

# Tracing anthropogenic contamination in the Pearl River estuarine and marine environment of South China Sea using sterols and other organic molecular markers

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## Abstract

5 $\beta$ -Coprostanol together with eight other sterols and unresolved complex mixtures (UCMs) were quantitatively investigated for surficial sediments and surface waters to assess the impacts of anthropogenic activities on the Pearl River estuarine and marine environment of South China Sea. The studied area extends from the Pearl River Estuary southward to the open sea. 5 $\beta$ -Coprostanol concentrations ranged from trace amounts to 53  $\mu\text{g g}^{-1}$  TOC in surficial sediments. The highest levels and highest percentages of coprostanol were found in the Pearl River estuary, especially in the inner estuary and those sites close to the submarine outfalls of Hong Kong. For waters, only in estuarine samples was coprostanol quantitatively detected, ranging from 11 to 299  $\text{ng L}^{-1}$ . Bimodal UCM “humps” were observed for most sediment samples, with concentrations ranging from 215 to 10,491  $\mu\text{g g}^{-1}$  TOC in sediments and from 2 to 26  $\mu\text{g L}^{-1}$  in waters, respectively. Progressive seaward declines in concentrations were found for both 5 $\beta$ -coprostanol and UCM in surficial sediments. Trace or no 5 $\beta$ -coprostanol was found in open-sea samples. Concentrations of coprostanol and UCM in surficial sediments are correlated. These results imply that there are obvious anthropogenic contaminations in the Pearl River estuary. The submarine outfalls in Hong Kong represent important sources of the sewage pollution to the Pearl River estuarine sediments evidenced by a combination of coprostanol concentration, diagnostic indices, sterol profiles and UCM. No obvious dispersion or transport of the sewage contamination occurred from the Pearl River estuary to the open South China Sea indicated by fecal sterol biomarkers.

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**Keywords:** The Pearl River estuary; The South China Sea; Sterol; UCM; Sediments and waters; Anthropogenic contamination

## 1. Introduction

Contaminations caused by human activities in the coastal and marine environment have been becoming an increasing concern in recent years (Maldonado

et al., 2000; Eganhouse and Sherblom, 2001; Carreira et al., 2004). Among those sewage contamination is especially important in tropical and subtropical Asia, because it is possibly related with many waterborne diseases that are widespread in these regions (Isobe et al., 2002). Coliform bacteria have been used as the indicator of sewage pollution for several decades. However, the method suffers from some major constraints, such as rapid biodegradation in marine environment and consequent hourly fluctuations in their number (Mudge and

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Lintern, 1999). In addition, fecal contamination from municipal sewage discharge cannot be unambiguously differentiated from those resulting from other animals' feces only by coliform bacteria (Leeming et al., 1996).

Organic molecular markers are organic compounds that maintain sufficient structural integrity for their sources to be recognized (Eganhouse, 1997). They have been proven useful tools for investigating the potential impacts of wastewater discharges to the oceans. 5 $\beta$ -Coprostanol, mainly coming from human feces (comprising 40–60% of the total fecal sterols excreted in human wastes) has been applied successfully to trace sewage pollution in diverse environments due to its source specificity and environmental stability (Grimalt et al., 1990; Vankatesan and Kaplan, 1990; Takada et al., 1994; Chalaux et al., 1995; Leeming et al., 1996; Nichols et al., 1996; Mudge and Lintern, 1999; Patton and Reeves, 1999; Maldonado et al., 2000; Eganhouse and Sherblom, 2001; Isobe et al., 2002; Peng et al., 2002; Carreira et al., 2004). Furthermore, Sterols in human feces show distribution profile considerably different from those in animals' excretion and plant sterols, therefore fingerprinting the fecal sterols together with other structurally related sterols can provide particularly useful information for source identification of fecal environmental pollution (Leeming et al., 1996; Isobe et al., 2002).

Petroleum and its refined products are another group of important contaminants in marine and coastal environments (Meyers and Ishiwatari, 1993; Wang et al., 1998; Rowland et al., 2001; Reddy et al., 2002; Readman et al., 2002). Petrogenic byproducts enter marine and estuarine environments from oil spills, urban runoff, atmospheric deposition as well as waste discharge. Chemical fingerprintings of terpanes, steranes, PAHs and isoprenoids and determination of diagnostic ratios have been successfully used to trace pollutions by petrogenic hydrocarbons in the environment (Page et al., 1998; Zakaria et al., 2000; Wang and Fingas, 2003). Also, unresolved complex mixtures (UCM), widely present in oils and refined products can provide useful information for differentiating and correlating source oils and contaminations (Wang et al., 1999; Rowland et al., 2001; Reddy et al., 2002). UCM, consisted of recalcitrant petroleum compounds, are usually more abundant than the resolved hydrocarbons and often dominate gas chromatograms of weathered petroleum. They are widespread in environmental samples (Rowland et al., 2001; Reddy et al., 2002). The potential toxicity of UCM is being of great ecological concern in recent years (Rowland et al., 2001). Generally, different sources and types of petroleum have different carbon number ranges and shapes of UCM. In some instances when samples have multiple sources of petroleum contamination, there can find

multiple UCM humps in the chromatograms (Meyers and Ishiwatari, 1993). Recently the comprehensive two-dimensional GC (GC $\times$ GC) technique has been developed to explore the chemical composition of UCM to evaluate the extent of UCM weathering and assess its toxicity (Rowland et al., 2001; Reddy et al., 2002).

Pearl River Estuary (PRE) is one of the largest estuaries in the world, covering an area of 8000 km<sup>2</sup> (Zhang et al., 2002). The estuary runs southward into the South China Sea. Within the estuary, there exists a south-westward coastal current (called the South China coastal current), originated from the counterclockwise Coriolis force in the Northern Hemisphere. Pearl River Delta (PRD) region is one of the most developed and highly industrialized in China, and even in Southeastern Asia. More than 20 million people live in this region. Important cities include Guangzhou, Hong Kong, Shenzhen, Zhuhai and Macao. Organic pollutants (including organochlorine pesticides (OCPs) and PAHs) detected in the estuarine and the marine sediments are possibly resulting from industrial, agricultural and municipal wastewater discharge, atmospheric deposition and urban runoff (Zhang et al., 2002; Mai et al., 2003). In a previous study, we reported as high as 919 ng g<sup>-1</sup> dry weight of coprostanol in sediments in the coast of Macao (Peng et al., 2002).

In recent years, the Hong Kong government has 25 submarine outfalls (including 12 screening, 9 biological, 2 primary, and 2 chemical) to release the municipal sewage into the South China Sea in order to have cleaner beaches and marine water (<http://www.epd.gov.hk/epd/environmentinhk/>). Hong Kong has a population of 6 million. The discharges totalize approximately 90 $\times$ 10<sup>8</sup> m<sup>3</sup>/year of treated effluent. It was reported that discharge of municipal wastewaters to the marine environment through submarine outfalls represents a potentially important source of large inputs of anthropogenically organic compounds due to sedimentation, resuspension and transportation by coastal currents (Maldonado et al., 2000). It is therefore important to study the fate of these organic pollutants in order to evaluate the environmental impacts of these effluent discharges. But little data has been reported so far about the possible effects of the discharge from these submarine outfalls on the PRE estuarine and marine environments of the South China Sea.

In this study, the distribution of fecal sterols and sterol profiles, total petroleum hydrocarbon (TPH) and UCM in the estuarine and marine sediments and surface waters of the South China Sea were quantitatively investigated. The result will be used to assess the anthropogenic impacts on the PRE estuarine and marine environments of South China Sea induced by sewage discharge and other human activities.

## 2. Method and material

### 2.1. Study area

The study area is located geographically between 113.4° and 114.8° E and 20.8° and 22.7° N, starting at the Pearl River mouth, and extending spokewise toward the open South China Sea. The water depth goes from 7 m to 85 m. We divided the area into four strata according to the distance from the inland water: inner estuary (navigational channel, surrounded by densely populated cities), outer estuary, inner shelf and outer shelf (Fig. 1).

### 2.2. Sampling

#### 2.2.1. Surficial sediment samples

Surficial sediments were collected by a stainless steel grab sampler during a voyage in July, 2002. The sampling sites were designed as such to cover four strata.

Top 5 cm sediments were scooped into solvent-rinsed aluminum jars, and then sealed in zip-lock polyethylene bags and transported on ice to the laboratory where they were stored at  $-20^{\circ}\text{C}$  until further analysis.

#### 2.2.2. Surface water samples

Surface water samples were collected with amber glass bottles (1.5 L) during a voyage from 19 to 23 January, 2003. Glass bottles were pre-rinsed with distilled acetone and then with de-ionic water. A few drops of hydrochloric acid (0.1 M) were added to each sample to prevent possible biodegradation. All water samples were stored at  $4^{\circ}\text{C}$  before being treated within two days after they were transported to the laboratory.

### 2.3. Analytical procedure

#### 2.3.1. Chemicals and apparatus

All glassware and apparatus were baked at  $450^{\circ}\text{C}$  or rinsed with redistilled methanol (MeOH), hexane and

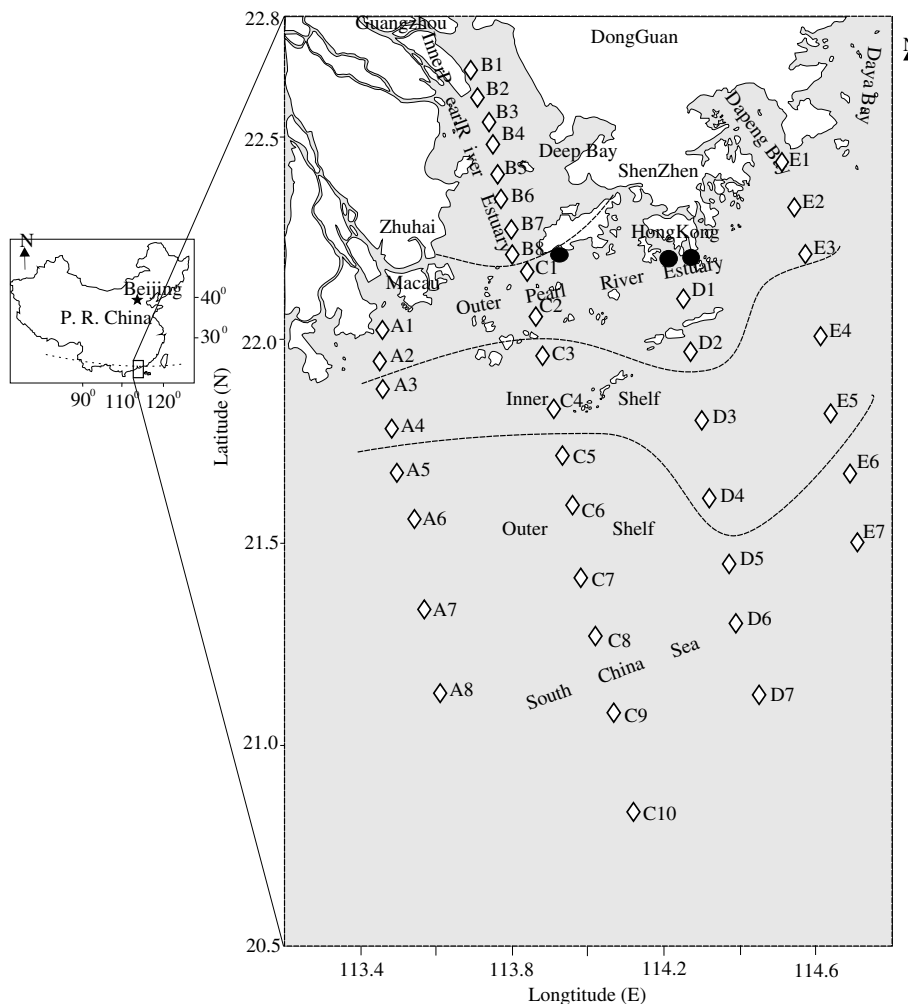


Fig. 1. The sketch map of the study area and sampling sites: (●) submarine outfalls of Hong Kong.

dichloromethane (DCM) prior to use. All solvents, DCM, hexane, MeOH and ethyl acetate were redistilled before use. Neutral silica gel and alumina (80–100 mesh) were treated with mixed solvent of MeOH and DCM (1:1) by Soxhlet extraction for 72 h, dried at 50 °C, activated at 180 °C and 250 °C overnight respectively and then deactivated with 5% (w/w) of deionized water. Anhydrous sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) was baked at 500 °C. Sterol standards (5 $\beta$ -coprostanol, coprostanone, cholesterol, ergosterol, campesterol, stigmasterol, lanostanol,  $\beta$ -sitosterol and stigmastanol), derivatization agent bis-trimethyl-silyl trifluoroacetamide (BSTFA), internal standards deuterated eicosane ( $\text{C}_{20}\text{D}_{42}$ ), and normal tetracosane ( $\text{C}_{24}\text{H}_{50}$ ) were purchased from Sigma (<http://www.sigma-aldrich.com>). Androstanol from Steraloids Inc. (<http://www.steraloids.com/>) was used as recovery surrogate. Authentic standard mixture of 9 sterols was prepared in DCM.

### 2.3.2. Elemental analysis of surficial sediments

Total organic carbon (TOC) and total nitrogen (TN) of the surficial sediments were determined using a CHN-O RAPID elemental analyzer (Heraeus, Germany) after thorough decalcification by hydrochloric acid.

### 2.3.3. Extraction

Sediments were lyophilized, thawed and homogenized before accelerated solvent extraction under 1500 Pa and 100 °C (ASE 300, Dionex, USA) with 100 mL of re-distilled DCM. A known amount of androstanol was added as the recovery surrogate prior to extraction. HCl-activated copper was used to remove elemental sulfur in the extracts. Previous studies have shown that esterified coprostanol comprises only 8–15% of total sterols in sewage and the proportion of esters in environmental samples is even smaller (Isobe et al., 2002). Saponification process was therefore omitted in this work. The extracts were reduced to about 2 mL by rotary evaporation and then the solvents were exchanged to hexane before concentrated to 1 mL.

Water samples were spiked with androstanol as the recovery surrogate and liquid–liquid extracted with 30 mL DCM three times using a Teflon separate funnel. The extracts were combined and concentrated to a small volume. Extra water in the extracts was removed with prebaked anhydrous sodium sulfate. The solvents were then exchanged to hexane and concentrated to 1 mL.

### 2.3.4. Purification and fractionation

The extracts were subjected to purification and fractionation by a combined silica gel–alumina column chromatography before quantification of PHC, UCM and sterols. Two fractions were separated from the column. The first fraction containing PHC and UCM was eluted with 30 mL of mixture of hexane and DCM

(7:3), and the sterols were eluted with 30 mL of ethyl acetate.

### 2.3.5. Derivatization

The sterol fractions were concentrated and transferred to 2-mL glass vials. The vials were subjected to a gentle nitrogen flow and evaporated just to dryness. One hundred microlitres (for sediment samples) or 50  $\mu\text{L}$  (for water samples) of BSTFA were added to derivatize the sterols to the corresponding trimethylsilyl ethers under 60 °C for 2 h. Samples were then kept in a desiccator at room temperature overnight prior to instrumental analysis.

### 2.3.6. Instrumental analysis and quantification

An HP 5890 GC-FID equipped with a DB-5 capillary column (30 m  $\times$  0.25 mm i.d. and 0.25  $\mu\text{m}$  film thickness) was used for analysis and quantification. The carrier gas is  $\text{N}_2$ . The injection port in splitless mode was maintained at 300 °C. For TPH and UCMs analysis, 1  $\mu\text{L}$  of each sample was injected after adding a known amount of  $\text{C}_{20}\text{D}_{42}$  as an internal standard. The oven temperature was held at 60 °C for 2 min, then programmed at 4 °C/min to 290 °C and a final held for 20 min. The detector temperature was set at 290 °C.

For sterols, the oven temperature was held at 60 °C for 2 min, then programmed at 4 °C/min to 100 °C, held for 1 min, and then at 2 °C/min to 290 °C with a final held of 20 min.

The authentic mixture of nine sterols was used as calibration standard. Identification and confirmation of compounds were achieved by comparison of relative retention time with authentic standards on GC–MS (HP6890 + Platform II). A known amount of  $\text{C}_{24}\text{H}_{50}$  was used as an internal standard. The quantification was achieved by seven-point calibration curves drawn for individual sterols on an HP Chemstation system. The calibration curves for each sterol showed high linearity ( $r^2 \geq 0.97$ ). Recovery of androstanol was 82–115% for sediment samples and 75–98% for water samples respectively. The reported results have not been corrected for recovery because the surrogate was added only to confirm the performance and feasibility of our analyses and methods.

### 2.3.7. Quality assurance and quality control (QA/QC)

The limit of quantification (LOQ) for individual sterol compounds ranged from 2–4  $\text{ng g}^{-1}$  (dry wt) for 25 g of sediment and 10  $\text{ng/L}$  for 1.5 L of water respectively. Procedure blanks and reagent blanks were carried out during the analyses. No significant amounts of target sterols were detected. Considering the high proportions of sand in some sediment samples, all concentration data of sediment samples was normalized with TOC.

### 3. Results and discussions

#### 3.1. Sewage contamination in the surficial sediments

Cholesterol and phytosterols (including ergosterol, campesterol, stigmasterol, lanostanol,  $\beta$ -sitosterol and stigmastanol) occurred in high concentration in most sediment samples. The highest levels of coprostanol

were found in estuarine sediments, especially in the inner estuary where serves as navigational channel and is surrounded by densely populated cities (sites B, Fig. 1), and those sites close to the submarine outfalls of Hong Kong (site C2, D1 and D2, Fig. 1). Clearly progressive seaward decline in concentration of coprostanol was found in surficial sediments (Fig. 2a), with the highest average in the outer estuary ( $25 \mu\text{g g}^{-1}$  TOC, Table 1), where the

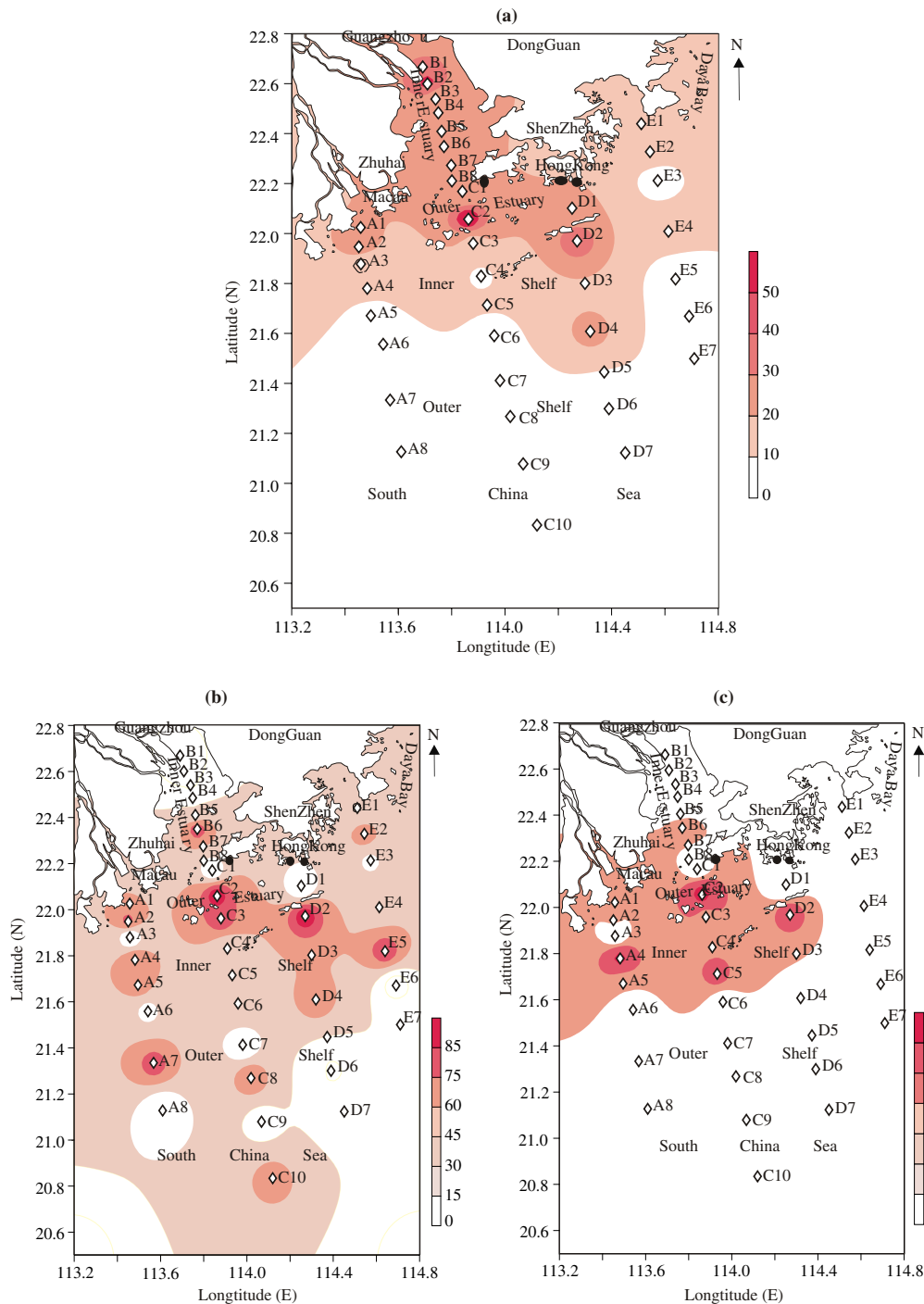


Fig. 2. The spatial distribution of sterols ( $\mu\text{g g}^{-1}$  TOC) in the Pearl River estuarine and marine sediments of South China Sea: (a) coprostanol; (b) cholesterol; (c) phytosterol; (●) submarine outfalls in Hong Kong.

submarine outfalls of Hong Kong are located. In contrast, no similar distributions were observed for other sterols (Fig. 2b and c).

It has been reported that some ratios of sterol compounds are useful indicators to trace and evaluate sewage contamination. Coprostanol as a percentage of total sterols can be used as an index of the degree of sewage pollution (Leeming et al., 1996; Maldonado et al., 2000). Ratios of coprostanol/cholesterol,  $C_{27}$  sterols/ $C_{29}$  sterols and coprostanol/phytosterol can also be used to examine sterols from different biogenic sources and to confirm the urban sewage source. Ratios of coprostanol/cholesterol <1 are reported to indicate a biogenic source and >1 to indicate a sewage source (Leeming et al., 1996; Patton and Reeves, 1999; Isobe et al., 2002).

Table 1 presents diagnostic indices of sterols which can be used to assess the contribution of human activities to pollution of the estuarine and marine sediments of the South China Sea. Coprostanol averages 11.5% and 15% in the sediments of inner and outer PRE respectively, which is much higher than those in the open-sea samples (5.2%). The largest coprostanol percentage was found at site D1 (39%), where is in the vicinity of one submarine outfall of Hong Kong. Generally, the estuary exhibits higher levels in ratios of coprostanol/cholesterol, with averages of 0.7 and 0.95 in the inner and outer PRE sediments respectively; while the open water sediments demonstrate lower ratios (<0.7, and with averages lower than 0.3). The highest ratio of coprostanol/cholesterol (2.6) was also found at site D1. Site B2 (in the inner estuary) had the second highest ratio of coprostanol/cholesterol (1.8). The distribution of ratios of  $C_{27}$ -sterols/ $C_{29}$ -sterols and coprostanol/phytosterol were similar to those of coprostanol percentage and ratios of coprostanol/cholesterol, with the highest values for the sediments at site D1 (3.6 and 0.95) and becoming smaller for the open-sea sediments.

Surface sediments represent the current contamination status well due to receiving direct input and surface runoff. It can be therefore concluded from the results described above that sewage contamination is obvious in the PRE sediments. However, it must be noted that the sewage contamination is confined to the estuary. No seaward transport or dispersion of fecal contaminants was observed, which can possibly be attributed to the south-westward coastal current in the estuary. The submarine outfalls represent significant point sources of sewage contamination of the surficial sediments in the PRE of the South China Sea.

### 3.2. Sewage contamination in the surface water

Water samples were extracted without removing the particulates. Hence, the extracts represented the sum of particle- and water-phase organics.

Table 1  
Distribution of organic molecular markers in the Pearl River estuarine and marine surficial sediments of South China Sea

Sampling sites	TOC (%)	Cop <sup>a</sup> ( $\mu\text{g g}^{-1}$ TOC)	%Cop	Cop/Cho <sup>b</sup>	Cop/Phyto <sup>c</sup>	$C_{27}/C_{29}$ <sup>d</sup>	TPH <sup>e</sup> ( $\text{mg g}^{-1}$ TOC)	UCM <sup>f</sup> ( $\text{mg g}^{-1}$ TOC)	% UCM
Inner estuary	0.06–0.94	17–44 (25) <sup>g</sup>	7–14.5 (11.5)	0.3–1.8 (0.7)	0.1–0.3 (0.15)	0.4–1.6 (0.7)	0.3–16.5 (8.4)	1.9–10.5 (5.5)	47.2–77.2 (68.5)
Outer estuary	0.37–1.02	7–53 (30)	6.7–38.6 (15)	0.5–2.6 (0.95)	0.1–0.95 (0.3)	0.5–3.6 (1.3)	1.2–6.7 (4.1)	1.0–4.8 (2.6)	66.6–81.5 (73.1)
Inner shelf	0.29–0.70	4–25 (12)	0.8–10.7 (5.2)	0.1–0.5 (0.3)	0.1–0.15 (0.1)	0.2–1.3 (0.6)	1.5–5.3 (3.2)	0.2–4.4 (2.0)	6.7–76 (56.7)
Outer shelf	0.17–0.64	nd–8 (3)	0–9.5 (5.2)	0–0.7 (0.2)	0.01–0.1(0.04)	0.2–0.7 (0.5)	0.7–5.4 (2.0)	0.5–2.3 (1.5)	42.3–76.8 (50.6)

nd: not detected.

<sup>a</sup> Cop: 5 $\beta$ -coprostanol.

<sup>b</sup> Cho: cholesterol.

<sup>c</sup> Phyto: phytosterol.

<sup>d</sup>  $C_{27}/C_{29}$ :  $C_{27}$ -sterols/ $C_{29}$ -sterols.

<sup>e</sup> TPH: total petroleum hydrocarbon.

<sup>f</sup> UCM: unresolved complex mixture.

<sup>g</sup> Values in parentheses are averages.

Coprostanol was quantitatively detected in six among 24 estuarine water samples analyzed, ranging from 11 to 299 ng/L, mainly collected in the inner PRE (sites B). The highest concentration coprostanol in waters was found at site B6 (299 ng/L). The coprostanol percentage relative to total sterols was in the range of 0.9–31%. Both the largest coprostanol percentage (31%) and largest ratio of coprostanol/cholesterol (1.01) were seen at site B1. This result suggests that the inner PRE water

is obviously impacted by fecal contamination, which possibly comes from direct discharge (from ships, boats, etc.) and urban runoff. The fecal contaminants show no transport or prolongation toward open South China Sea.

As a whole, the concentrations of coprostanol in the Pearl River estuarine and marine environment of the South China Sea are comparable to those in Key West (UK) sediments from the vicinity of the outfall

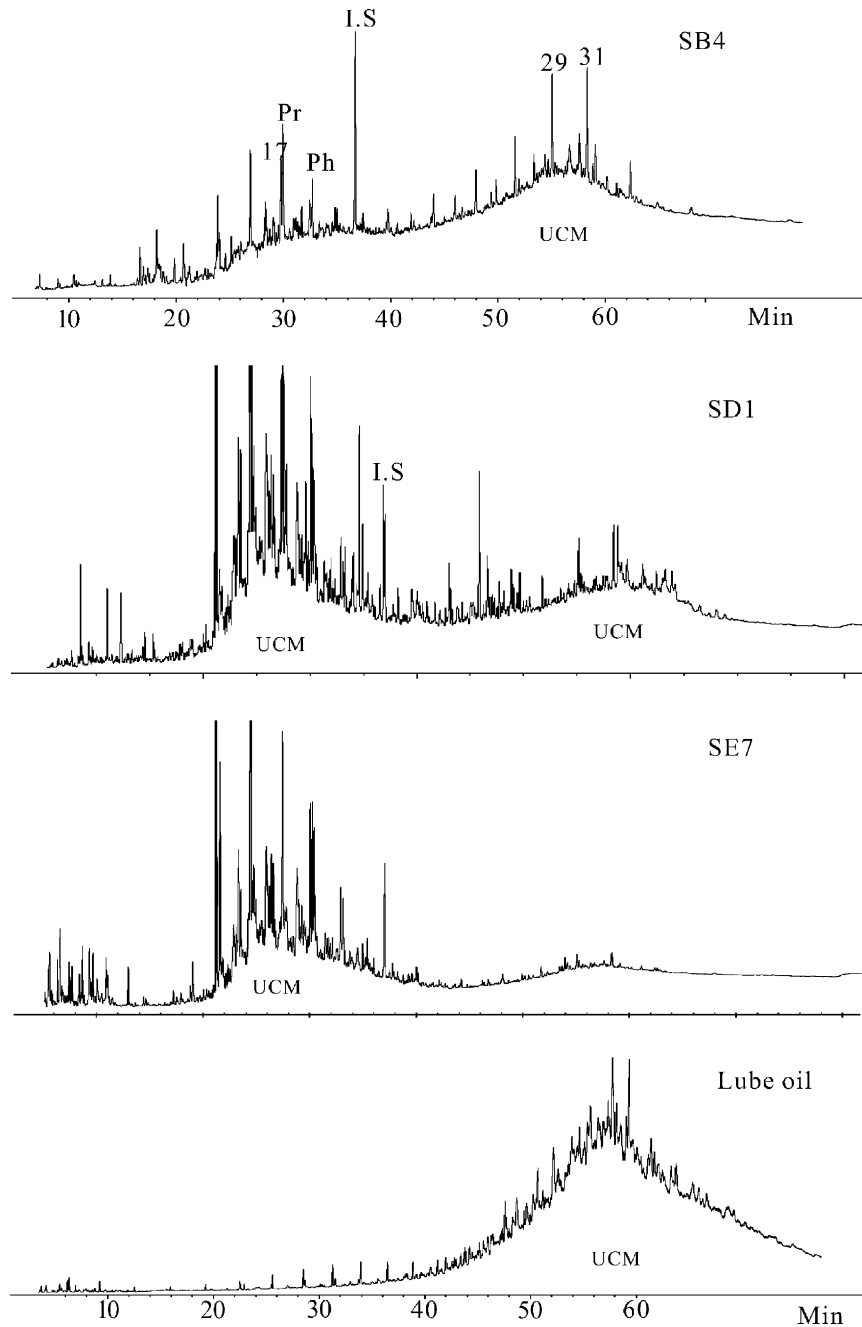


Fig. 3. GC-FID chromatograms of hydrocarbons in the estuarine and marine sediments of South China Sea. I.S. represents internal standard; numbers above peaks indicate the carbon chain length of *n*-alkanes; Pr, pristane; Ph, phytane; UCM, unresolved complex mixture; SB4, SD1, SE7, sediment samples.

(30–340 ng g<sup>-1</sup> dw), Boston Harbor (USA) sediments impacted by combined sewer overflow (7.3–255 μg g<sup>-1</sup> OC) (Eganhouse and Sherblom, 2001), Tokyo Bay (Japan) sediments (2.7–21.0 (average 5.7) μg g<sup>-1</sup> OC), and those of Malaysia and Vietnam (29 and 14.5 μg g<sup>-1</sup> OC respectively) (Isobe et al., 2002).

### 3.3. Hydrocarbon distributions in surficial sediments

The distributions of hydrocarbon fractions of surficial sediments are characterized by bimodal (unimodal in some samples) UCM “humps”, with peaks at *n*-C<sub>16</sub> to *n*-C<sub>18</sub> and *n*-C<sub>29</sub> to *n*-C<sub>31</sub> (Fig. 3). Concentrations of

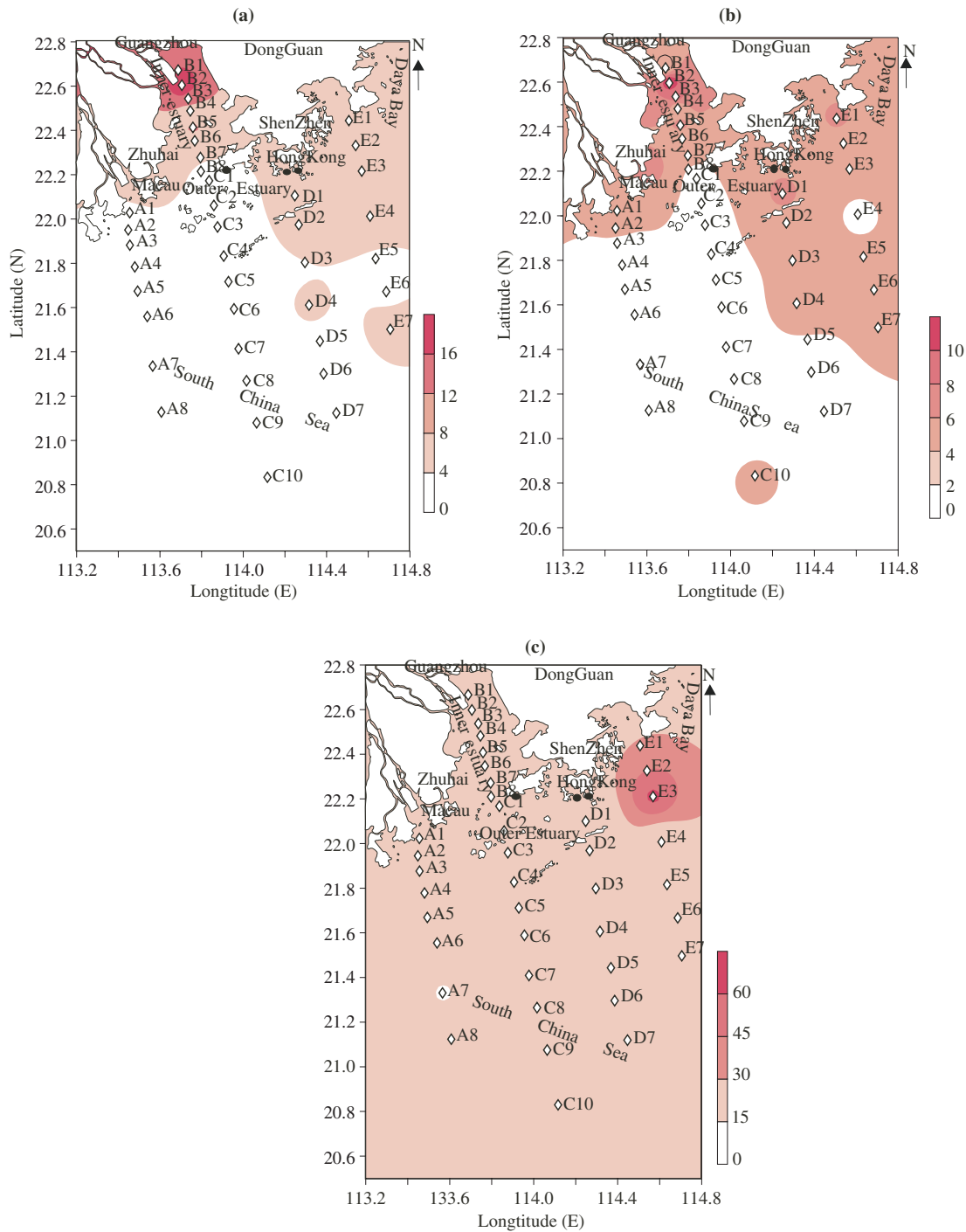


Fig. 4. Distributions of TPH and UCM in sediment (mg g<sup>-1</sup> TOC) and water samples (μg L<sup>-1</sup>) of Pearl River estuary and South China Sea: (a) TPH in sediments; (b) UCM in sediments; (c) UCM in waters.



total petroleum hydrocarbon (TPH) range from 0.3 mg/g TOC to 16.5 mg/g TOC, with UCM representing from 42% to 81% of the total hydrocarbons (Table 1). Progressive seaward declines in concentrations were found for both TPH and UCM in surficial sediments (Fig. 4, Table 1). High levels of both TPH and UCM were detected in the inner estuarine sites (sites B) and those close to submarine outfalls (site C1, site C2, site D1 and site D2). It was found that concentrations of UCM were related to some degree with that of 5 $\beta$ -coprostanol in the sediments ( $R^2 = 0.44$ ). Generally, the UCM concentration in the sediments of PRE and the South China Sea (0.2–10.5 mg/g TOC) is comparable to that in Tokyo Bay sediments (418–8419 (average 6426)  $\mu\text{g g}^{-1}$  OC).

From the shapes of UCM “humps”, it can be found that at least two petroleum pollution sources are present in the sediments of PRE and the South China Sea. In order to identify the sources of the petroleum contamination in the sediments of PRE and the South China Sea, we collected and analyzed nine suspected source oils and petroleum products, including four crude oils produced in China, one crude oil from Thailand, one from Russia, one from Yemen, one diesel and one lube oil. Lube oil seems to be a significant source of petroleum pollution, especially in the estuarine sediment (Fig. 3), and its contribution becomes progressively smaller as going far toward the open sea. The unambiguous identification of the sources of oil pollution in the sediments in this region is beyond this work, we will not discuss in detail here.

### 3.4. Hydrocarbon distributions in surface waters

The hydrocarbons in the water samples differ greatly from those in the sediments (Fig. 5). No preference of odd carbon hydrocarbons over even carbon hydrocarbons (CPI) was observed, indicating the presence of petroleum hydrocarbons. UCM in the carbon range of  $n\text{-C}_{17}$  to  $n\text{-C}_{28}$ . The distribution profiles of the UCM in waters are different from those in sediments. The concentration of UCM was in the range of 2–62  $\mu\text{g L}^{-1}$ . On the other hand, UCM concentrations were relatively low in estuarine waters, whereas most open-sea samples showed significant UCM accumulations (Fig. 5). The petroleum-related contamination in the surface waters collected in January 2003 was considerably different from those in the surficial sediments. It may imply that there presented a temporal contamination source in the surface water of the South China Sea at that time, which possibly came from ships in this region.

## 4. Conclusion

The PRE of the South China Sea is obviously impacted by anthropogenic activities such as sewage dis-

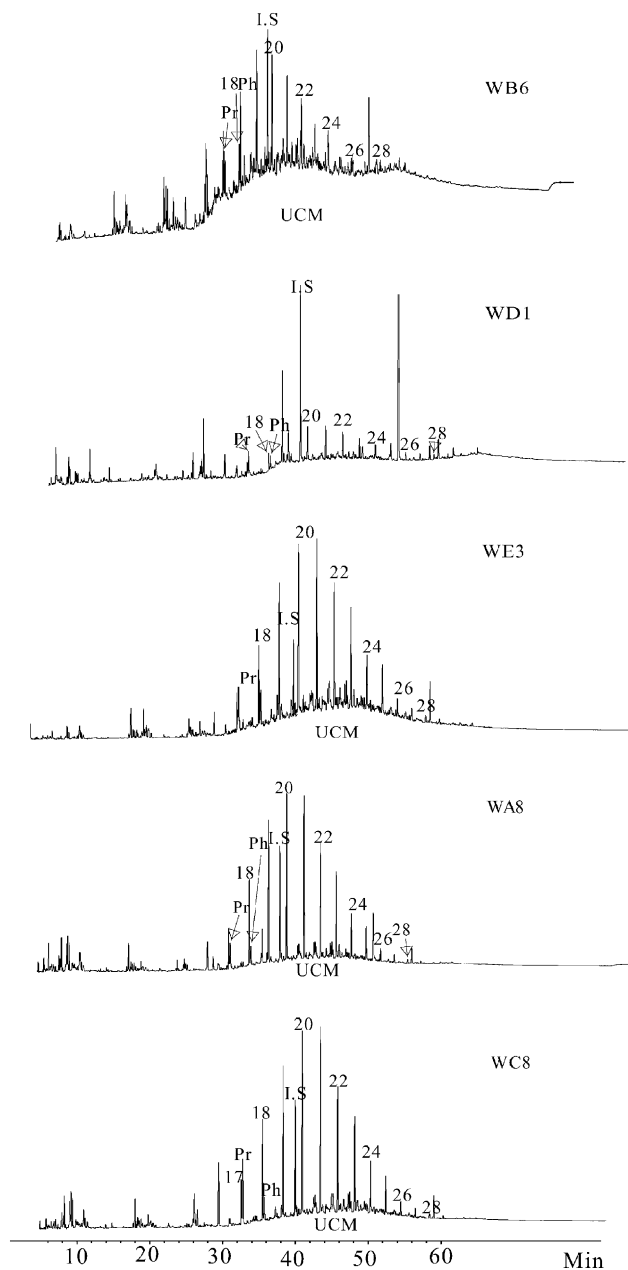


Fig. 5. GC-FID chromatograms of hydrocarbon fractions in water samples of Pearl River estuary and South China Sea. I.S. represents internal standard; numbers above peaks indicate the carbon chain length of  $n$ -alkanes; Pr, pristane; Ph, phytane; UCM, unresolved complex mixture; WB6, WD1, WE3, WA8, WC8, water samples.

charges, shipping and urban runoff, evidenced by distribution of 5 $\beta$ -coprostanol together with sterol profiles, TPH and UCM hydrocarbons. High levels of coprostanol and UCM accumulation in the sites close to the submarine outfalls imply that the submarine outfalls in Hong Kong represent important sources of anthropogenic contamination to the PRE sediments.

Lube oil, which is widely used by ships and boats, is a significant source of petroleum-related pollution in the

sediments of the PRE and the South China Sea, especially in the PRE.

Progressive seaward declines were found for the concentrations of 5 $\beta$ -coprostanol, UCM and TPH, and the coprostanol percentages relative to the total sterols. Trace amounts of or no quantifiable 5 $\beta$ -coprostanol were detected in the open-sea sediments and waters. The contaminants in the sediments of the PRE and the South China Sea are dominated by local sources and largely confined to the PRE. No significant dispersion or seaward transport of those anthropogenic contaminations was indicated by organic molecular markers analyzed.

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### References

- Carreira, R.S., Wagener, A.L.R., Readman, J.W., 2004. 19 Sterols as markers of sewage contamination in a tropical urban estuary (Guanabara Bay, Brazil): space–time variations. *Estuarine, Coastal and Shelf Science* 60, 587–598.
- Chaloux, N., Takada, H., Bayona, J.M., 1995. Molecular markers in Tokyo bay sediments: sources and distribution. *Marine Environmental Research* 40, 77–92.
- Eganhouse, R.P., 1997. Molecular markers in environmental geochemistry American Chemical Society Symposium Series 671. American Chemical Society, Washington, DC.
- Eganhouse, R.P., Sherblom, P.M., 2001. Anthropogenic organic contaminants in the effluent of a combined sewer overflow: impact on Boston Harbor. *Marine Environmental Research* 51, 51–74.
- Grimalt, J.O., Fernandel, P., Bayona, J.M., Albaiges, J., 1990. Assessment of fecal sterols and ketones as indicators of urban sewage inputs into coastal waters. *Environmental Science and Technology* 24, 357–363.
- Isobe, K.O., Tarao, M., Zakaria, M.P., Chiem, N.H., Minh, L.Y., Takada, H., 2002. Quantitative application of fecal sterols using gas chromatography–mass spectrometry to investigate fecal pollution in tropical waters: Western Malaysia and Mekong delta, Vietnam. *Environmental Science and Technology* 36 (21), 4497–4507.
- Leeming, R., Ball, A., Ashbolt, N., Nichols, P., 1996. Using faecal sterols from humans and animals to distinguish faecal pollution in receiving waters. *Water Research* 30, 2893–2900.
- Mai, B., Qi, S., Zeng, E.Y., Yang, Q., Zhang, G., Fu, J., Sheng, G., Peng, P., Wang, Z., 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, 4855–4863.
- Maldonado, C., Venkatesan, M.I., Philips, C.R., Bayona, J.M., 2000. Distribution of trialkylamines and coprostanol in San Pedro Shelf sediments adjacent to a sewage outfall. *Marine Pollution Bulletin* 40, 680–687.
- Meyers, P.A., Ishiwatari, R., 1993. Lacustrine organic geochemistry—an overview of indicators of organic matter sources and diagenesis in lake sediments. *Organic Geochemistry* 20 (7), 867–900.
- Mudge, S.M., Lintern, D.G., 1999. Comparison of sterol biomarkers or sewage with other measures in Victoria Harbour, BC, Canada. *Estuarine, Coastal and Shelf Science* 48, 27–38.
- Nichols, P.D., Leeming, R., Rayner, M.S., Latham, V., 1996. Use of capillary gas chromatography for measuring fecal-derived sterols—application to stormwater, the sea-surface microlayer, beach greases, regional studies, and distinguishing algal blooms and human and non-human sources of sewage pollution. *Journal of Chromatography A* 733, 497–509.
- Page, D.S., Boehm, P.D., Douglas, G.S., Bence, A.E., Burns, W.A., Mankiewicz, P.J., 1998. Petroleum Sources in the Western Gulf of Alaska/Shelikoff Strait Area. *Marine Pollution Bulletin* 36 (12), 1004–1012.
- Patton, D., Reeves, A.D., 1999. Sterol concentrations and temporal variations on the North Shore Mudflats of the Firth of Tay, Scotland. *Marine Pollution Bulletin* 38, 613–618.
- Peng, X., Zhang, G., Mai, B., Min, Y., Wang, Z., 2002. Spatial and Temporal Trend of Sterol Pollution in Macao Estuary, Southern China. *Marine Pollution Bulletin* 45 (1–12), 295–299.
- Readman, J.W., Fillmann, G., Tolosa, I., Bartocci, J., Villeneuve, J.-P., Catinni, C., Mee, L.D., 2002. Petroleum and PAH contamination of the Black Sea. *Marine Pollution Bulletin* 44, 48–62.
- Reddy, C.M., Eglinton, T.I., Hounshell, A., White, H.K., Xu, L., Gaines, R.B., Frysinger, G.S., 2002. The West Falmouth oil spill after 30 years: the persistence of petroleum hydrocarbons in marsh sediments. *Environmental Science and Technology* 36, 4754–4760.
- Rowland, S., Donkin, P., Smith, E., Wraige, E., 2001. Aromatic hydrocarbon “humps” in the marine environment: unrecognized toxins? *Environmental Science and Technology* 35, 2640–2644.
- Takada, H., Farrington, J.W., Bothner, M.H., Hohanson, C.G., Tripp, B.W., 1994. Transport of sludge-derived organic pollution to deep-dea sediments at Deep Water Dump Site 106. *Environmental Science and Technology* 26, 1062–1072.
- Vankatesan, M.I., Kaplan, I., 1990. Sedimentary coprostanol as an index of sewage addition in Santa Monica Basin, Southern California. *Environmental Science and Technology* 24, 208–214.
- Wang, Z., Fingas, F., 2003. Development of oil hydrocarbon fingerprinting and identification techniques. *Marine Pollution Bulletin* 47, 423–452.
- Wang, Z., Fingas, M., Elenkinsopp, S., Sergy, G., Landriault, M., Sigouin, L., Lambert, P., 1998. Study of the 25-year-old Nipisi oil spill: persistence of oil residuals and comparisons between surface and subsurface sediments. *Environmental Science and Technology* 32, 2222–2232.
- Wang, Z., Fingas, M., Page, D.S., 1999. Oil spill identification. *Journal of Chromatography A* 843, 369–411.
- Zakaria, M.P., Horinouchi, A., Tsutsumi, S., Takada, H., Tanabe, S., Ismail, A., 2000. Oil Pollution in the Straits of Malacca, Malaysia: application of molecular markers for source identification. *Environmental Science and Technology* 34, 1189–1196.
- Zhang, G., Parker, A., House, A., Mai, B., Li, X., Kang, Y., Wang, Z., 2002. The sedimentary records of HCH and DDT in the Pearl River estuary, South China. *Environmental Science and Technology* 36, 3671–3677.