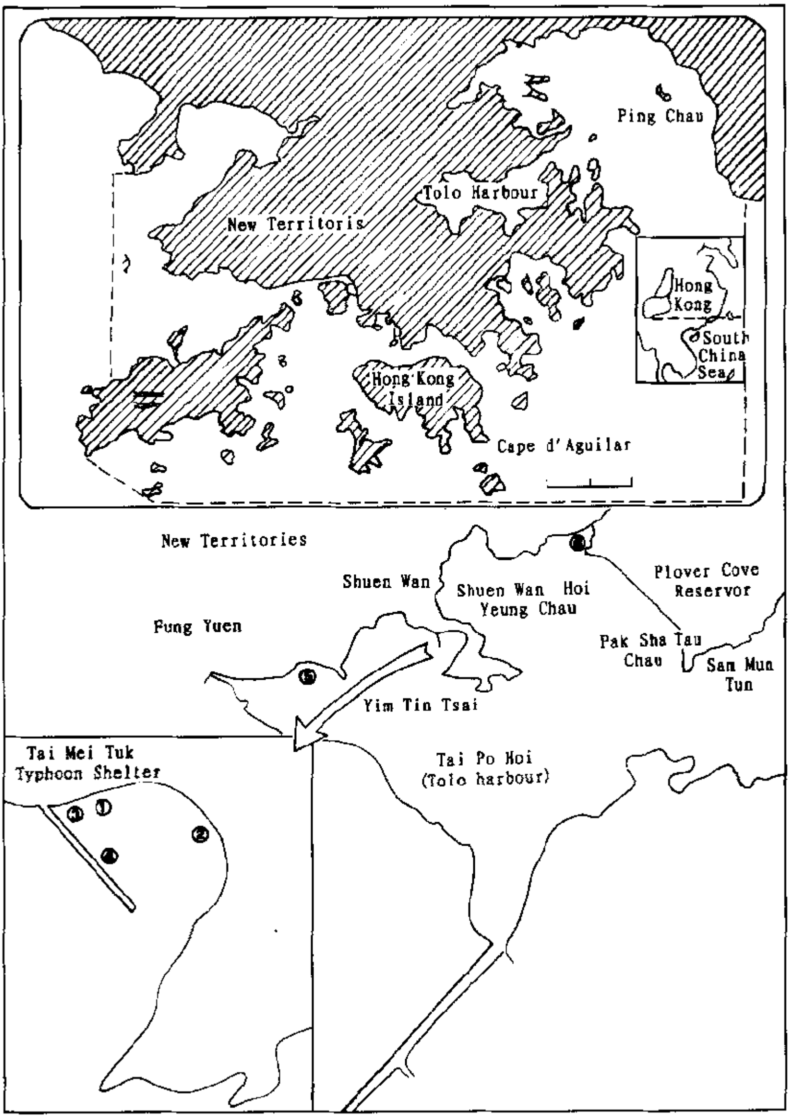

Fig.1 Sampling locations

Ekman grab sampler. Amphipods, crabs and hermit crabs were found among the collected sediments (water depth was about 1.2m).

Site 2: The sediments at this site were observed to be relatively inorganic. Filamentous green algae, primarily *Enteromorpha*, were collected along with the sediment (water depth was about 0.9 m). Sediments were collected using the epipelic suction sampler (Fig.2).

Site 3: Water depth was about 0.75 m. Numerous benthic invertebrates were found at this site and sediments were collected using the epipelic suction sampler.

Site 4: Water depth was about 3 m. The sediment was black and gave off a strong hydrogen sulphide smell. No invertebrates were found. Sediment at site 4 was collected using an Ekman grab sampler.

Site 5: Water depth was 0.8m. The area was near the Tai Po Industrial Estate storm drain which was a double storm drain out-fall located about 500m north of the Motorola Building. Sediments were collected at this and all remaining sites using an epipelic suction sampler.

Site 6: Water depth was less than 0.75 m. The sediment was quite coarse grained, brownish-yellow sand.

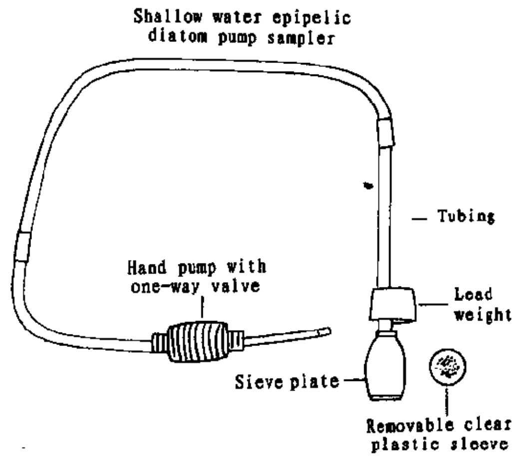

Fig.2 The shallow water epipelic suction sampler

Site 7 was located about 300 m northwest of the ferry dock on Ping Chau Island in about 0.1m of water. The sediments at this site were coarse grained sands which were relatively free of organic matter except for some filamentous green algae.

Site 8 was located about 300 m southeast of the ferry dock on Ping Chau Island(Fig.1). The water depth at this site was about 0.1m. The sandy sediments at site 8 were slightly finer than those at site 7 and filamentous green algae was also present.

Site 9 was located about 1.3 km southeast of the ferry dock on Ping Chau Island (Fig.1). The water depth at this site was about 0.8 m. The sandy sediments at site 9 were quite fine and a flocculant organic layer was present at the sand-water interface.

Site 10, 11, and 12 were located at Cape d'Aguilar in shallow water (0.1—0.4 m). No filamentous green algae were present at these sites. All three of these sites (Fig.1) had coarse sandy sediments.

Samples were collected at low tide to make sediment sample conditions as uniform as possible. Salinity, conductivity, dissolved oxygen, oxidation-reduction potential (ORP), pH, water depth, temperature and total dissolved solids were measured at each site using a YSI Model 6000 Environmental Monitoring System(Campbell Scientific Inc.).

3 Results

With the exception of site 4 where the sediments were anaerobic and had an oxidation-reduction potential (ORP) of −154(Tables 1 and 2), the diatoms were active when viewed live under the microscope. Secchi transparencies were nearly always less than 1.5 m and the compensation depth was generally less than 3.5 m. A list of the diatom species found at each site was complied but is not included because of its large size. This list is available from the author if desired.

Table 1 Water chemistry data for mud-water interface samples collected at sites 1—6

Parameter units	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Conductivity, mS	50.1	50.0	48.9	49.2	49.6	49.8
Total dissolved solids, ppm	32.5	32.6	32.5	31.8	31.7	31.9
Salinity, ppt	32.7	32.7	32.7	31.9	31.8	26.0
Dissolved oxygen, % sat.	79	48.6	92	42.9	37	84.1
ORP(mV) E_h	85	84	73	−154	33	37
pH	7.55	7.51	7.49	7.61	7.59	7.55
T, ℃	27.2	27.2	27.3	27.1	27.2	27.3
Depth, m	0.9	0.7	0.6	1.3	1.2	0.7

Table 2 Water chemistry data for mud-water interface samples collected at sites 7—12

Parameter units	Site 7	Site 8	Site 9	Site 10	Site 11	Site 12
Conductivity, mS	50.1	50.0	50.9	50.2	50.6	50.8
Total dissolved solids, ppm	32.5	32.6	32.5	32.8	32.7	32.9
Salinity, ppt	32.7	32.7	32.7	32.9	32.8	32.7
DO, % sat.	90.4	98.6	92.0	92.1	90.7	91.3
ORP, (mV) E_h	77	79	73	81	83	79
pH	7.48	7.37	7.56	7.96	7.46	7.66
T, ℃	27.3	27.2	27.3	27.0	27.3	27.3
Depth, m	0.1	0.1	0.8	0.3	0.4	0.1

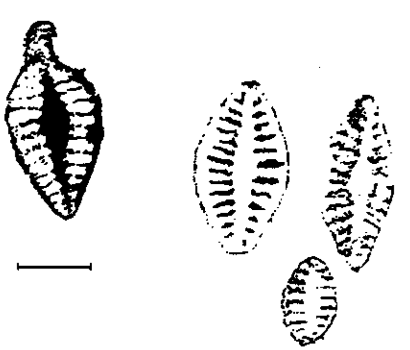

Fig.3 *Achnanthes hauckiana* Grun. collected from site 6 where it occurred with deformities (left side) and without deformities (right side). The solid line represents a distance of 15μm (left side) and 20μm (right side)

3.1 Deformed diatoms

Normal cells of *Achnanthes hauckiana Grun* (Jin, 1985) were easily distinguished by their symmetry (Fig.3), whereas deformed cells (Fig.3) lacked this bilateral symmetry. Normal (upper inset) and deformed *Diatoma species* (Fig.4), were recognizable because deformed individuals displayed a " wavy" pattern of longitudinal lines in which the valve ornamentation rather than the valve cell wall was the critical trait. Deformed individuals of *Fragilaria capucina* (Fig.5) , displayed a lack of longitudinal symmetry. Both it and *Achnanthes hauckiana Grun*. illustrated external cell wall deformities while *Diatoma* (*Fig*.4) represented abnormal cell wall ornamentation deformities.

Sediment toxicity, as indicated by the "Microtox"

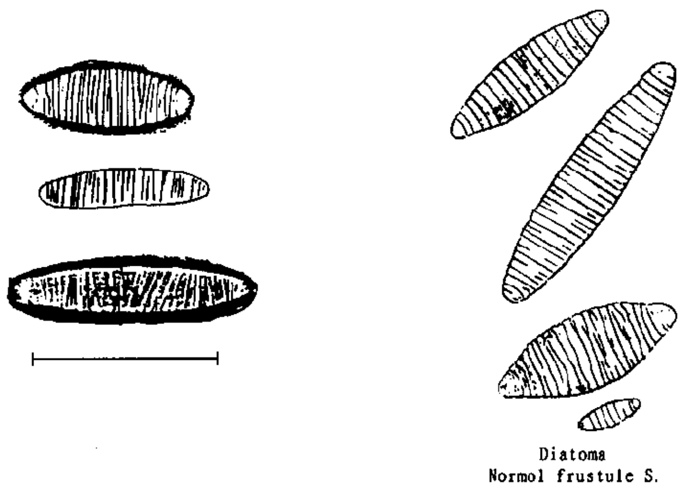

Fig. 4 Three deformed individuals of *Diatoma* collected from site 6 (left side) each display a slightly different pattern of abnormal valve ornamentation. The four normal individuals at the right are from Jin *et al*. 1986. The solid line represents a distance of 45μm (left + right sides)

Fig. 5 *Fragilaria capucina* from site 6 displaying a valve deformity. The normal *F. capucina* at the right was observed at the same site; the solid line represents a distance of 30μm (left side) and 25μm (right side)

test, was examined at each of the study site (Table 3) to determine if a relationship between sediment mutagenicity and diatom deformity frequency existed. Diatom diversity and species richness at the twelve study sites were also calculated (Table 3). Diatom diversity (H) where $H = -\Sigma(p_i \mathrm{Log}_2 p_i)$ and p_i = the number of diatoms in the ith species divided by the total number of diatoms counted in the sample. The results were calculated on a count of 3000 diatom frustules per site. The number of deformed diatoms observed at each site ranged from none at sites 1, 2, 7—12, one at site 3, 4 and 5 to three at site 6 (Table 3). 3000 diatom frustules were counted at each site, 1000 for each of the 3 replicate slides.

The concentrations of 7 elements (Table 4) were analyzed to determine whether sites that exhibited high levels of heavy metals also exhibited elevated microtox and diatom deformity levels. Heavy metal concentrations exceeded U.S. EPA guidelines at sites 4 and 5 for nickel, lead, zinc, copper and chromium (EPD, 1994).

Table 3 Results of the sediment microtoxicity tests

Sample site number[1]	EC_{50}%, W/V[2]	Descending toxicity ranking[3]	Number of deformities[4]	Diversity[5], H	Species richness (#/3000)
Site 1	5.5931	6	0	3.19	20
Site 2	3.2105	5	0	3.65	26
Site 3	2.6446	4	1	3.22	18
Site 4	0.1455	2	1	3.14	15
Site 5	0.0328	1	1	2.70	15
Site 6	1.5846	3	3	3.09	17
Site 7	5.6241	7	0	3.74	42
Site 8	5.8844	10	0	3.77	39
Site 9	5.7558	8	0	3.61	41
Site 10	5.8964	11	0	3.59	38
Site 11	5.8978	12	0	3.45	40
Site 12	5.8793	9	0	3.65	35

1. Site locations are provided in Fig. 1. Sites 7—12 were "controls";
2. EC_{50}-effective concentration causing a 50% decrease in bioluminescence;
3. Represents sediments with the highest acute toxicity based on the microtox solid phase test;
4. The number of deformed benthic diatoms per thousand observed;
5. The total number of benthic diatom taxa observed in a count of 3000 diatom frustules.

Table 4 Concentrations of seven common sediment contaminants

Contaminant[1]	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site7—12
Cadmium, mg/kg	<0.5	<0.5	<0.5	0.75	1.1	<0.5	<0.5—0.8
Chromium, mg/kg	2.0	3.1	2.3	32	42	2.2	<2—3.7
Copper, mg/kg	16	25	30	260	440	20	24—35
Iron, g/kg	14	8.1	1.0	19	27	5.3	5—8.6
Nickel, mg/kg	1.8	2.2	1.8	18	31	2.8	<1 2.4
Lead, mg/kg	39	34	26	110	130	22	25—38
Zinc, mg/kg	31	35	34	330	450	32	30—36

1. Sediment contaminant determinations were carried out by the Environmental Protection Department, Wanchai, Hong Kong. All concentrations are given as ppm(mg/kg dry weight of sediment)

Site No. 5 (Tai Po Industrial Estate discharge area) had the highest microtox toxicity levels (Table 3) and the highest levels of heavy metals(Table 4). Site No. 6 was associated with the illegal dumping of acidified wastes from a one mean operation involving the reclamation of precious metals from discarded computer parts and other electronic equipment. Site 6 had the highest diatom deformity levels (one per thousand) versus one per three thousand at sites 3, 4 and 5. This site, which is a river mouth site, exhibited the lowest salinities(Table 1; 26 parts per thousand) and thus, osmotic factors may have played a role in deformity induction at this site(Schmid, 1990). The sediments collected at site 6 were quite coarse (mean particle size of 0.2—0.3cm) by comparison to samples from the other sites. It is well documented that the bulk of the total metals measured in sediment tends to be associated with the finer particles, which have a greater surface area(Hong, 1993). It is possible that organisms collected at site 6, including the diatoms, were exposed to metal contaminants in the pore water or in the water column on a regular basis, but that the metals did not absorb or accumulate in these coarser sediments.

In order to determine whether or not there were significant differences between clean and contaminated sediment sites in the number of diatom deformities, the Fisher exact probability test was used (Fisher, 1953). A total of 6 deformities were observed at the Tolo Harbour contaminated sediment sites while no deformities were observed at the "control" sites. What is the probability that all 6 deformities would occur by chance alone for contaminated sites while none occurred at the "clean" sites? According to the Fisher exact probability test, the probability of this occurring by chance alone is 0.01562. Thus, there was less than 2 chances in a hundred that all six deformed diatoms would have been observed at the contaminated sites and none at the clean sites. Because all three slides were coded, these observations were not affected by subjective bias during the counting of the samples.

4 Discussion

4.1 Contaminated sediments

The presence of hazardous pollutants in water and sediments has resulted in some of today's most troublesome environmental problems. Many contaminants tend to accumulate not only in the sediment, but also in living organisms and these accumulations are often linked to such things as morphological deformities, reproductive and immune system failures and population collapse (Dickman, 1989; William, 1980; Yu, 1984).

There was a significant correlation($P < 0.05$, $r_s = 0.652$; Siegal, 1956) between sediment toxicity as reflected by the microtox test and benthic diatom diversity(Table 3). Site 5 displayed the highest heavy metal concentrations, the lowest diversity and the highest microtox acute toxicity levels. Site 6, by contrast, had some of the lowest heavy metal concentrations, intermediate microtox and diversity levels and high deformity frequencies relative to the other five Tolo Harbour sites (Table 3 and Table 4). It is possible that mutagenic or teratogenic substances other than heavy levels may have been present at site 6 or that sporadic discharges of heavy metals that reacted with the developing diatoms and then washed off the creek sediments into the sea were responsible for the elevated deformity frequencies at this site, but this remains only spiculation. It is also true that because site 6 is a freshwater site, the pH of the water is lower than at the other sites which are all marine sediments. Heavy metals are more bioavailable at lower pH than they are in the sea water sites where pH is high(Krebs, 1983).

The microtox test for acute toxicity was correlated($P < 0.05$) with diatom species richness as assessed by the Spearman rank correlation test(Siegel, 1956). The deformity frequencies and microtox tests were not significantly correlated. This was not unexpected as in numerous studies conducted by Quillardet (Quillardet, 1985), the substances which were most toxic were not necessarily the most mutagenic.

The heavy metal levels of the sediments were not correlated with the microtox tests. It is possible that substances other than heavy metals were the primary toxic chemicals but since no tests for toxic organic compounds in sediments was carried out, it is not possible to critically evaluate this possibility.

4.2 Diatoms as continuous monitors

Because benthic diatoms remain in place for a number of months, they act as continuous monitors during that period(sensu Buikeman, 1978; Dickman, 1980). This is a decided advantage to using diatoms rather than microtox bacteria in situations where toxic and/or mutagenic discharges are infrequent(i.e. sporadic).

4.3 Diatom deformities

Benthic diatom deformities have been correlated with the presence of genotoxic and teratogenic chemicals in aquatic sediments in a number of countries(Adersen, 1991; Baber, 1981; Dickman, 1992; Yang, 1993). William *et al*.(William, 1980) described the effect of eleven heavy metals on the morphology of *Thalassiosira aestivalis*. The metals, present at μg/L concentrations, were divided into three groups on the basis of their toxic effects. In the first group the presence of either Cu, Zn, or Ge increased the chain length and normal separation of cells failed to occur. In the second group, Hg, Cd and Pb, there was some disruption of cell division and elongated or bent cells occurred. In the third group, Cr, Ni, Se and Sb were without effect at or below 1 μg/L.

For deformities to occur, heavy metal concentration must be high enough to cause the formation of abnormal frustules, but low enough to allow at least for one division(Pickett-Heaps, 1990). This range of concentrations is often quite narrow (Braeck, 1976). For example, morphological aberrations occur in *Thalassiosira pseudonana* over a narrow range of Cu^{2+} ion activities($10^{-8.3}$ to $10^{-8.6}$ mol/L; Braeck, 1976). Abnormal cell morphology observed in samples from nature must have used with caution as an indicator of heavy metal pollution because the abnormalities can also be caused by a variety of other factors including nutrient limitation(William, 1980).

In the present study, there was a long history of elevated nutrients in Tolo Harbour in contrast to Ping Chau Island and Cape d'Aguilar (EPD, 1994; Hong, 1993; Hodgkiss, 1995) and therefore, nutrient limitation is unlikely to be a critical factor. As a result, if deformity frequencies were associated with limited nutrients, more deformities should have been reported for Ping Chau Island and Cape d'Aguilar than for Tolo Harbour.

Species diversity and species richness levels at the twelve sites were closely correlated with one another and inversely correlated with sediment toxicity as reflected by our microtox tesults. The r_s for the inverse relationship between microtox toxicity and species richness was 0.809 which was significant at the 0.01 level of probability (Spearman rank correlation test, Siegal, 1956).

The station with the highest heavy metal concentrations(Station 5) displayed the highest sediment toxicity as indicated by the solid phase microtox test(Table 3). The highest number of deformed diatoms occurred at site 6 where heavy metal concentrations were elevated above the control sites but were not as high as those observed at sites 4 and 5.

The microtox sediment toxicity levels were inversely correlated with species richness($P < 0.05$). In my opinion, the data presented in this paper do not permit an unequivocal conclusion about the use of diatom deformity frequencies as a means of assessing the mutagenic/teratogenic impact of anthropogenic substances on a marine benthic diatom community. In addition, the large number of diatoms that must be evaluated for deformities makes this method extremely labour and time intensive and few scientists are likely to adopt it as a standard screening tool for contaminated sediments. If the counting procedure could be automated then the method might be viewed as promising and deserving of further study.

Acknowledgements——I am grateful to Prof. John Hodgkiss, Dr. Tore Lindstom and Mr. Tom Glenwright for reading over the manuscript and to Philippe Jaouen, Ivan Tsoi, Gina Sequeira and Alfred Lai of the University of Hong Kong for their assistance in taking and analyzing some of the samples and to Ann Neller and the staff of the Chemical Waste and Ecotoxicology Laboratories of Hong Kong Environmental Protection Department for their technical assisstance. I am also grateful to the Director of the Environmental Protection Department of Hong Kong for permission to cite the EPD heavy metal data in this paper. Funds to support this research were provided by a University of Hong Kong-CRCG grant (337/023/0003).

References

Ames B N, McCann J, Yamasaki E. Mutation Research, 1975, 31:347—364

Andersen N A, Tuchman M L. J Great Lakes Research, 1991, 17:144—149

Barber H G, Carter J R. Microscopy, 1981, 34:214—226

Beckman. Microtox system operating manual. Beckman Instruments, Inc., Carlsbad, California. 1982

Beebe LE, Fox S D, Issaq H J, Anderson L M. Environmental Toxicology and Chemistry, 1990, 10:757—764

Braeck G S, Jensen A. J Exp Mar Biol Ecol, 1976, 25:37—50

Buikeman A L Jr, Herricks E E. JWPCF, 1978, 50:1637—1648

Cheng Haodi, Gao Yahui, Liu Shicheng. Nanodiatoms from Fujian coast. Beijing:China Ocean Press. 1993.91

Dickman M D, Smol J, Steele P. Water Pollution Research Canada, 1980, 15:17—31

Dickman M, Que Lan, Mattews B. Water Pollution Research Journal of Canada, 1989, 24:47—79

Dickman M D, Yang J R, Brindle I D. Water Pollution Research Journal of Canada, 1992, 25:131—159

Enviro-Chem Engineering and Laboratory Co. Ltd. Report on marine sediment analyses in Victoria Harbour. Doc Ref. 93/MS-110-R1, 95-97 Des Voeux Road West, Hong Kong. 1994

Environmental Protection Department of Hong Kong(EPD). Marine water quality in Hong Kong, Monitoring section, Waste and Water Service Group, Environmental Protection Department, Hong Kong Govt. 1994:236

Fisher R A, Yates F. Statistical tables for biological, agricultural and medical research. London: Oliver and Boyd. 1953:125

Fordham C L, Reagan D P. Environmental Toxicology and Chemistry, 1991, 10:949—960

Germain J. Flora des Diatomees D'eaux douce et saumatre. Paris, France. 1981:444

Hodgkiss I J. Environmental research in Pearl River and coastal areas(Eds. by C. K. Wong, K. H. Chu Q. C., Chen & X. L. Ma). Guangzhou: Guangdong Higher Education Press. 1995:192

Janz D M, Metcalf C D. Environmental Toxicology and chemistry, 1991, 10:917—924

Jin D, Cheng Z, Lin J, Liu S. Marine benthic diatoms in China. Beijing: China Ocean Press. 1985:313

Jin D, Cheng Z, Lin J, Liu S. Marine benthic diatoms in China. Beijing: China Ocean Press. 1986:527

Krebs F. Gewasserschutz, Wasser, Abwasser, 1983, 63:173—230

Lam C W Y, Kong M F, Liu P H. Marine water quality in Hong Kong. Environmental Protection Department. Marine Section. Water Quality Group, Hong Kong Government. 1987: 109

Metcalfe C D, Sonstegard R A, Qulliam M A. Bull Environ Contam Toxicol, 1985, 35: 240—248

Microbics Corporation 1992. Microtox manual: A toxicity testing handbook. Carlsbad, CA. 1992. 3

Patrick R, Reimer C. The diatoms of the United States, Vol. I. Monograph of the Academy of Natural Sciences of Philadelphia. Philadelphia Penn USA. 1966

Patrick R, Reimer C. The diatoms of the United States, Vol. II. Monograph of the Academy of Natural Sciences of Philadelphia. Philadelphia Penn USA. 1975

Pickett-Heaps J, Schmid A-M M, Edgar L A. Progress in phycological research, Biopress Ltd. 1990. 7

Quilardet P C, de Bellecobme, Hofung M. Mutation Research, 1985, 147: 79—95

Siegel S. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Co. Inc. New York. 1956. 312

William H T, Hollibaugh J T, Seibert D L R. Phycologia, 1980, 19(3): 202—209

Yang J R, Duthie H C. Hydrobiologia, 1993, 269/270: 57—66

Yu D. Pollution solutions. Private Sector Committee Environment Centre, 83 Tat Chee Avenue Kowloon, Hong Kong, 1994: 3

(Received for review March 31, 1997. Accepted April 15, 1997)