

Genotoxicity of the sediments collected from Pearl River in China and their polycyclic aromatic hydrocarbons (PAHs) and heavy metals

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Abstract The accelerated industrialization and urbanization in the last three decades around the Pearl River Delta within Guangdong Province in China have led to serious concerns about the impacts on the aquatic environment. In the present study, the genotoxicity of the sediments collected from the Pearl River was evaluated by micronucleus (MN) assay with *Vicia faba* root tip cells, and the 16 EPA priority polycyclic aromatic hydrocarbons (PAHs) and heavy metals (HMs, including Cr, Cu, As, Se, Cd, Hg, and Pb) in the sediments were determined respectively by GC-MS, inductively coupled plasma mass spectrometry, and inductively coupled plasma atomic emission spectrometry. The results showed that there were significant increases of MN frequencies observed in the sediment-exposed groups, compared with the

negative group ($P < 0.05$, $P < 0.01$), indicating that the sediments clearly had genotoxicity to the *V. faba* root cells. The total concentrations of the priority PAHs (250–13,656 ng g⁻¹, dry weight) and HMs (As, 22,770–36,639 μg kg⁻¹; Cr, 39,333–133,343 μg kg⁻¹; Cu, 36,145–159,270 μg kg⁻¹; Pb, 51,210–166,642 μg kg⁻¹; Cd, 475.4–1,818.9 μg kg⁻¹; Hg, 59.9–460.8 μg kg⁻¹; and Se, 331.7–1,250.4 μg kg⁻¹, dry weight) were close to those obtained from other urbanized and industrialized areas, which have been considered moderately polluted. There was a clear positive correlation between MN potency and the molar concentrations of Hg and Pb in the sediments (Hg, $r = 0.94$; Pb, $r = 0.91$), suggesting that Hg and Pb were the most important factors that posed the sediments higher genotoxicity to *V. faba* root cells. Our results suggested that both biological and chemical approaches are necessary to be included in a battery of tests to assess the eco-environmental risks of sediments.

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Keywords Pearl River · Sediment · Micronucleus (MN) assay · Polycyclic aromatic hydrocarbons (PAHs) · Heavy metals (HMs)

Introduction

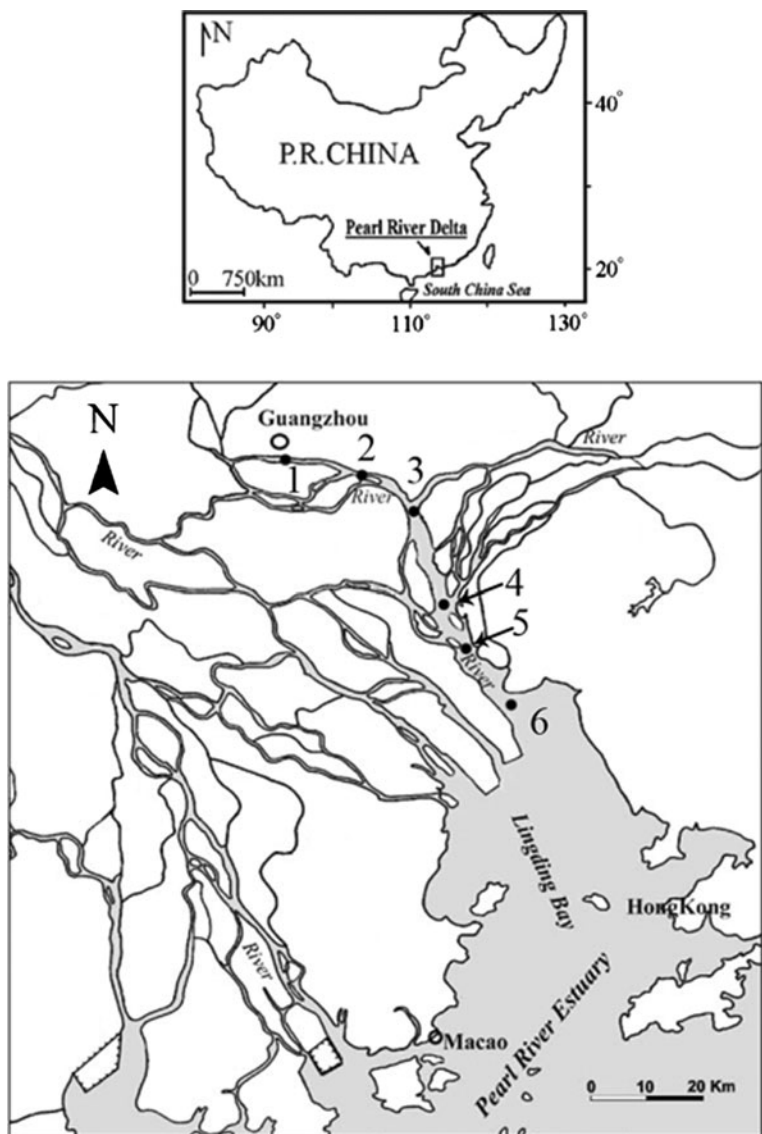
The protection of environment, and in particular the aquatic ecosystem, has become a matter of considerable concern over the past few decades (Wadhia and Clive

Thompson 2007). In recent years scientists have become increasingly concerned about the tropical and subtropical regions of Asia, which are highly populated and generally under rapid economic development and urbanization (Mai et al. 2003). The tropical mild to high temperatures and heavy rainfalls are able to rapidly dissipate pollutants into the atmosphere and aquatic systems from points of usage or discharge (Mai et al. 2003); moreover, high temperatures and increased metabolic rates may enhance their damages to biota (Nasci et al. 1999).

The Pearl River is one of the largest rivers in China, its deltaic region is characterized by a great

number of tributaries and streams, forming a complicated watershed called Pearl River Delta, covering an area of about 8,000 km², within Guangdong Province in Southern China (Fig. 1). Approximately 3.13×10^{11} m³ of freshwater annually flows into the South China Sea (Mai et al. 2002). The water of the Pearl River is used for public and industrial water supply, irrigation, waste disposal, navigation, and watering animals as well as sports and leisure. The industrialization and urbanization have accelerated in the last three decades around Pearl River Delta, as a result of the drastic economic reform initiated in early 1980s. Explosive increases

Fig. 1 Map showing sampling locations in the Pearl River



in industrial and agricultural productivities and growing population have led to serious concerns about whether the environment is adequately protected and the natural resources are properly utilized (Mai et al. 2002). Particular focus is currently being placed on the aquatic environment.

Due to their inherent capacity to absorb contaminants, aquatic sediments are regarded as particularly sensitive to anthropogenic impacts (Kosmehl et al. 2008). Sediments represent a vast sink for contaminants in aquatic systems and can serve as a reservoir of toxic contaminants that continually threaten the health and viability of aquatic biota, and subsequently may provide a starting point for entry into the aquatic food web (Vigano et al. 1995; Chen and White 2004; Geffard et al. 2003; Coughlan et al. 2002; Vigano et al. 2001). Thus, analyses of sediment samples are an important tool for assessing impact of anthropogenic activities on aquatic systems (Mai et al. 2002).

Recent studies of sediment cores and surficial bed sediments in the Pearl River Delta have indicated that the inputs of polycyclic aromatic hydrocarbons (PAHs) (Liu et al. 2005) and heavy metals (HMs) (Gan et al. 2010) parallel the increase in industrialization around the Pearl River Delta. Mai et al. (2002) and Liu et al. (2002) respectively reported that partial areas of the Pearl River have higher sedimentary PAH and/or HMs concentrations, forming “higher risk area”. However, chemical measurements are not per se indicators of toxicity (Vigano et al. 2001). Moreover, literature has documented the difficulty of predicting a toxicological hazard for the aquatic community of a given area using only data on either water or sediment chemistry (Vigano et al. 2001). Therefore, bioassay strategies should be developed with a focus on the most important (eco)toxicological effects. The biological endpoints are chosen according to their importance of known biological targets. Genotoxicity and/or disruption of the genome are the first targets of concern (Schramm et al. 1999). MN assay in *Vicia faba* root cells, which was validated and its protocol was standardized through a program under the International Program on Chemical Safety, is highly sensitive and capable of detecting mutagens, clastogens, and carcinogens from the environment, and showed excellent correlations with tests in the mammalian systems and human lymphocytes systems (Ma 1999; Grant 1994). It has been recommended for use in mutation screening or monitoring by the Royal

Swedish Academy of Sciences, Committee 17 of the Environmental Mutagen Society and the World Health Organization (Grant 1994).

A relatively small number of studies were concerned with establishing causal etiological links between genotoxic and/or carcinogenic effects observed in situ and exposure to contaminated sediments. Establishing such links requires sophisticated ex situ exposures (Chen and White 2004). By the ex situ approach, the crude sample is tested, without any previous treatment, under laboratory conditions by means of a well-known and standardized bioassay. Such an approach has been successfully applied using both plants and aquatic animals. Furthermore, the ex situ approach has the advantage that most of the variables are under control and that the “real” genotoxicity of the complex mixture can be studied (Minissi et al. 1998).

In order to understand and to assess the impact of drastically increased anthropogenic activity on the aquatic ecosystem of the Pearl River, initial efforts are much needed to determine the genotoxicity of the sediments and their contaminants. We attempted to fulfill this objective by measuring the genotoxicity of the sediments collected from the Pearl River by MN assay in *V. faba* root cells in ex situ way, the contents of 16 EPA priority PAHs by gas chromatography–mass spectrometry, and the HMs by the inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). The relationships between the genotoxicity of the sediments and their PAHs and HMs were also discussed. The aim is to provide data on their potential risks on the aquatic environment, and to provide policy maker with better information for planning of sound environmental actions.

Materials and methods

Sample collection

Six sediment samples were collected along the main watercourse of the Pearl River in March 2003, using a stainless grab sampler (Fig. 1, Table 1). Top 20-cm sediments were scooped using a precleaned stainless steel scoop into solvent-rinsed aluminum jars. A previous study obtained the sediment accumulation

Table 1 List of sampling stations

Station	Latitude/longitude	Water depth (m)
1	23°06.27' N/113°17.39' E	4.2
2	23°05.35' N/113°26.05' E	9.7
3	23°02.03' N/113°30.03' E	12.0
4	22°57.43' N/113°32.40' E	6.7
5	22°53.02' N/113°34.18' E	14.0
6	22°48.11' N/113°35.18' E	17.2

rate of 1.87 cm/year for a sediment core (ZJ-9) in Nam Van Estuary (Zhang et al. 2002); hence a 0–20-cm layer of sediments represented approximately 10 years of deposition. All the samples were transported on ice to a laboratory where they were stored at -20°C until analyzed.

Measurement of C, H, and N elements of sediment

Eight to ten milligrams of powder of freeze-dried sediment samples were determined for C, H, and N elements with a CHN-O RAPID elemental analyzer (Heraeus, Germany). Every sample was measured in triplicate. Acetanilide was used as external standard (instrument detection limit = $10 \pm 0.2 \mu\text{g g}^{-1}$ for carbon).

Micronucleus assay in *V. faba* root cells

The protocol published by Ma et al. (1995) was adopted for this study and only a brief description is presented here with minor modifications. *V. faba* seeds (provided by the School of Life Sciences, Huazhong Normal University, P. R. China) were stored at 4°C in dry conditions until use. Before soaking, seeds were disinfected by a short immersion (3 min) in a 5% calcium hypochlorite solution and then thoroughly rinsed five times with distilled water. Seeds soaked in 48-h aerated tap water for 20 h were removed of their coats to germinate between two moist filter papers for 4 days at $22 \pm 2^{\circ}\text{C}$. After removing the tips of the primary roots, the seedlings were transferred to Hoagland's solution maintained at $22 \pm 2^{\circ}\text{C}$ for 4 days to let secondary roots grow. When newly secondary roots reached 1.0–1.50 cm in length, every eight seedlings were treated with the sediments abovementioned for 24 h in darkness at $22 \pm 2^{\circ}\text{C}$. As a

negative control, synthetic clay was mixed up with aerated tap water (Minissi et al. 1998). Potassium dichromate solution ($\text{K}_2\text{Cr}_2\text{O}_7$, 0.01 g/L) was used as the positive (Feng et al. 2007). After a post-exposure recovery period of 44 h, roots were isolated and fixed (at 4°C during 16 h) in freshly prepared 3:1 ethanol/glacial acetic acid mixture. Then, root tips were immersed in distilled water for 5 min and hydrolyzed in 5 M HCl at $22 \pm 2^{\circ}\text{C}$ for 1 h. After subsequent staining in orcein (1%) for 5 min, root tips were prepared for enumerating micronucleated cells. Micronucleated cells were scored on at least 1,000 cells/root and for one root/seedling under a microscope (Eclipse 80i, Nikon, Japan; $\times 400$). Only micronuclei distinctly separated from the main nucleus were scored (Feng et al. 2007). MN frequency (MN%) was calculated as follows:

$$\text{MN}\% = \frac{\text{number of cells containing micronucleus}}{\text{total number of cells counted}} \times 1,000 \quad (1)$$

Statistical differences between control and exposed groups were determined with Dunnett's *t* test (Feng et al. 2007).

PAHs analysis

The analytical procedure used for extraction, separation, and measurement of PAHs in sediment samples was detailed elsewhere (Mai et al. 2002, 2003), and

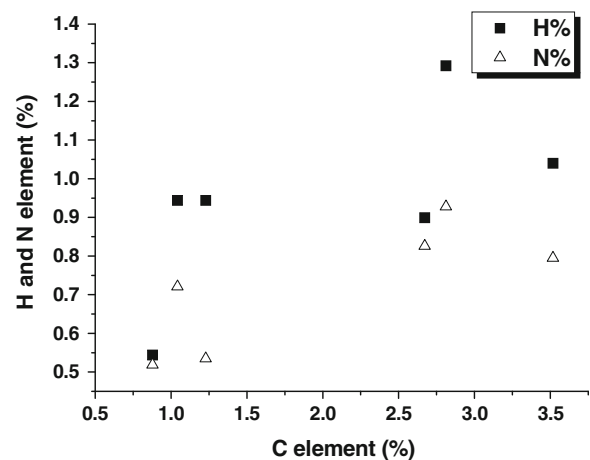


Fig. 2 The correlation between C and H and N elements in the sediments from the Pearl River

Table 2 Micronucleus frequencies in *V. faba* root cells exposed by the sediments from the Pearl River

Tested materials	Numbers of cells observed	Micronucleus frequencies (‰)
S1	8,550	7.5±2.0**
S2	9,042	8.8±2.9**
S3	9,051	8.8±2.8**
S4	10,050	6.2±1.8**
S5	10,067	4.6±1.5*
S6	8,378	5.5±1.7*
Negative control	9,154	2.5±1.0
Positive control	10,522	15.5±2.6**

* $P < 0.05$; ** $P < 0.01$ (significant differences between negative control and exposed groups by Dunnett's *t* test)

only a brief description is given here. A mixture of deuterated PAH compounds (naphthalene- d_8 , acenaphthene- d_{10} , phenanthrene- d_{10} , chrysene- d_{12} , and perylene- d_{12}) as recovery surrogate standards was added to all the samples prior to extraction. Freeze-dried sediment samples were Soxhlet-extracted with methylene chloride. The extracts were concentrated, solvent-exchanged to hexane, and purified using a 1:2 alumina/silica column chromatography. The first fraction, containing aliphatic hydrocarbons, was eluted with 15 mL of hexane. The second fraction containing PAHs was collected by eluting 5 mL of hexane and 70 mL of methylene chloride/hexane (30:70). The PAH fraction was con-

centrated to 0.4 mL under a gentle N_2 stream. A known amount of internal standard (hexamethylbenzene) was added to the extract prior to instrumental analysis.

Concentrated of PAHs were determined with a Hewlett-Packard 5890 series gas chromatograph/5972 mass spectrometer in the selective ion monitoring mode, and chromatographic separation was provided by a 50 m×0.32 mm-i.d. (0.17 μ m film thickness) DB-5MS capillary column (J&W Scientific, Folsom, CA). Detailed instrumental conditions were described previously (Mai et al. 2002, 2003). Quantification was done using the internal calibration method (five-point calibration). Surrogate recoveries were 58±12% for naphthalene- d_8 , 75±10% for acenaphthene- d_{10} ,

Table 3 The concentrations (ng g⁻¹ dry wt.) of the priority PAHs in the sediments from the Pearl River

	The numbers of sediment samples					
	1	2	3	4	5	6
Naphthalene	21.6	20	20.8	15	14.2	21.4
Acenaphthylene	170	229.2	129.6	23.2	8.6	16.6
Acenaphthene	3.2	4.8	3.2	1	1	0.6
Fluorene	7.2	10.8	8	0.8	1.8	2
Phenanthrene	200.4	25.6	18	5.4	3.2	4.2
Anthracene	173.2	243.6	111.2	28.4	13	17.6
Fluoranthene	11.6	200	10	2.6	1.2	1.4
Pyrene	18.8	29.2	14.8	4.4	2	2.2
Benzo (a) anthracene	28.4	2,390.8	23.2	5	1.8	15.8
Chrysene	18	3,060	15.2	4.4	1	24.8
Benzo (b) fluoranthene	513.6	2,049.2	239.2	127.2	16.8	165
Benzo (k) fluoranthene	139.6	480.8	67.6	33.8	5.4	42.6
Benzo (a) pyrene	127.6	1,634	84.8	41	5.4	84.2
Indeno (1,2,3-cd) pyrene	205.2	2,611.6	198.8	199.2	89.4	226.8
Dibenz (a,h) anthracene	16.8	520	149.6	36.4	15.8	26.2
Benzo (g,h,i) perylene	64.8	146.4	70.4	215.2	69.4	231.8
ΣPAHs	1,720	13,656	1,164.4	743	250	883.2

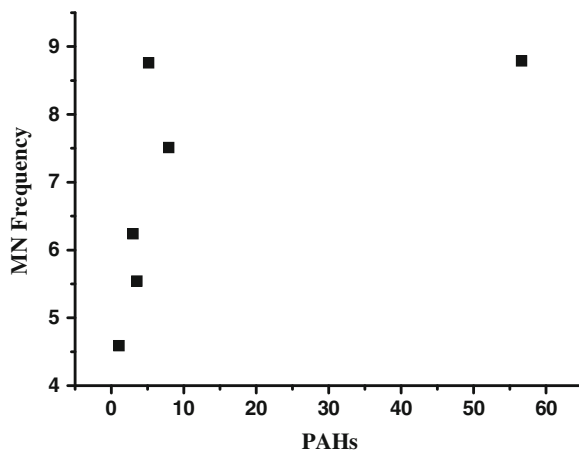


Fig. 3 The correlation between MN frequencies (%) and molar concentrations of the total priority PAHs ($\mu\text{mol kg}^{-1}$, dry wt.) in the sediments from the Pearl River

$93\pm 8\%$ for phenanthrene- d_{10} , $94\pm 10\%$ for chrysene- d_{12} , and $90\pm 9\%$ for perylene- d_{12} with sediment samples ($n=6$).

For each batch of six field samples, a procedural blank (solvent with a clean GF/F filter), a spiked blank (16 PAH standards spiked sample into solvent with a clean GF/F filter), a matrix spiked sample (16 PAH standards spiked sample into sediment), a matrix spiked duplicate, a sample duplicate, and National Institute of Standards and Technology (NIST; Gaithersburg, MD) 1941 reference sample were processed. The relative percent difference for individual PAHs identified in paired duplicate sample ($n=4$) was all $<15\%$. Recoveries of all the PAHs in the NIST 1941 sample were between 80% and 120% of the certified values ranged from 0.2 to 2 ng g^{-1} (dry wt.) for 10 g of sediment. Actual reporting limits were adjusted based on the sample sizes used.

Table 4 The concentrations ($\mu\text{g kg}^{-1}$ dry wt.) of heavy metals in the sediments from the Pearl River

	The numbers of sediment samples					
	1	2	3	4	5	6
As	36,639	24,420	47,265	26,390	22,770	28,334
Cr	115,523	69,647	133,343	46,101	39,333	52,811
Cu	153,931	97,034	159,270	54,648	36,145	50,069
Pb	122,819	166,642	114,185	68,208	51,210	60,927
Cd	1,381.7	1,809.5	2,630.9	1,818.9	475.4	789.7
Hg	190.8	404.1	460.8	93.1	59.9	69.5
Se	1,206.7	929.2	1,250.4	1,173.3	331.7	654.4

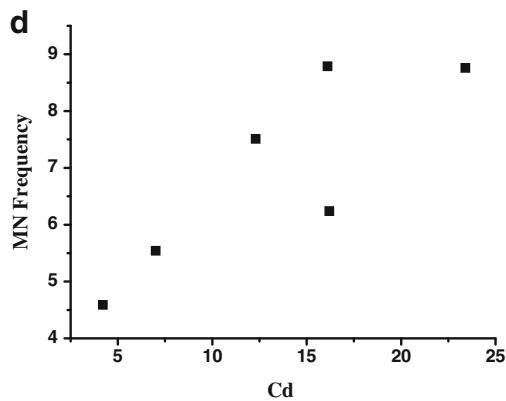
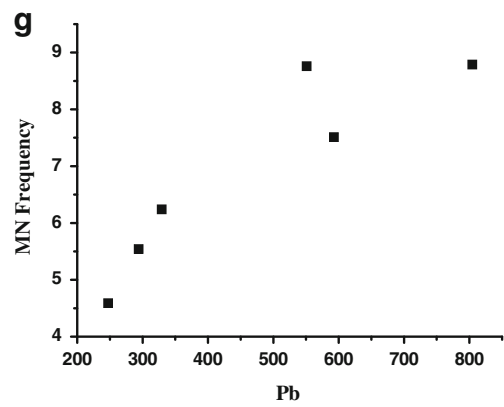
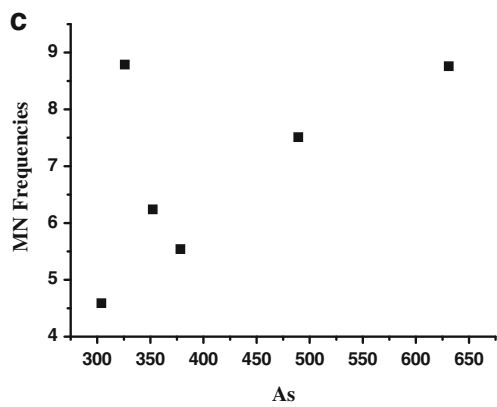
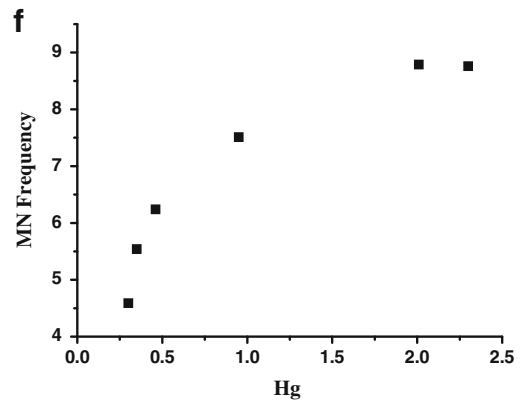
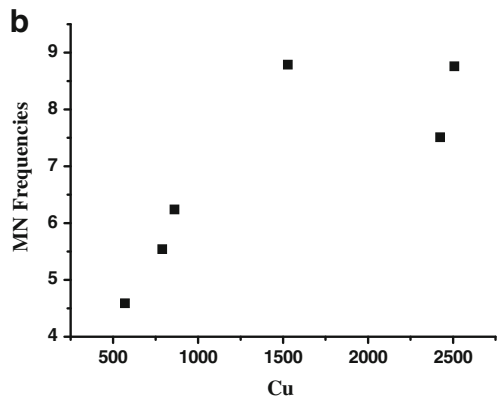
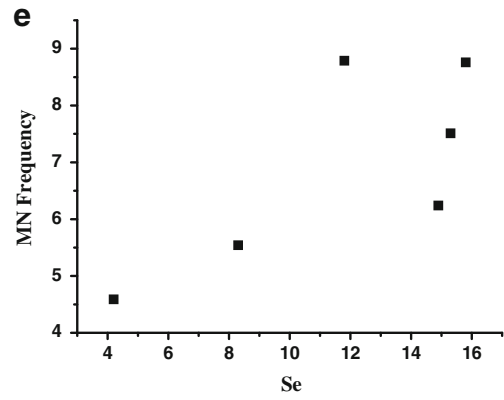
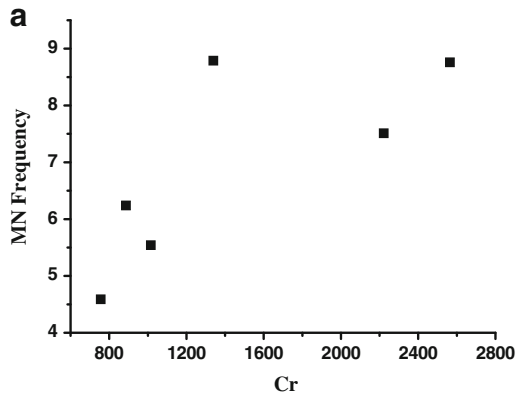
Fig. 4 a–g The correlation between MN frequencies (%) and molar concentrations of heavy metals ($\mu\text{mol kg}^{-1}$, dry wt.) in the sediments from the Pearl River

Heavy metals analysis

The freeze-dried sediment samples were sent to the Instrumental Analysis and Research Center of Sun Yat-Sen University for microwave digestion. The metal elements in the sediments were determined by Perkin Elmer Elan 6000 ICP-MS and Varian Vista ICP-AES in the Laboratory of Isotope Geochronology and Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, with external standard calibrations (Wei et al. 2003b, a). Analytical precision was better than 5% for the metal elements except Cd (better than 10%). Several Chinese rock standards (GSR-1, GSR-2, GSR-3, GSR-4, GSR-5, and GSR-6) and some Chinese and USGS sediment standards (GSD-9, GSD-12, and MAGI), which have different mineralogy and a fairly large span of element concentrations (Wei et al. 2003a), were repeatedly measured along with the samples for quality control. Accuracy was within $\pm 5\%$ for the metal elements except Cd ($\pm 15\%$).

Results and discussion

The weight percent of C, H, and N elements in the sediments collected from the Pearl River were shown in Fig. 2. The clear correlation between C and H and N elements suggested that they were mainly in organic speciation in the sediments (C/H, $r=0.63$; C/N, $r=0.79$; and H/N, $r=0.78$).



Many of the pollutants found in the aquatic environment are known genotoxic and carcinogenic substances. These substances may interact, directly or after metabolic activation, with DNA or DNA processing machinery and thereby induce the cytogenetic alterations in the cells of organisms (Chen and White 2004). MN is an important biomarker of cytogenetic damages in the cells of organisms (Fenech 1998). In this study, the results of MN assay with *V. faba* were listed in Table 2. There were significant increases of MN frequencies observed in the sediment-exposed groups, compared with the negative group ($P < 0.05$, $P < 0.01$), indicating that the sediments clearly had genotoxicity to the *V. faba* root cells, and that the sediment-associated genotoxic substances are available for exposure to biota. Baumann (1998) had reported there was a cause-and-effect relationship between exposure to genotoxins in sediment and water and neoplasm epizootics in wild fish populations. Recent studies have also shown that exposure to genotoxic substances in environments can enhance the frequency of heritable recessive lethal mutations, and the accumulation of such deleterious mutations can contribute to the decline of small populations via a phenomenon known as mutational meltdown (Wirgin and Waldman 1998; Chen and White 2004). Thus, study of cancer epizootics in fish in the Pearl River and the ecological implications of in situ are of great value for further research.

The 16 USEPA priority PAHs have been used to evaluate anthropogenic pollution levels in environment (Mai et al. 2002). The individual concentrations measured of the 16 priority PAHs were listed in Table 3. The total PAHs concentrations ranging from 250 to 13,656 ng g⁻¹ (dry weight) were close to those obtained from other urbanized and industrialized areas, such as Brisbane River (3,940–16,110 ng g⁻¹), Georges River (56–21,400 ng g⁻¹), Tamar Estuary (430–14,070 ng g⁻¹), and Casco Bay (215–14,400 ng g⁻¹), which have been considered moderately polluted (Mai et al. 2002). Nevertheless, these are relatively low as compared to those found in highly polluted areas, such as Boston Harbor (483–718,000 ng g⁻¹), Chesapeake Bay (555–178,000 ng g⁻¹), and New Bedford Harbor (14,000–170,000 ng g⁻¹) (Mai et al. 2002).

As Mai (Mai et al. 2002) have reported, the PAH composition pattern were different among the sample stations in this study suggested that the PAH influx to the river is dominated by local ongoing inputs from

different sources. Moreover, the spatial distribution of PAHs also suggested that sediment contamination in this study area were dictated more predominantly by anthropogenic inputs than by natural process. Noticeably, the second sediments contained a substantially higher concentration of PAHs (13,656 ng g⁻¹) than other sediments (250–1,720 ng g⁻¹), and showed the highest concentrations for most of the PAH classes in this study. Because this sample station is near the Zhongshan Dock, an important dock of Guangzhou city, which may converge many pollutants from different sources, such as industrial emission and effluents, municipal runoff, and harbor traffic. However, the fifth sample station is located in a rural area, so the PAH content was the lowest in this study.

Many researchers had attempted to relate measured mutagenic or carcinogenic potential with PAH contamination (Chen and White 2004). Some researchers had reported that PAHs were the predominant contributors to Σ TEQs in sediments, contributing 10–240 times greater TEQs than those by PCDDs/DFs (Nakata et al. 2003). Moreover, White (2002) and Landrum et al. (2003) reported that the genotoxicity of the priority PAHs is additive, acting at the same molar concentration whether present as individual compounds or in mixture. Therefore, the mutagenic risk posed by mixtures of the priority PAHs can reasonably be estimated as the sum of the risks posed by the mixture components.

In this study, MN frequencies were compared with the total measured priority PAHs in Fig. 3. Although there was a tendency of MN frequencies in *V. faba* root cells increasing with the increase of PAH concentrations in the sediments, the correlation between MN potency and PAH contamination was not very strong. This suggested that the priority PAHs might only contribute to a portion of the genotoxicity of the sediments to *V. faba* root cells, and the rates of their contribution are variable in the different sites.

In the aquatic sediments, HMs also are among the major groups of genotoxic pollutants that may pose serious threat to human as well as environmental well being (Patra et al. 2003). In this study, the concentrations of the HMs in the sediments from the Pearl River were listed in Table 4. The concentrations of As (22,770–36,639 $\mu\text{g kg}^{-1}$), Cr (39,333–133,343 $\mu\text{g kg}^{-1}$), Cu (36,145–159,270 $\mu\text{g kg}^{-1}$), and Pb (51,210–166,642 $\mu\text{g kg}^{-1}$) were one to two

orders of magnitude higher than those of Cd (475.4–1,818.9 $\mu\text{g kg}^{-1}$), Hg (59.9–460.8 $\mu\text{g kg}^{-1}$), and Se (331.7–1,250.4 $\mu\text{g kg}^{-1}$) in the sediments. The first, second, and third sites, which are in or near Guangzhou, had the highest concentrations of the total and/or individual HM in this study. The fifth site had the lowest HM concentrations. Huang and Wai (2004) indicated that the sources and inputs of HMs to the Pearl River were complex, mainly including wastewater metallurgical industry; electroplate industry and corrosion of metal equipment in ports, boats, and ships; overland runoff of mining areas upstream; and so on. In this study, the variable HM compositions in the sediments also indicated that the HM influx to these sediments was complex and variable (Table 4).

The HMs (Cr, Cu, As, Se, Cd, Hg, and Pb), which were determined in the sediments in this study, have the DNA-damaging effects on plant cells (Majer et al. 2002; White and Claxton 2004). The correlations between the molar concentrations of these HMs and MN frequencies were compared in the Fig. 4a–g. There was a clear tendency of MN frequencies in *V. faba* root cells increasing with the increase of the HM concentrations in the sediments. Except As element ($r=0.58$), the coefficient of the correlation between the molar concentrations of other HMs and MN frequencies in *V. faba* root cells were more than 0.7 (Cr: $r=0.75$, Cu: $r=0.82$, Se: $r=0.74$, Cd: $r=0.85$, Hg: $r=0.94$, and Pb: $r=0.91$). This suggested that these HMs may have higher contribution to the genotoxicity of the sediments than that of the priority PAHs. Noticeably, Hg and Pb had the highest correlation with the MN frequencies, indicating that they may be the most important factors that posed the sediments higher genotoxicity to *V. faba* root cells. Although Hg had the lowest molar concentrations among these HMs in the sediments, however, it may contribute to a more portion of genotoxicity of the sediments and become of great concern on the Pearl River. This also suggested that combining the biological and chemical approaches is necessary to assess the eco-environmental risks of sediments.

However, it is currently difficult to establish definitive causal etiological links between observed genotoxic effects exposure to the contaminated sediments and these HMs and/or the priority PAHs. Because the sediments were of complex mixture,

they may contain many kinds of contaminants that can induce the damage of DNA or chromosomes in the plant cells. Moreover, many factors, such as the site-specific physical, chemical, and mineralogical characteristics of sediments and physical–chemical nature of contaminants, have great effects on the bioavailability of contaminants in the sediments (Baumard et al. 1998; Geffard et al. 2003). The presence of other toxic organic contaminants and metals in the sediments may also have adverse effects on the *V. faba* root cells. Therefore, it is also important to carry out further investigation on how these factors affect the genotoxicity of the sediments.

Conclusion

The sediments collected from the Pearl River clearly had genotoxicity to the *V. faba* root cells. The total measured priority PAHs concentrations and the contents of HMs in the sediments were close to those obtained from other urbanized and industrialized areas, which have been documented moderately polluted. The HMs had a higher contribution to the genotoxicity of the sediments than that of the priority PAHs. Hg and Pb may be the most important factors that posed the sediments higher genotoxicity to *V. faba* root cells, although the definitive causal etiological links have not been established. It is of great interest to further investigate the presence of other toxic organic contaminants and/or heavy metals in the sediments and their bioavailability. Our results suggested that both biological and chemical approaches are necessary to assess the eco-environmental risks of sediments.

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References

- Baumann, P. C. (1998). Epizootics of cancer in fish associated with genotoxins in sediment and water. *Mutation Research*, 411(3), 227–233.

- Baumard, P., Budzinski, H., & Garrigues, P. (1998). PAHs in Arcachon Bay, France: Origin and biomonitoring with caged organisms. *Marine Pollution Bulletin*, 36(8), 577–586.
- Chen, G., & White, P. A. (2004). The mutagenic hazards of aquatic sediments: A review. *Mutation Research*, 567(2–3), 151–225.
- Coughlan, B. M., Hartl, M. G., O'Reilly, S. J., Sheehan, D., Morthersill, C., van Pelt, F. N., et al. (2002). Detecting genotoxicity using the Comet assay following chronic exposure of Manila clam *Tapes semidecussatus* to polluted estuarine sediments. *Marine Pollution Bulletin*, 44(12), 1359–1365.
- Fenech, M. (1998). Important variables that influence baseline micronucleus frequency in cytokinesis-blocked lymphocytes—A biomarker for DNA damage in human populations. *Mutation Research*, 404, 155–165.
- Feng, S., Wang, X., Wei, G., Peng, P., Yang, Y., & Cao, Z. (2007). Leachates of municipal solid waste incineration bottom ash from Macao: Heavy metal concentrations and genotoxicity. *Chemosphere*, 67, 1133–1137.
- Gan, H., Liang, K., & Zheng, Z. (2010). Background values, contamination assessment and zoning of heavy metals in sediments of the Pearl River Estuary. *Earth and Environment*, 38(3), 344–350.
- Geffard, O., Geffard, A., His, E., & Budzinski, H. (2003). Assessment of the bioavailability and toxicity of sediment-associated polycyclic aromatic hydrocarbons and heavy metals applied to *Crassostrea gigas* embryos and larvae. *Marine Pollution Bulletin*, 46(4), 481–490.
- Grant, W. F. (1994). The present status of higher plant bioassays for the detection of environmental mutagens. *Mutation Research*, 310(2), 175–185.
- Huang, S., & Wai, O. W. H. (2004). A review of heavy metal pollution in the Pearl River Estuary. *Journal of Hydrodynamics, Ser. B*, 16(4), 367–378.
- Kosmehl, T., Hallare, A. V., Braunbeck, T., & Hollert, H. (2008). DNA damage induced by genotoxicants in zebrafish (*Danio rerio*) embryos after contact exposure to freeze-dried sediment and sediment extracts from Laguna Lake (The Philippines) as measured by the comet assay. *Mutation Research*, 650(1), 1–14. doi:10.1016/j.mrgentox.2007.09.009.
- Landrum, P. F., Lotufo, G. R., Gossiaux, D. C., Gedeon, M. L., & Lee, J. H. (2003). Bioaccumulation and critical body residue of PAHs in the amphipod, *Diporeia* spp additional evidence to support toxicity additivity for PAH mixtures. *Chemosphere*, 51(6), 481–489. doi:10.1016/S0045-6535(02)00863-9.
- Liu, F., Yan, W., Wang, W., Gu, S., & Chen, Z. (2002). Pollution of heavy metals in the Pearl River Estuary and its assessment of potential ecological risk. *Marine Environmental Science*, 21(3), 34–38.
- Liu, G. Q., Zhang, G., Li, X. D., Li, J., Peng, X. Z., & Qi, S. H. (2005). Sedimentary record of polycyclic aromatic hydrocarbons in a sediment core from the Pearl River Estuary, South China. *Marine Pollution Bulletin*, 51(8–12), 912–921.
- Ma, T. H. (1999). The international program on plant bioassays and the report of the follow-up study after the hands-on workshop in China. *Mutation Research*, 426(2), 103–106.
- Ma, T. H., Xu, Z., Xu, C., McConnell, H., Rabago, E. V., Arreola, G. A., et al. (1995). The improved Allium/Vicia root tip micronucleus assay for clastogenicity of environmental pollutants. *Mutation Research*, 334(2), 185–195.
- Mai, B., Fu, J., Sheng, G., Kang, Y., Lin, Z., Zhang, G., et al. (2002). Chlorinated and polycyclic aromatic hydrocarbons in riverine and estuarine sediments from Pearl River Delta, China. *Environmental Pollution*, 117, 457–474.
- Mai, B., Qi, S., Zeng, E. Y., Yang, Q., Zhang, G., Fu, J., et al. (2003). Distribution of polycyclic aromatic hydrocarbons in the coastal region off Macao, China: Assessment of input sources and transport pathways using compositional analysis. *Environmental Science and Technology*, 37(21), 4855–4863.
- Majer, B. J., Tschерko, D., Paschke, A., Wennrich, R., Kundi, M., Kandeler, E., et al. (2002). Effects of heavy metal contamination of soils on micronucleus induction in *Tradescantia* and on microbial enzyme activities: A comparative investigation. *Mutation Research*, 515(1–2), 111–124.
- Minissi, S., Caccese, D., Passafiume, F., Grella, A., Eleonora, C., & Rizzoni, M. (1998). Mutagenicity (micronucleus test in *Vicia faba* root tips), polycyclic aromatic hydrocarbons and heavy metal content of sediments collected in Tiber river and its tributaries within the urban area of Rome. *Mutation Research*, 420(1–3), 77–84.
- Nakata, H., Sakai, Y., Miyawaki, T., & Takemura, A. (2003). Bioaccumulation and toxic potencies of polychlorinated biphenyls and polycyclic aromatic hydrocarbons in tidal flat and coastal ecosystems of the Ariake Sea, Japan. *Environmental Science and Technology*, 37(16), 3513–3521.
- Nasci, C., Da Ros, L., Campesan, G., van Vleet, E. S., Salizzato, M., Sperti, L., et al. (1999). Clam transplantation and stress-related biomarkers as useful tools for assessing water quality in coastal environments. *Marine Pollution Bulletin*, 39, 255–260.
- Patra, J., Sahoo, M. K., & Panda, B. B. (2003). Persistence and prevention of aluminium- and paraquat-induced adaptive response to methyl mercuric chloride in plant cells in vivo. *Mutation Research*, 538(1–2), 51–61.
- Schramm, K.-W., Hofmaier, A., Klobasa, O., Kaune, A., & Kettrup, A. (1999). Biological in vitro emission control. *Journal of Analytical and Applied Pyrolysis*, 49, 199–210.
- Vigano, L., Arillo, A., De Flora, S., & Lazorchak, J. (1995). Evaluation of microsomal and cytosolic biomarkers in a seven-day larval trout sediment toxicity test. *Aquatic Toxicology*, 31, 189–202.
- Vigano, L., Arillo, A., Falugi, C., Melodia, F., & Polesello, S. (2001). Biomarkers of exposure and effect in flounder (*Platichthys flesus*) exposed to sediments of the Adriatic sea. *Marine Pollution Bulletin*, 42(10), 887–894.
- Wadhia, K., & Clive Thompson, K. (2007). Low-cost ecotoxicity testing of environmental samples using microbioassays for potential implementation of the water framework directive. *Trends in Analytical Chemistry*, 26(4), 300–3006.
- Wei, G., Liu, Y., Li, X., Shao, L., & Liang, X. (2003a). Climatic impact on Al, K, Sc and Ti in marine sediments:

- evidence from ODP site 1144, South China Sea. *Geochemical Journal*, 37, 593–602.
- Wei, G., Liu, Y., & Li, X. J. (2003b). High-resolution elemental records from the South China Sea and their paleoproductivity implications. *Paleoceanography*, 18, 1054–1065.
- White, P. A. (2002). The genotoxicity of priority polycyclic aromatic hydrocarbons in complex mixtures. *Mutation Research*, 515(1–2), 85–98.
- White, P. A., & Claxton, L. D. (2004). Mutagens in contaminated soil: A review. *Mutation Research*, 567(2–3), 227–345.
- Wirgin, I., & Waldman, J. R. (1998). Altered gene expression and genetic damage in North American fish populations. *Mutation Research*, 399(2), 193–219.
- Zhang, G., Parker, A., House, A., Mai, B., Li, X., Kang, Y., et al. (2002). Sedimentary records of DDT and HCH in the Pearl River Delta, South China. *Environmental Science and Technology*, 36(17), 3671–3677.