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Ecotoxicological Assessment of Persistent Organic and Heavy Metal Contamination in Hong Kong Coastal Sediment

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Abstract. The aim of the present project is to determine the feasibility of measuring hepatic cytochrome P4501A1 (CYP1A1) and metallothionein (MT) mRNA in fish as an integrative measurement of persistent organic pollutants (POPs) and heavy metal contamination in sediment arising in Hong Kong. Sediment samples were collected from different sites including Victoria Harbour (VS6), Yim Tin Tsai (YTT) at Tolo Harbour, Mai Po marshes (MPM) at Deep Bay and Southern Waters (SS6) of coastal waters. The samples were analyzed for total and extractable concentrations of Cd, Cu, Ni, Zn and Pb, as well as PCBs and PAHs. In addition, biomarker responses were studied in tilapia exposed experimentally to coastal sediment for 7 days. Using RT-PCR technique, hepatic CYP1A1 and MT mRNA were measured. Three control groups were used including one negative control group maintained in seawater only, while the second and third positive control groups in seawater but intraperitoneally injected with either β-naphthoflavone (40 μg/g body wt) or cadmium chloride (10 μg/g body wt), respectively. The chemical data showed that VS6, YTT and MPM were classified as "Class C Sediment" according to the sediment quality criteria defined by the Hong Kong Environmental Protection Department, indicating the sites were heavily polluted. The exposure of tilapia to the sediment induced hepatic CYP1A1 (VS6 > YTT > MPM > SS6) and MT (VS6 > MPM > YTT > SS6) levels. The induction patterns were comparable to the levels of POPs and metal contamination in the sediment, indicating that the biomarker responses could be used to differentiate low to high levels of contamination among sediment.

Keywords: RT-PCR, CYP1A1, metallothionein, tilapia, POPs

Introduction

Hong Kong is situated on the south of Guangdong Province, consisting Hong Kong Island, the Kowloon Peninsula, the New Territories and 235 outlying islands. It has a population of approximately 6.5 million. The City generates more than 100 million m^3 of marine mud due to various urban and industrial developments, such as new airport construction and land reclamation along coastal areas (Hong Kong Environmental Protection Department 1998a). The western areas (Deep Bay) of the territory are heavily affected by the runoff from the Pearl River (308 billion m^3.year-1) (Mortan and Wu 1975), while the eastern part (Tolo Harbour) receives substantial pollutant loads from the industrial areas and two sewage treatment plants (Hodgkiss and Yim 1995). A transition zone, Victoria Harbour and adjacent waters are heavily polluted by coastal reclamation (i.e. sediment dredging activities) and urban runoffs (Mortan 1989). The Southern Waters are comparatively cleaner. In general, Hong Kong coastal water has been heavily polluted during the past 20 years, leading to a dramatic decline in water quality. With the increase in public concern of health hazard and in particular of seafood contamination, pollution action plans detailing sewage system modification, effluent export scheme and legislative control on all effluents have been implemented to improve and maintain the coastal water quality (Hong Kong Environmental Protection Department 1998a). However, recent studies indicated that the level of heavy metal contamination in coastal environment is still persistent (Blackmore 1998). In addition, report on the levels of persistent organic pollutants (POPs) in Hong Kong coastal sediment revealed a moderate polychlorinated biphenyls (PCBs) (5-66 μg/kg dry weight) and polynuclear aromatic hydrocarbons (PAHs) (39-2787 μg/kg dry weight) contamination (Hong Kong Environmental Protection Department 1998b). Contamination of green-lipped mussels (*Perna viridis)*

in Victoria Harbour by organochlorine pesticides: 2,2-bis(p-chlorophenyl)-1, 1, 1-trichloroethane, their metabolites (up to 2043 μg/kg dry weight) and hexachlorocyclohexane (up to 92 μg/kg dry weight) has also been reported (Phillips 1985). Furthermore, our previous study indicated that PCB concentration in muscle (17.1 μg/kg wet weight) and viscera (54.4 μg/kg wet weight) of grey mullet collected from Mai Po exceeded the guideline value (10 μg/kg wet weight) for human intake (Liang *et al.* 1999), indicating a possible risk in local seafood consumption. Hence, environmental assessment is necessary to monitor the aquatic ecosystem before life system collapsed.

To date, the monitoring system is mainly based on chemical analysis. It is widely used to determine the amount and types of contaminant present in the samples (water, sediment and aquatic biota) collected from the environment. It is, however, not possible to monitor all, both of anthropogenic and natural origins (Oost *et al.* 1996). In addition, the impact of these chemicals on the aquatic ecosystem cannot be determined. Therefore, biomonitor should be included for a complete environmental assessment to determine any possible toxicological effects of multiple contaminant exposure. Many biomonitors have been used to study water pollution in Hong Kong (Cheung *et al.* 1997; Blackmore 1998; Wong *et al.* 1999), however the use of molecular approach to study the sublethal physiological effects of coastal sediment exposure in fish is limited.

The aim of this study was to evaluate the biological impact of coastal sediment on fish. Chemical analysis on sediment was conducted to determine the levels of contamination as well as to identify the species of potential pollutants. In addition, the induction of CYP1A1 and MT gene expressions was determined to represent the integrative measurement of POPs and heavy metals exposures respectively. In

summary, the ecotoxicological impact assessment of coastal sediment using a combined chemical and biological approach was conducted to characterize the contamination pattern, and its toxicological potential.

Materials and Methods

Animals

Tilapia (*Oreochromis mossambicus*) weighing between 10-12 g were reared in glass tanks supplied with aerated seawater for at least 2 weeks. The water was filtered using submersible pumps to ensure that the water in each tank was constantly recirculated. The fish were then transferred to tanks containing 50 g/L coastal sediment collected from Victoria Harbour (VS6), Yim Tin Tsai (YTT), Mai Po Marshes (MPM) or Southern Waters (SS6) (Figure 1). At the end of the exposure period (7 days), eight fish were sampled from each treatment group. The fish were anaesthetised in 0.05% tricaine methanesulfonate and killed by spinosectomy according to the animal care regulations of the University. Liver tissues (~ 0.1 g) were removed rapidly, and placed in tubes containing 1 ml of TRIZOL (Gibco, BRL) solution for subsequent RNA isolation. In addition, three control groups were used; one negative control group was maintained in seawater, while the second and third positive control groups were held in seawater but were intraperitoneally injected with either β-naphthoflavone (β-BNF) (40 μg/g body wt) or cadmium chloride ($CdCl_2$) (10 μg/g body wt).

RNA Isolation and RT-PCR

Tissues were homogenized in TRIZOL Reagent using a glass-Teflon homogenizer according to the manufacturer's instructions. Briefly, after 5 min room temperature incubation (20 °C), chloroform was added for phase separation. The upper aqueous

phase was collected and the RNA was precipitated by mixing with isopropyl alcohol. The RNA pellet was washed once with 75 % ethanol and the RNA pellet was air-dry and was finally redissolved in RNase-free water. A_{260}/A_{280} ratios were between 1.6-1.8. RT-PCR was performed using Ready-To-Go™ RT-PCR beads (Amersham Pharmacia Biotech). Briefly, total RNA was diluted to 1 μg/μl in RNase-free water, mixed with 0.5 μg of pd $(T)_{12-18}$ and 47 μl of RNase-free water to a final volume of 49 μl in a reaction tube containing RT-PCR bead. The reaction was incubated at 42 °C for 30 min, followed by 95 °C for 5 min to inactivate the reverse transcriptase and to completely denature the template. One of the gene specific primer sets (CYP1A1: 5'-ATGCGGATCCTTCACCATYCCICACWGCAC-3' and 5'-ATGCGGATCCARGAA GAGGAAGACCTC-3' (Campbell and Delvin 1996); MT: 5'-ATGGATCCGTGCGA ATGC-3' and 5'-AGACTCCTCACTGGCAGCA-3' (Chan 1994) was added to give a final volume of 50 μl. The number of PCR cycle was varied to determine the optimal number that would allow detection of the appropriate messages, while still keeping amplification for these genes in the log phase. After optimization, reactions were run for 25 cycles with 60 °C (CYP1A1) or 56 °C (MT) annealing cycle (1 min), 72 °C extension cycle (1 min 30 s), and a 95 °C denaturing cycle (50 s), plus final incubation at 72 °C for 3 min. Control amplifications were done either without RT or without RNA. Following PCR amplification, the reaction products were run at 100 V on a 1 % agarose gel with 0.5 μg/ml ethidium bromide. Amplification from primers, CYP1A1 and MT produces a fragment of 270 and 190 bp in length respectively. The total band volumes of amplified products were calculated by gel-documentation software (GelWorks 1D, UVP). All glass- and plastic-ware were treated with diethyl pyrocarbonate and autoclaved.

Chemical Analyses

The total and extractable concentrations of heavy metals including Cd, Cu, Ni, Zn and Pb were determined. For total metal analysis, 0.1 g of freeze-dried sediment was digested using 60% perchoric acid, conc. nitric acid and conc. sulphuric acid (Allen, 1989). For the preparation of extractable metal fractions, the procedures employed are those reported by Miller and McFee (1983). Briefly, 1 g of freeze-dried sediment was mixed with 50 ml of 1 M potassium nitrate solution by shaking at 200 rpm for 16 h. The mixtures were centrifuged at 4,000 rpm for 20 min at 4 °C. The supernatant was then stored in an acid-treated plastic bottle at 4 °C. The total and extractable heavy metal contents were measured by atomic absorption spectrophotometry (AAS: Varian Spectro AA-20 model), according to the methods described by Allen (1989). The samples were stored at 4 °C for no more than one week. Three replicates were conducted for each sample and the arithmetic mean and standard deviation were calculated.

The persistent organic pollutants including polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs) were analyzed using Gas Chromatograph/Mass Spectrophotometer (GC/MS). The extraction, column purification, detection and quantification procedures followed the methods described in APHA (1993). Freeze dried sediment was passed through 1 mm mesh sieve to separate the stones, leaves and dead invertebrates. It was then ground into fine powder using a mortar and a pestle. About 10 g of sediment was extracted by 80 ml acetone:n-hexane (1:1, v/v) in a water bath for 12 h using a soxhlet apparatus. For PCBs analysis, one further cleanup step was done by washing with conc. sulfuric acid, copper powder. Thereafter, interference compounds for PCBs and PAHs were removed by passing through a microflorisil column.

Statistical Analysis

All data were presented as mean ± standard deviation. Variation in metals, PCBs and PAHs concentrations in sediment collected from different sites were tested by ANOVA followed by Duncan's Multiple Range Test. The levels of MT and CYP1A1 mRNA in the liver tissues were analysed by ANOVA to test if different sediment exposure cause significant quantitative changes in the gene expressions. The level of significance was set at P value < 0.05.

Results

Heavy metals analyses

Figure 2 shows the concentrations of heavy metal in (a) total and (b) extractable fractions of sediment samples collected from various sites. Sediment of VS6 had the highest concentrations of total (Cd: 1.02; Cu: 199.5; Ni: 36.9; Zn: 197.2 and Pb: 151.1 μg/g dry weight) and extractable (Cu: 5.67; Ni: 3.7; Zn:3.1 and Pb: 4.4 μg/g dry weight) heavy metals ($P < 0.05$) in comparing to other sampling sites. In YTT, high levels of Cd (total: 0.8; extractable: 0.24 μg/g dry weight) and Pb (total: 83.1; extractable: 2.56 μg/g dry weight) were found whereas in MPM, high levels of Cu (total: 98.6; extractable: 2.4 μg/g dry weight) and Zn (total: 128.4; extractable: 1.84 μg/g dry weight) were detected. Comparatively, SS6 was cleaner but with a high level of Ni (total:20.17; extractable 1.2 μg/g dry weight).

PCBs and PAHs analyses

The PCBs and PAHs analyses are presented in detail in Table 1. It was found that the sediment of VS6 contained high level of PCBs (63.76 ppb), followed by YTT (18.7 ppb), MPM (2.42 ppb) and SS6 (0.21 ppb). The analysis of total PAHs from VS6

sediment revealed the presence of 8502.62 ppb of total PAHs, with 7803.6 ppb of total low molecular weight PAHs and 699.02 ppb of total high molecular weight PAHs. All samples contained procarcinogenic groups of PAHs, including benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene and dibenzo(a,h)anthracene.

MT and CYP1A1 mRNA expression

For the detection of MT mRNA, the MT-F/MT-R primer set amplifies a ~190 bp fragment from MT mRNA (Fig. 3a). The band was detected in the Cd-treated control (3.27 ± 0.44^{a}), followed by VS6 (3.24 ± 0.56^{a}), MPM (2.0 ± 0.42^{b}) and YTT (1.17 ± 0.31^{c}) but barely detectable in the seawater control and SS6 (0.35 ± 0.04^{d} and 0.31 ± 0.06^{d}, respectively).

For CYP1A1 mRNA measurement, the CYP1A1-F/CYP1A1-R primer set amplifies a ~270 bp fragment from CYP1A1 mRNA (Fig. 3b). The band was strongly detected in the samples obtained from the β-BNF treated fish (3.1 ± 0.29^{ab}) but weakly in the samples from the seawater controls (1.7 ± 0.11^{c}). This band was also evident in the samples of VS6 (3.3 ± 0.42^{a}) with the intensity of the band decrease from YTT (2.7 ± 0.34^{b}), MPM (2.3 ± 0.17^{b}) and SS6 (2.1 ± 0.16^{c}).

Discussion

A major worldwide environmental issue is the introduction of persistent toxic substances including heavy metals and persistent organic pollutants (POPs) into our environment (Galal-Gorcher 1993; Herrman 1993). These substances are characterized by their persistence or longevity before degradation, thereby retaining high chronic toxicity (Hansen 1998). Toxic heavy metals such as Cd and Hg in

aquatic habitats are known to cause various adverse effects on fish, including premature hatching, growth retardation, developmental abnormalities and increased mortality (Berg 1970; Beholt 1976; Roch and Maly 1979). Sublethal concentrations of Zn in water could also delay sexual maturity and reproduction in fish (Hogstrand and Wood 1996). POPs such as PAHs, PCBs, polychlorinated dibenzo-p-dioxins/dibenzofurans and other chlorinated hydrocarbons are extremely toxic (Ramamoorthy 1997; Tyler *et al.* 1998; DeRisi *et al.* 1997; Kelce *et al.* 1995; Perera 1997). Their bioaccumulation in aquatic organisms could pose high risk of adverse health effects to both the accumulating organisms and their higher trophic consumers, including human (Ip and Phillips 1989; Ip 1990; Guo *et al.* 1997; Leonards *et al.* 1998; Lunden and Noren 1998). It is known that these chemicals affect growth and development, immune function, neurological function, reproduction, and induce mutations and cancers in mammals (White *et al.* 1994; Arnold *et al.* 1996). Although the deterioration in aquatic ecosystem is alarming, methods for environmental monitoring have not been fully developed.

Chemical analysis (i.e. gas chromatography/mass spectrometry and atomic absorption spectrophotometry) on the levels of POPs and heavy metals in environmental samples (i.e. water, sediment and biota) provide invaluable information on the severity of contamination (Cheung *et al.* 1997; Wong *et al.* 1999; Liang *et al.* 1999). Hence, the first part of the present study was conducted to assess the chemical properties of the sediment collected from various coastal waters. The results showed that the levels of contamination in various sediment samples were consistent with past reports (Hong Kong Environmental Protection Department 1998b). YTT and VS6 were contaminated with low to moderate levels of PCBs and PAHs, in which high chlorinated PCBs and some of the procarcinogenic high molecular weight PAHs (i.e.

benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene and dibenzo(a,h)anthracene) were detected (Stein *et al.* 1990). In addition, high levels of heavy metal were found in the sediment of VS6, YTT and MPM as compared to SS6. The levels of extractable heavy metal, however, were not directly correlated with total heavy metal present. One possible explanation for this discrepancy is some of the heavy metal may exist in insoluble organic and/or inorganic complexes which could not be readily extractable (Jenne and Zachara 1987; Martin *et al.* 1987; Forstner 1990; Burton 1992). Nevertheless, based on the present results and the sediment quality criteria defined by the Hong Kong Environmental Protection Department (Lau and Rootham 1993), the sediment qualities of VS6, YTT and MPM were classified as "Class C sediment" meaning these are heavily polluted sites, while SS6 was classified as "Class A" with relatively cleaner water. Although, the chemical data provided information on the concentration of contaminant in coastal sediment, yet the bioavailability and toxicity is difficult to be measured due to the complexity of sediment-contaminant interactions (Schlekat *et al.* 1992). Better understanding on their related toxicity can be provided by a direct measurement on the biological and physiological responses of biota to the bioavailable contaminant.

The use of biomarkers to assess the exposure of aquatic organisms to environmental contaminant has received increasing attention in the past decade. Together with chemical analysis, biomarker responses could be considered as a supplementary approach to determine the biological impacts of environmental contaminant (Everaarts *et al.* 1994; Oost *et al.* 1997). Since biological responses are mediated by the interaction between the toxicant and biochemical receptor, biomakers have the potential to act as an integrative measurement at the suborganism level to indicate pollution status, before harm to the biota occur (Payne *et al.* 1987). Indeed,

biomarker studies have now been incorporated into a number of national monitoring programs for marine water (WHO 1993; Burgeot *et al.* 1996). Among specific biomarkers, the activities/contents of CYP1A1 and MT are widely used in environmental monitoring (Payne *et al.* 1987; Campbell and Devlin 1997; Peters *et al.* 1997; Machala *et al.* 1997; Oost *et al.* 1997). Heavy metal is one of the most potent agents for MT induction (Hamer 1986). The induction of hepatic MT is implicated in cellular protection and play crucial role in metal detoxification (Olsson 1993). On the other hand, PCBs and PAHs induce CYP1A1 (Stegeman 1981; Kleinow *et al.* 1987). The expression of the CYP1A1 is considered to encode proteins, acting to insert oxygen onto lipophilic chemicals to produce the more polar hydroxylated and epoxidated products, and enter into the animal's excretion pathways (Parke *et al.* 1990). Hence, in the second part of this study, we have incorporated the use of RT-PCR to determine hepatic MT and CYP1A1 mRNA levels in fish, upon sediment exposure. This method has some definite advantages over the traditional 7-ethoxyresorufin O-deethylase assay (Klotz *et al.* 1984) and the differential pulse polarography (Olafson and Olsson 1991) commonly used to measure activity or protein contents of CYP1A1 and MT in an indirect way. The detection of CYP1A1 and MT at mRNA levels, in the present study, however can provide a direct measurement of their corresponding gene expression. The sensitivity of this assay also enable us to determine the induction of CYP1A1 and MT mRNA levels using as little as 0.01 – 0.1 g of tissue samples.

The results of this study demonstrated that juvenile tilapia experimentally exposed to sediment exhibited significant increase in hepatic expression of MT and CYP1A1 (except in SS6) in comparison to the seawater controls. Hepatic MT and CYP1A1 mRNA levels in fish exposed to sediment from VS6, MPM and YTT increased up to

3.6 to 9 folds following the 7-day exposure period. Since Zn, Cd and Cu are potent MT-inducer (Gedamu and Zafarullah 1993), fish exposed to sediment of VS6 and MPM which contained high levels of Cd, Zn and Cu, also produced higher levels of hepatic MT mRNA. For CYP1A1 induction, the highest level of mRNA detected was in fish exposed to VS6 sediment, which was contaminated with the highest amount of PCBs and PAHs. Since PCBs and PAHs are not very soluble in water (Neilson 1994), the induction of hepatic CYP1A1 gene expression in sediment exposed fish may be due to the ingestion of PCBs/PAHs-contaminated suspended solid, resulting in their absorption, accumulation and stimulation in fish. In addition, the induction of CYP1A1 in the presence of procarcinogens (i.e. benzo(a)pyrene, indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene and dibenzo(a,h)anthracene) could signify the possible process of carcinogenesis in fish since the chemicals could be converted by CYP1A1 into active carcinogens (Varanasi *et al.* 1989; Okey 1990; Stegeman and Hahn 1994). Previous studies have indicated similar increases (1 – 6 folds) in CYP1A1 in fish exposed to coal dust (Campbell and Delvin 1997). Hence, the levels of MT and CYP1A1 induction observed in this study corresponds well with the levels of induction noted previously in fish exposed to environmental samples.

Conclusion

In the present study, ecotoxicological impact assessments using a combined approach of chemical and biological investigations of field samples were conducted. It gives an integrative measurement on the adverse effect of contaminant, providing a specific and sensitive way to assess the potential impact of environmental contaminant to fish. Chemical analysis defined VS6, YTT and MPM as the same class of sediment, however, RT-PCR assay could further characterize the levels of heavy metal toxicity

as VS6 > MPM > YTT > SS6 while POP toxicity as VS6 > YTT > MPM > SS6. This method therefore can detect and differentiate sediment containing low to high levels of contamination. Therefore the use of RT-PCR assay is a valuable tool to assess the biological impacts of environmental toxicants and to improve the present system of sediment quality criteria.

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References

Allen SE (1989) Chemical Analysis of Ecological Material, 2nd Ed. Blackwell Sci. Pub. Oxford.

APHA (1993) Standard method for the examination of water and wastewater, 17th Ed. American Public Health Association, American Water Works Association and Water Pollution Control Federation. Washington, DC

Arnold SF, Klotz DM, Collins BM, Vonier PM, Guillette LJ Jr, McLachlan JA (1996) Synergistic activation of estrogen receptor with combinations of environmental chemicals. Science 272:1489-1492

Beholt DA, Leonhard EN, Christensen GM, Fiandt JT (1976) Toxic effects of cadmium on three generations of brook trout (*Salvelinus fontinalis*). Trans Am Fish Soc 105:550-560

Berg A (1970) Studies on the metabolism of calcium and strontium in freshwater fish. II. Relative contribution of direct and intestinal absorption in growth conditions. Mem. 1st Ital Idrobiol Dott Marchi 26:241-255

Blackmore G (1998) An overview of trace metal pollution in the coastal waters of Hong Kong. Sci Total Environ 214:21-48

Burton GA (1992) Sediment toxicity evaluations: their niche in ecological assessments. Environ Sci Tech 26:2068-2074

Burgeot T, Bocquene G, Porte C, Dimeet J, Santella RM, Garcia de la Parra LM, Pfhol-Leszkowicz A, Raoux C, Galgani F (1996) Bioindicators of pollutant exposure in the northwestern Mediterranean Sea. Marine Ecology Progress Series 131:125-141

Chan KM (1994) PCR-cloning of goldfish and tilapia metallothionein complementary DNAs. Biochem Biophys Res Comm 205: 368-374

Campbell PM, Devlin RH (1996) Expression of CYP1A1 in livers and gonads of Pacific salmon: quantitation of mRNA levels by RT-PCR. Aquat Toxicol 34: 47-69

Campbell PM, Devlin RH (1997) Increased CYP1A1 and ribosomal protein L5 gene expression in a teleost: The response of juvenile chinook salmon to coal dust exposure. Aquatic Toxicol 38:1-15

Cheung YH, Neller A, Chu KH, Tam NFY, Wong CK, Wong YS, Wong MH (1997) Assessment of sediment toxicity using different trophic organisms. Arch Environ Contam Toxicol 32:260-267

DeRisi JL, Iyer VR, Brown PO (1997) Exploring the metabolic and genetic control of gene expression on a genomic scale. Science 278: 680-686

Everaarts JM, Sleiderink HM, des Besten PJ, Halbrook RS, Shugart LR (1994) Molecular responses as indicators of marine pollution: DNA damage and enzyme induction in Limanda limanda and Asterias rubens. Health Perspec 102:37-43

Forstner U (1990) Inorganic sediment chemistry and elemental speciation. In: Baudo R, Gisey J, Muntau H (eds) Chemistry and toxicity of in-place pollutions. Lewis Publishers, Boca Raton, FL

Galal-Gorchev H (1993) Dietary intake, levels in food and estimated intake of lead, cadmium, and mercury. Food Addit Contam 10:115-28

Gedamu L, Zafarullah M (1993) Molecular analysis of rainbow trout metallothionein and stress protein genes: structure, expression and regulation. In: Hochachka PW, Mommsen TP (eds) Biochemistry and molecular biology of fishes, vol 2. Elsevier Science Publishers, BV, pp 241-258

Guo YL, Ryan JJ, Lau BPY, Yu ML, Hsu CC (1997) Blood serum levels of PCBs and PCDFs in Yucheng Women 14 years after exposure to a toxic rice oil. Arch Environ Contam Toxicol 33:104-108

Hamer DH (1986) Metallothionein. Ann Rev Biochem 55:913-951

Hansen JC (1998) The human health programme under AMAP. AMAP human health group. Arctic monitoring and assessment program. Int J Circumpolar Health 57:280-91

Herrman JL (1993) The role of the World Health Organization in the evaluation of pesticides. Regul Toxicol Pharmacol 17:282-286

Hodgkiss IJ, Yim WWS (1995) A case study of Tolo Harbour, Hong Kong. In: McComb AJ (ed) Eutrophic Shallow Estuaries and Lagoons. Boca-Raton, USA CRC-Press, pp 41-57

Hogstrand C, Wood CM (1996) The Physiology and toxicology of zinc in fish. In: Taylor EW (ed) Toxicology of aquatic pollution, physiological, molecular and cellular approaches. Cambridge, University Press, pp 61-84

Hong Kong Environmental Protection Department (1998a) Environment Hong Kong. A Review of 1997. Government Printer. Hong Kong

Hong Kong Environmental Protection Department (1998b) Marine Water Quality in Hong Kong. Results for 1997 from the marine monitoring program of the Environmental Protection Department. Monitoring Section, Water Policy and Planning Group, Environmental Protection Department, Hong Kong Government, Hong Kong

Ip HMH, Phillips DJH (1989) Organochlorine chemicals in human breast milk in Hong Kong. Arch Environ Contam Toxicol 18: 490-494

Ip HMH (1990) Chlorinated pesticides in foodstuffs in Hong Kong. Arch Environ Contam Toxicol 19:291-296

Jenne EA, Zachara JM (1987) Factors to consider in conducting laboratory sorption/desorption tests. In: Dickson KL, Maki AW, Brungs WA (eds) Fate and effects of sediment-bound chemicals in aquatic systems. Pergamon Press, New York, pp 83-98

Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM (1995) Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. Nature 375:581-585

Kleinow K, Melancon M, Lech JJ (1987) Biotransformation and induction: implications for toxicity, bioaccumulation and monitoring of environmental xenobiotics in fish. Environ Health Perspect 61: 105-119

Klotz AV, Stegeman JJ, Woodin BR, Snowberger EA, Thomas PE, Walsh C (1984) An alternative 7-ethoxyresorufin O-deethylase activity assay: a continuous visible spectrophotometric method for measurement of cytochrome P450 monoxygenase activity. Anal Biochem 140: 138-145

Lau MM, Rootham RC (1993) A stratgy for the management of contaminated dredged sediment in Hong Kong. J Environ Manag 38:99-114

Leonards PEG, Broekhuizen S, de Voogt P, Van Straalen NM, Brinkman UAT, Cofino WP, van Hattum B (1998) Studies of bioaccumulation and biotransformation of PCBs in mustelids based on concentration and congener patterns in predators and preys. Arch Environ Contam Toxicol 35:654-65

Liang Y, Wong MH, Shutes RBE, Revitt DM (1999) Ecological risk assessment of polychlorinated biphenyl contamination in the Mai Po Marshes nature reserve, Hong Kong. Wat Res: 33: 1337-1346

Lunden A, Noren K (1998) Polychlorinated naphthalenes and other organochlorine contaminants in Swedish human milk, 1972-1992. Arch Environ Contam Toxicol 34:414-423

Machala M, Nezveda K, Petrivalsky M, Jarosova A, Piacka V, Svobodova Z (1997) Monooxygenase activities in carp as biochemical markers of pollution by polycyclic and polyhalogenated aromatic hydrocarbons: choice of substrates and effects of temperature, gender and capture stress. Aquatic Toxicol 37:113-123

Martin JM, Nirel P, Thomas AJ (1987) Sequential extraction techniques: promises and problems. Mar Chem 22:313-342

Miller WP, McFee WW (1983) Distribution of cadmium, zinc, copper and lead in soils of industrial northwestern Indiana. J Environ Qual 12:29-33

Morton B, Wu SS (1975) The hydrology of the coastal waters of Hong Kong. Environ Res 10: 319-347

Morton B (1989) Pollution of the coastal waters of Hong Kong. Mar Pollut Bull 20: 310-318

Neilson AH (1994) Persistence: Environmental aspects. In: Organic chemicals in the aquatic environment: distribution, persistence, and toxicity. Lewis Publishers, London, pp 183-213

Okey AB (1990) Enzyme induction in the cytochrome P-450 system. Pharma Ther 45:241-298

Olafson RW, Olsson PE (1991) Electrochemical detection of metallothionein. In: Riordan JF, Vallee BL (eds) Methods in enzymology. Academic Press Inc, San Diego, pp 205-213

Olsson PE (1993) Metallothionein gene expression and regulation in fish. In: Hochachka PW, Mommsen TP (eds) Biochemistry and molecular biology of fishes, vol 3. Elsevier Science Publishers, Amsterdam, pp 258-278

Oost RVD, Goksoyr A, Celander M, Heida H, Vermeulen NPE (1996) Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*). II. Biomarkers: pollution-induced biochemical responses. Aquatic Toxicol 36:189-222

Oost RVD, Vindimian E, Brink, PJVD, Satumalay K, Heida M, Vermeulen NPE (1997) Biomonitoring aquatic pollution with feral eel (*Anguilla anguilla*). III. Statistical analyses of relationships between contaminant exposure and biomarkers. Aquatic Toxicol 39:45-75

Parke DV, Ioannides C, Lewis DFV (1990) The role of the cytochromes P450 in the detoxication and activation of drugs and other chemicals. Can J Physiol 69:537-549

Payne JF, Fancey LL, Rahimtula AD, Edward LP (1987) Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. Comp Biochem Physiol 86: 233-245

Perera FP (1997) Environment and cancer: who are susceptible? Science 278: 1068-1073

Peters LD, Morse HR, Waters R, Livingstone DR (1997) Responses of hepatic cytochrome P450 1A and formation of DNA-adducts in juveniles of turbot (*Scophthalmus maximus* L.) exposed to water-borne benzo[a]pyrene. Aquatic Toxicol 38:67-82

Phillips DJH (1985) Organochlorines and trace metals in green-lipped mussels *Perna viridis* from Hong Kong waters: a test of indicator ability. Mar Ecolog Prog Ser 21: 251-258

Ramamoorthy K (1997) Potency of combined estrogenic pesticides. Science 257: 405-406

Roch M, Maly EJ (1979) Relationship of cadmium-induced hypocalcemia with mortality in rainbow trout (*Salmo gairdneri*) and the influence of temperature on toxicity. J Fish Res Board Can 36:1297-1303

Schlekat CE, GeeMc BL, Reinharz E (1992) Testing sediment toxicity in Chesapeake bay with the amphipod *Leptocheirus plumulosus*: an evaluation. Environ Toxicol Chem 11:225-236

Stegeman JJ (1981) Polynuclear aromatic hydrocarbons and their metabolism in the marine environment. In: Gelboin HV, Pop Ts'o (eds) Polycyclic hydrocarbons and cancer. Academic Press, New York, pp 1-60

Stegeman JJ, Hahn ME (1994) Biochemistry and molecular biology of monooxygenases. In: Malins DC, Ostrander GK (eds) Aquatic toxicology: molecular, biochemical and cellular perspectives. CRC Press, Boca Raton, FL, pp 87-207

Stein JE, Reichert WL, Nishimoto M, Varanasi U (1990) Overview of studies on liver carcinogenesis in English sole from Puget Sound; evidence for a xenobiotic chemical etiology II: Biochemical studies. Sci Total Environ 94:51-69

Tyler CR, Jobling S, Sumpter JP (1998) Endocrine disruption in wildlife: a critical review of the evidence. Crit Rev Toxicol 28:319-61

Varanasi U, Reichert WL, Stein J (1989) 32P-postlabelling analysis of DNA adducts in liver of wild english sole (*Parophrys vetulus*) and winter flounder (*Pseudopleuronectes americanus*). Cancer Res 49:1171-1180

White R, Jobling S, Hoare SA, Sumpter JP, Parker MG (1994) Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology 135: 175-182

WHO (1993) Biomarkers and risk assessment: concepts and principles. Environmental health criteria 155. World Health Organization, Geneva

Wong CKC, Cheung RYH, Wong MH (1999) Toxicological assessment of coastal sediments in Hong Kong using a flagellate, *Dunaliella tertiolecta*. Environ Pollut 105:175-183

Table 1. Concentration of PCBs in sediment collected from Victoria Harbour (VS6), Yim Tin Tsai (YTT), Mai Po Marshes (MPM) and Southern Waters (SS6).

Samples	VS6	YTT	MPM	SS6
PCBs (ppb)				
Mono-PCBs	N.D.	N.D.	N.D.	N.D.
Di-PCBs	N.D.	0.43 ± 0.02	N.D.	N.D.
Tri-PCBs	N.D.	2.0 ± 0.12	N.D.	N.D.
Tetra-PCBs	2.28 ± 0.12^{b}	6.52 ± 0.43^{a}	N.D.	N.D.
Penta-PCBs	9.41 ± 0.53^{a}	4.87 ± 0.28^{b}	1.16 ± 0.07^{c}	0.21 ± 0.01^{d}
Hexa-PCBs	23.26 ± 1.34^{a}	3.12 ± 0.17^{b}	0.68 ± 0.05^{c}	N.D.
Hepta-PCBs	25.28 ± 2.11^{a}	1.76 ± 0.09^{b}	0.58 ± 0.02^{c}	N.D.
Octa-PCBs	2.77 ± 0.12	N.D.	N.D.	N.D.
Nona-PCBs	0.48 ± 0.03	N.D.	N.D.	N.D.
Deca-PCBs	0.28 ± 0.02	N.D.	N.D.	N.D.
Total PCBs	63.76	18.7	2.42	0.21

N.D. = Not detectable, less than the minimum detection level.

Data with the same superscript in the same row are not significantly different according to the results of one-way ANOVA followed by Duncan's Multuple Range Test ($p < 0.05$).

Table 2. Concentration of PAHs in sediment collected from Victoria Harbour (VS6), Yim Tin Tsai (YTT), Mai Po Marshes (MPM) and Southern Waters (SS6).

	VS6	YTT	MPM	SS6
Low molecular weights PAHs				
Naphthalene	7679.4 ± 354^{a}	287.95 ± 15^{d}	6082.1 ± 256^{b}	4554.6 ± 220^{c}
Acenaphthylene	8.74 ± 0.32^{a}	4.81 ± 0.21^{b}	N.D.	N.D.
Acenaphthene	5.91 ± 0.35^{a}	1.54 ± 0.09^{c}	N.D.	3.45 ± 0.18^{b}
Fluorene	8.45 ± 0.48^{b}	4.78 ± 0.18^{c}	14.78 ± 0.85^{a}	4.06 ± 0.11^{c}
Phenanthrene	48.19 ± 1.1^{b}	62.16 ± 2.8^{a}	28.07 ± 1.7^{c}	11.65 ± 0.45^{d}
Anthracene	52.88 ± 2.2^{a}	8.09 ± 0.25^{b}	8.28 ± 0.18^{b}	N.D.
Total LMW PAHs	7803.6	369.33	6133.26	4573.73
High molecular weight PAHs				
Fluoranthrene	95.76 ± 4.2^{b}	116.99 ± 5.4^{a}	28.78 ± 0.8^{c}	11.64 ± 0.44^{d}
Pyrene	119.98 ± 4.6^{a}	114.80 ± 5.3^{a}	44.56 ± 1.7^{b}	15.17 ± 0.91^{c}
Benzo(a)anthracene	55.81 ± 2.44^{a}	50.59 ± 1.32^{b}	13.0 ± 0.24^{c}	3.95 ± 0.12^{d}
Chrysene	64.33 ± 2.13^{a}	61.58 ± 3.11^{a}	24.33 ± 1.5^{b}	5.75 ± 0.18^{c}
Benzo(b)fluoranthene	81.02 ± 2.7^{a}	71.06 ± 4.2^{b}	27.33 ± 1.56^{c}	7.22 ± 0.53^{d}
Benzo(k)fluoranthene	61.30 ± 2.94^{a}	53.66 ± 2.14^{b}	13.31 ± 0.75^{c}	3.78 ± 0.14^{d}
Benzo(a)pyrene	76.12 ± 3.8^{a}	55.42 ± 3.5^{b}	21.86 ± 1.3^{c}	3.91 ± 0.12^{d}
Indeno(1,2,3-cd)pyrene	67.44 ± 2.3^{a}	55.91 ± 2.1^{b}	19.01 ± 0.34^{c}	4.47 ± 0.16^{d}
Dibenzo(a,h)anthracene	13.88 ± 0.58^{a}	11.11 ± 0.37^{b}	4.09 ± 0.18^{c}	N.D.
Benzo(g,h,i)perylene	63.38 ± 2.9^{a}	52.55 ± 3.1^{b}	24.98 ± 1.3^{c}	5.90 ± 0.38^{d}
Total HMW PAHs	699.02	643.67	221.25	61.79
Total PAHs	8502.62	1013	6354.51	4635.52

N.D. = Not detectable, less than the minimum detection level.

Data with the same superscript in the same row are not significantly different according to the results of one-way ANOVA followed by Duncan's Multuple Range Test ($p < 0.05$).

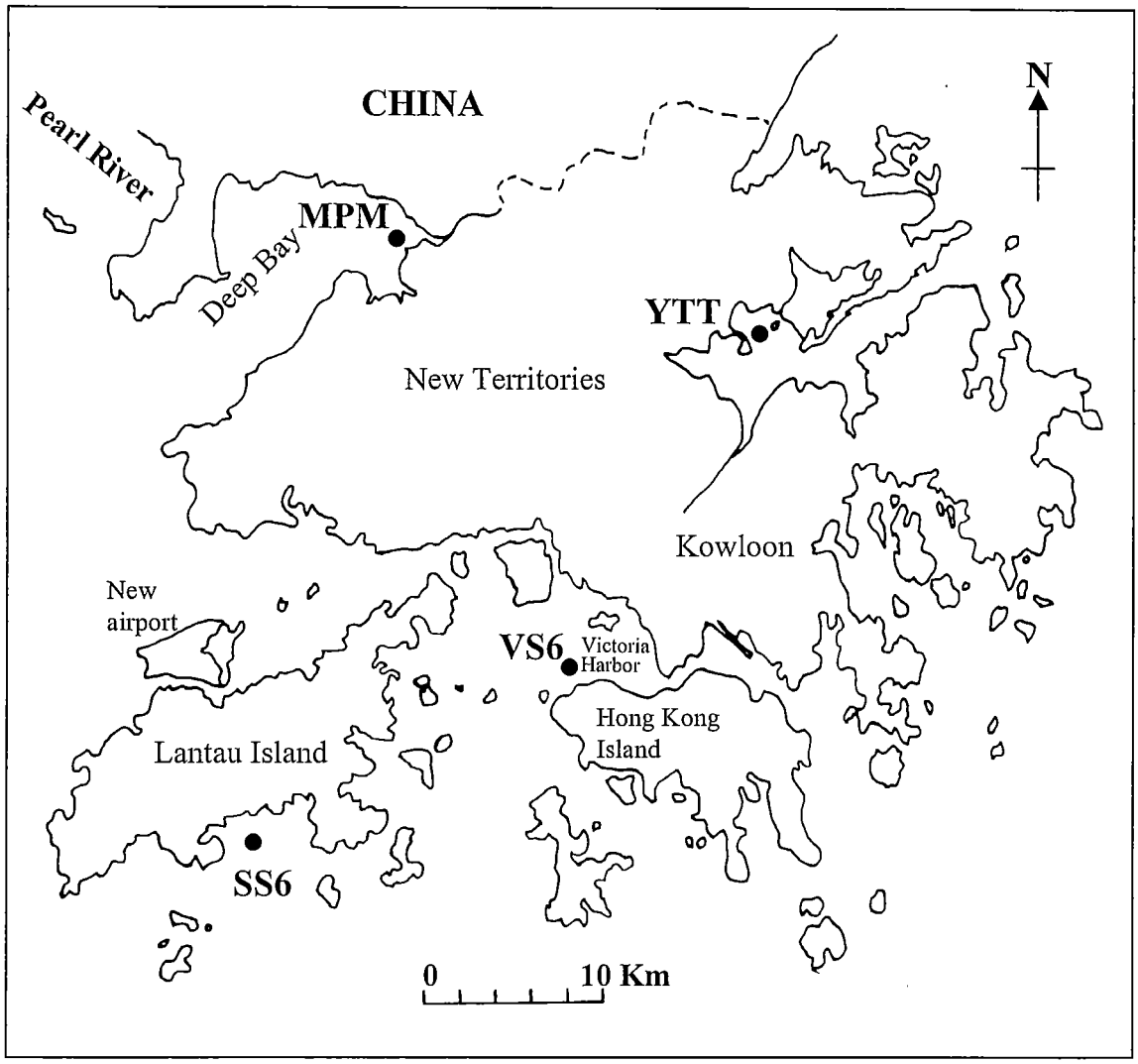

Fig. 1. Map of Hong Kong showing locations where sediment sample were collected (1) VS6: Victoria Harbour; (2) YTT: Yim Tin Tsai; (3) MPM: Mai Po Marshes and (4) SS6: Southern Waters.

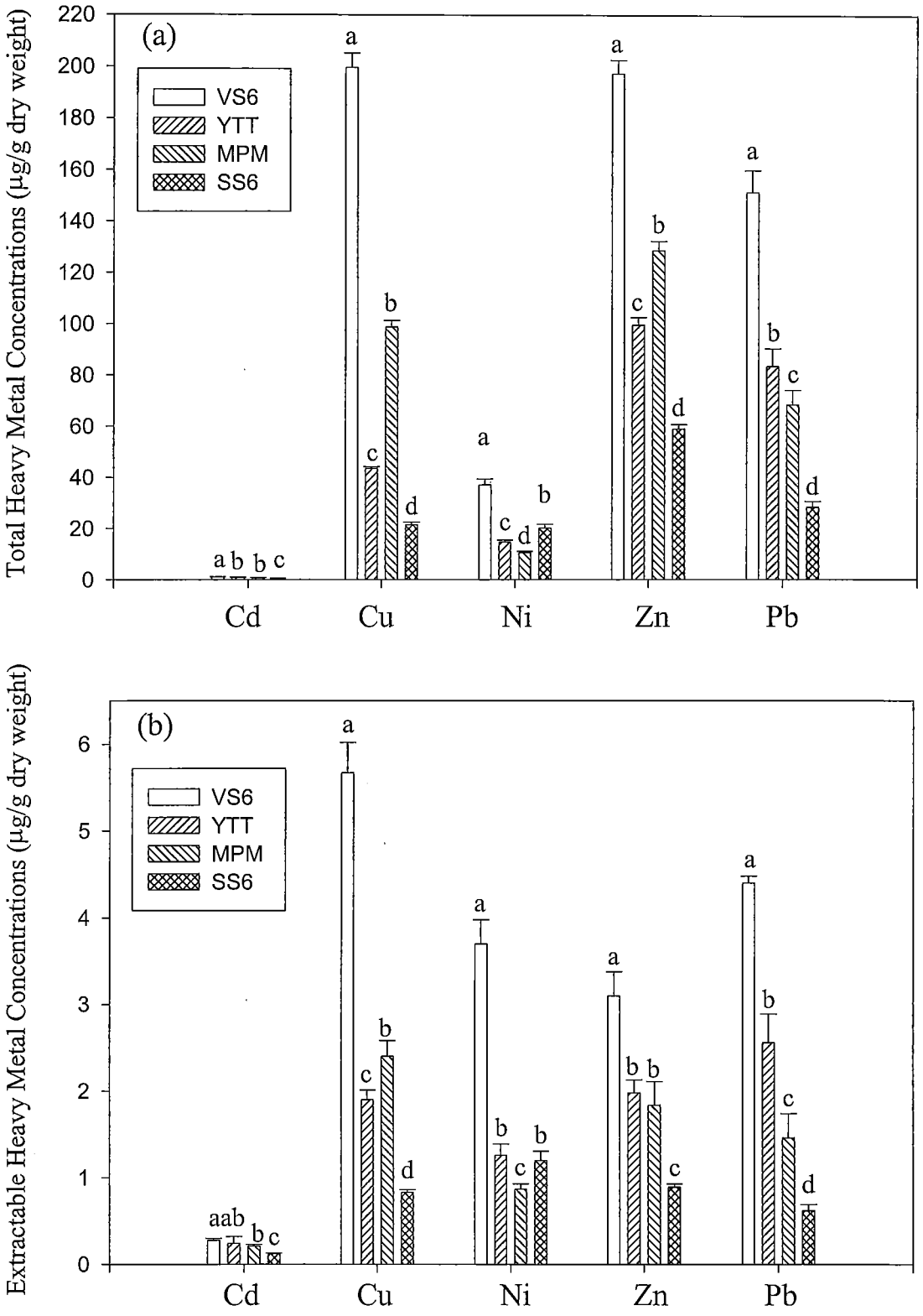

Fig. 2. (a) Total and (b) extractable heavy metal concentrations of sediments. Columns with the same letter are not significant different according to Duncan's multiple range test ($p < 0.05$). VS6: Victoria Harbour; YTT: Yim Tin Tsai; MPM: Mai Po Marshes; SS6: Southern Waters.

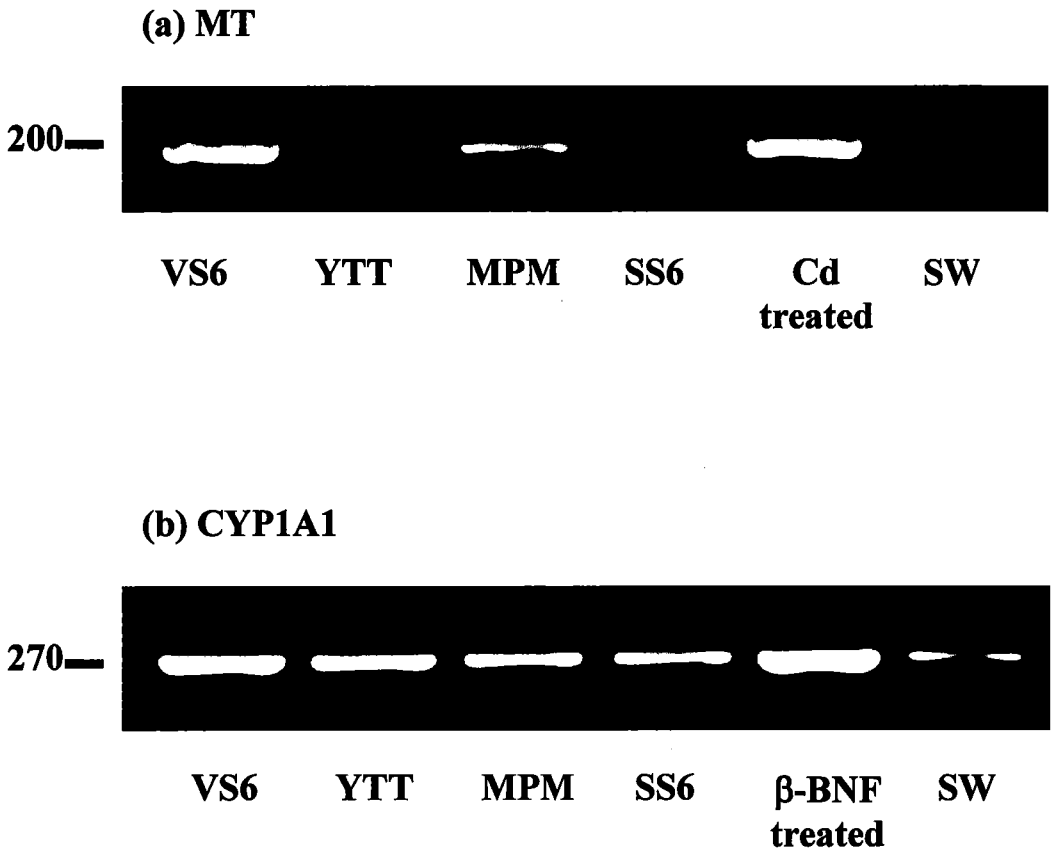

Fig. 3. Photographs of agarose gel showing representative RT-PCR results of (a) MT (~190 bp) and (b) CYP1A1 (~270 bp) using RNA isolated from the livers of the following treatment groups: Negative controls held in seawater only (SW); Positive controls injected with 10 μg/g $CdCl_2$ (Cd treated) or 40 μg/g β-BNF (β-BNF treated); Fish exposed to the sediment of Victoria Harbour (VS6), Yim Tin Tsai (YTT), Mai Po Marshes (MPM) and Southern Waters (SS6).