DOCTORAL THESIS

# Use of food waste feeds for culturing low trophic level fish (grass carp, bighead carp and mud carp): persistent toxic substances

Cheng, Zhang

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2014

# Use of Food Waste Feeds for Culturing Low Trophic Level Fish (Grass carp, Bighead carp and Mud carp): Persistent Toxic Substances

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**A thesis submitted in partial fulfillment of the requirements**

**for the degree of**

**Doctor of Philosophy**

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**Hong Kong Baptist University**

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# DECLARATION

I hereby declare that this thesis represents my own work which has been done after registration for the degree of PhD at Hong Kong Baptist University, and has not been previously included in a thesis or dissertation submitted to this or other institution for a degree, diploma or other qualification.

Signature: ____________________

Date: March 2014

# ABSTRACT

This study aimed at using different types of food wastes as major sources of protein to replace the fish meal used in fish feeds to produce quality fish. The major objectives were to (1) investigate the variations of metalloid/metals, polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) in the fish ponds (pond mud and water), and food wastes used as fish feeds; (2) analyze bioaccumulation and biomagnification of pollutants in the food chains; and (3) evaluate the potential health risks of exposure (to these pollutants) via dietary intake of fish fed with food waste feeds.

The traditional fish farming model was used to culture low trophic level fish: a filter feeder (bighead, *Aristichthys nobilis*), a herbivore (grass carp, *Ctenopharyngodon idellus*) and a bottom feeder (mud carp, *Cirrhina molitorella*), which are more environmental friendly as they can utilize more solar energy. Furthermore, low-trophic level fish are less susceptible to the accumulation of toxic chemicals. Two types of food wastes (mainly containing cereal (Food Waste A) and meat waste meal (Food Waste B)) were used as the major source of protein to replace the fish meal in fish feed to culture fish.

The concentrations of metalloid (arsenic (As)), metals (mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb), zinc (Zn), copper (Cu) and nickel (Ni)) in water, suspended particulate matter and sediment of the 3 experimental fish ponds located in Sha Tau Kok Organic Farm were monitored (bi-monthly during the first half year and tri-monthly during the second half year) and the results were similar to or lower than those in the commercial fish ponds around the Pearl River Delta (PRD) region. Results of the health risk assessments indicated that human consumption of grass carp (a herbivore) which fed

food waste feed pellets would be safer than other fish species (mud carp, bighead carp and largemouth bass). There were no or lower magnifications, and low concentrations of metalloid/metals contained in the ponds indicated that the practice of traditional pond management by draining pond water regularly can provide a better fish pond habitat for birds and other wildlife. Furthermore, the use of food waste instead of fish meal (mainly consisted of contaminated trash fish) further reduced Hg accumulation in the cultured fish.

During October 2011 - December 2012, the concentrations of PAHs and OCPs in three experimental fish ponds were monitored (bi-monthly during the first half year and tri-monthly during the second half year). The results were similar to or lower than those obtained in commercial fish ponds around the PRD region. The mean concentrations of ΣPAHs and ΣOCPs in sediment and fish collected from the experimental fish ponds during the $2^{nd}$ half year (May 2012 to December 2012) were significantly higher ($p<0.05$) than those in the $1^{st}$ half year (October 2011 to April, 2012). ΣPAHs and ΣDDTs in the two species of fish (grass carp and bighead carp) were significantly increased ($p<0.05$) with time, and PAHs and DDTs in grass carp and bighead carp fed with commercial fish feed pellets (control group) were significantly higher ($p<0.05$) than the fish fed with food waste pellets (Food Waste A and Food Waste B). Fruit, vegetables, bone meal and meat products were the major sources of PAHs and OCPs contamination for producing Food Waste A and Food Waste B. No significant increases in PAHs and DDTs concentrations with trophic levels were observed in the experimental ponds, showing that PAHs were not biomagnifed in the omnivorous food chains (plankton, grass carp, bighead carp and mud carp). DDTs were lower magnifications than those predatory food chains (plankton, trash fish, and largemouth bass) in farmed ponds. There was a very low cancer risk for

PAHs and DDTs exerted on humans via consumption of bighead carp, grass carp and mud carp (fed with food waste and commercial pellets). Furthermore, the use of food waste instead of fish meal (mainly consisted of contaminated trash fish) further reduced accumulation of PAHs and DDTs in the cultured fish.

The present results revealed that recycling of food waste for cultivating low trophic level fish (mainly bighead carp and grass carp) is feasible, which will also ease the disposal pressure of the large volume of food waste, a common problem encountered in densely populated cites such as Hong Kong.

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# TABLE OF CONTENT

# LIST OF TABLES

# LIST OF FIGURES

# ABBREVIATIONS

| | |
|---|---|
| Ace | Acenaphthene |
| Acel | Acenaphthylene |
| An | Anthracene |
| As | Arsenic |
| AT | Average exposure time |
| BaA | Benz[a]anthracene |
| BaP | Benzo[a]pyrene |
| BbkF | Benzo[b]fluoranthene |
| BCR | The European Community Bureau of Reference |
| BghiP | Benzo[ghi]perylene |
| BH | Bighead carp |
| BMF | Biomagnification factors |
| BSAF | Biota-sediment accumulation factor |
| C | Concentration |
| Cd | Cadmium |
| Chry | Chrysene |
| Cr | Chromium |
| CRM | Certified reference materials |
| Cu | Copper |
| DahA | Dibenz[a,h]anthracene |

| | |
|---|---|
| DCM | Dichloromethane |
| DDD | Dichlorobischlo rophenylethane |
| DDE | Dichlorodiphenyldichloroethylene |
| DDT | Dichlorodiphen yltrichloroethane |
| DR | Daily consumption rate |
| dw | Dry weight |
| ED | Exposure duration |
| EF | Enrichment factor |
| EF | Exposure frequency |
| ERM | Effects Range-Median |
| Fe | Iron |
| Fl | Fluorene |
| FlA | Fluoranthene |
| FW A | Food waste A |
| FW B | Food waste B |
| GC | Grass carp |
| GZ | Guangzhou |
| GC-CVAVFS | Gas chromatography – cold vapor atomic fluorescence spectrometry |
| GY | Gaoyao |
| HCB | Hexachlorobenzene |
| HCHs | Hexachlorocyclohexanes |
| Hg | Mercury |

| | |
|---|---|
| HI | Hazard index |
| HK | Hong Kong |
| $I_{geo}$ | Geoaccumulation Index |
| IP | Indeno(1,2,3-cd)pyrene |
| JECFA | The Joint FAO/WHO Expert Committee on Food Additives |
| LB | Largemouth bass |
| LOD | Limit of detection |
| MD | Mud carp |
| MeHg | Methylmercury |
| Mn | Manganese |
| MP | Mai Po |
| Nap | Naphthalene |
| Ni | Nickel |
| NIST | National Institute Standards and Technology |
| OCPs | Organochlorine pesticides |
| PAHs | Polycyclic aromatic hydrocarbons |
| Pb | Lead |
| PBDE | Bromated flame retardant |
| PhA | Phenanthrene |
| PRD | Pearl River Delta |
| PTDI | Provisional Tolerable Daily Weekly Intake level |
| PW | Pok Wai |

| | |
|---|---|
| Py | Pyrene |
| RfD | Recommended reference doses |
| S/N | Signal-to-noise ratio |
| SD | Shunde |
| SPE | Solid-phase extraction |
| SPM | Suspended particulate matter |
| STK | Sha Tau Kok |
| THg | Total Mercury |
| TL | Trophic level |
| TMF | Trophic magnification factors |
| TOC | Total Organic Carbon |
| USEPA | United states Envrionmental Protection Agency |
| WHO | Word Health Organization |
| ww | Wet weight |

# CHPAPTER 1 GENERAL INTRODUCTION

## 1.1 Food Wastes

Food waste is any food substance, raw or cooked, which is discarded, or intended or required to be discarded (USEPA, 2012b). When the food waste is disposed to the landfill, it would rot quickly, and produce substantial quantities of methane gas ($CH_4$) which is a potent greenhouse gas with 21 times the global warming potential of carbon dioxide ($CO_2$) (Gustavsson et al., 2011). Food waste is a global problem, about 1.3 billion tonnes per year, and one-third of the edible parts of food produced for human consumption, which impacts the environment and society (Figure 1.1). Food is wasted throughout the food supply chain, from initial agricultural production down to final household consumption. In medium-and high-income countries, food is to a great extent wasted at the consumer level. In low-income countries, food is mainly lost during the early and middle stages of the food supply chain; much less food is wasted at the consumer level (Gustavsson et al., 2011). In developed countries, food wastage is very serious, e.g. per capita food wasted by consumers in sub-Saharan Africa and South/Southeast is about 10-15 times lower than in North America and Europe (Gustavsson et al., 2011). In the U.S., it has been noted that, on average, 27% of all edible food goes to waste (USEPA, 2012b). In 2010, more than 33 million tonnes of food waste was generated in USA (USEPA, 2012b), more than any other material category (Figure 1.1). Food waste accounted for almost 14 % of the total municipal solid waste (MSW) stream, less than 3 % of which was recovered and recycled in 2010 (USEPA, 2012b). Food waste is a problem for not only the environment, but also the economy. It is currently costing us billions of dollars every year to both produce this food and to dispose of the waste (Hall et al., 2009; Gustavsson et al., 2011). The amount of food wastes generated in Hong Kong has increased in recent years. The quantity of disposed food waste from commercial and industry sectors has been increasing, from 400 tonnes per day in 2002 to 1,056 tonnes per day in 2011, and it comprised of 37% (about 330,000 tonnes) of the municipal solid waste loads at landfills (about 900,000 tonnes) in 2011 (EPD, 2011). In Hong Kong, the remaining capacities of the three existing landfills will be exhausted by 2018 (EPD, 2011).

In order to reduce the loading of local landfills, the policy framework of the Hong Kong Government on Municipal Solid Waste Management (2005-2014) mainly focused on waste reduction through producer responsibility schemes, waste charging and landfill disposal bans. In addition, the reuse, recovery, and recycling of wastes have been strongly advocated (EPD, 2005). A pilot plant, the Waste Recycling Centre at Kowloon Bay, with a 4 tonne/day capacity, was commissioned in 2008 to handle food waste from the Olympic equestrian events. A trial operation for the recycling of source-separated food wastes generated from selected commercial and industrial sectors including restaurants, hotels, wholesale markets and generators of the catering, food production, bakery and bean curd industries was subsequently commenced (EPD, 2009). Based on the results obtained from the trial, larger facilities (Siu Ho Wan, Lantau Island, to start operation in mid-2010s, and Shaling, North District in the late 2010s) will handle 200 tonnes (mostly food waste) per day each for the production of biogas and some 20 tonnes of compost every day (EPD, 2008).

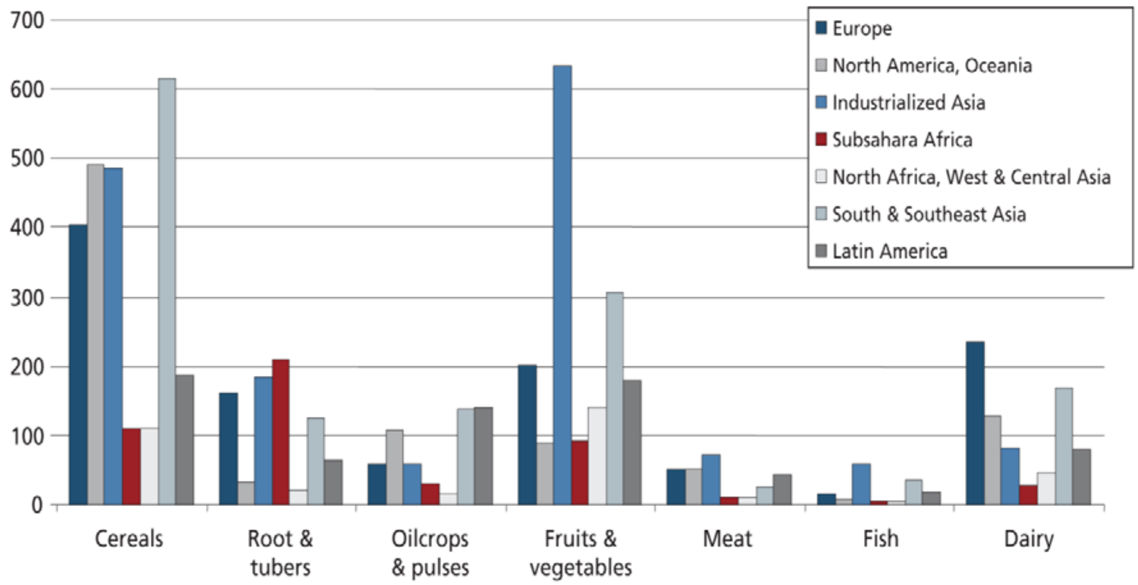



Figure 1.1 Production volumes of food waste of each commodity group, per region (million tonnes) (Gustavsson et al., 2011).

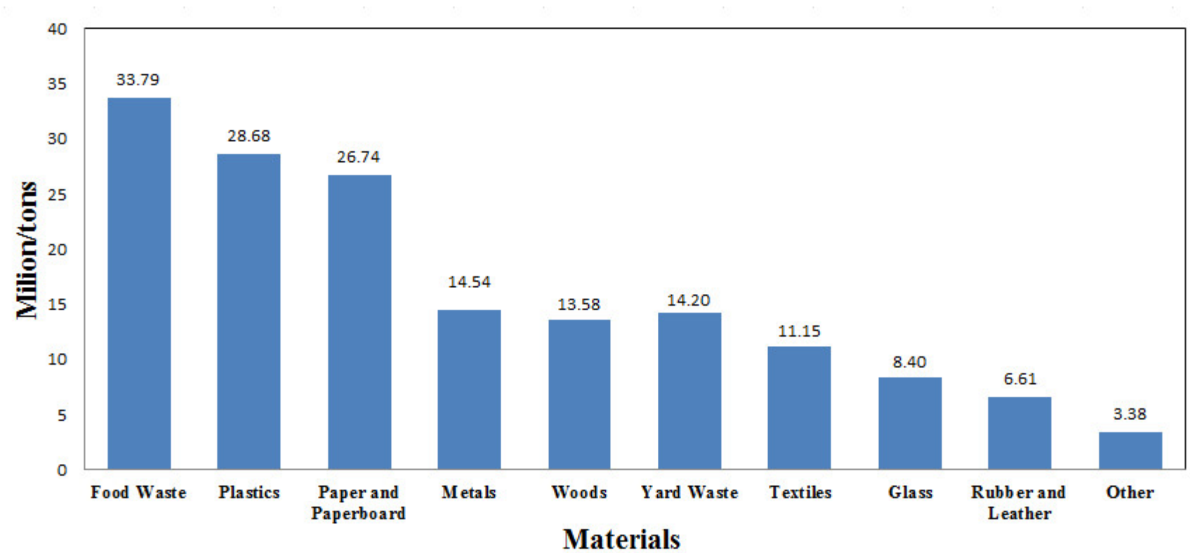



Figure 1.2 Municipal solid waste discarded (by material) in 2010 in the USA. (USEPA, 2012b)

Trash fish and compound feed are two major types of fish feeds commonly used in China. Trash fish are mostly wild, various in species, and captured from deep sea (Weatherley and Cogger, 1977), usually have low commercial value by virtue of low quality, small size, and low consumer preference and, therefore, are often used to directly feed fish in China. It was estimated that trash fish production amounted to over 5 million tonnes in China in 2001 (FAOUN, 2006), and almost all trash fish captured are exclusively applied to produce livestock and fish compound feeds in the form of fish meal or fish oil or used directly to feed fish. By 2013, China alone would require 4 million tonnes of trash fish to sustain marine cage aquaculture (FAOUN, 2006). On the other hand, compound feed was widely used in recent years in China. The amount of aquaculture compound feeds produced was only 0.85 million tonnes in 1991 but increased rapidly in recent years. In 2004, the total production of aquaculture feeds amounted to about 9 million tonnes in China, yet the amount of compound feeds used in aquaculture was still less than one-third of the total fish feed need (Fang, 2012).

Fish meal is the major component of fish feeds used in aquaculture. However, fish meal is usually made from small fish caught by capture fishery industry. Fish resources, captured from nature, has levelled off in the past 20 years, and therefore alternative sources of protein for making fish feed to replace declining fish stocks would be an important topic in aquaculture industry. Pollutants can be accumulated in trash fish. It has been demonstrated that the use of trash fish as fish feeds was more polluted than using pellet feeds, based on the concentrations of POPs such as OCPs, bromated flame retardant (PBDEs) and PCBs detected in fish fed by trash fish (Guo et al., 2009b). In addition, our previous studies also showed that trash fish used in PRD contained higher levels of Hg than pellet feeds (Cheng et al., 2011; Liang et al., 2011). In European counties, fish meal derived from the trash fish for production of pellet feeds also contained high concentrations of bromated flame retardant (PBDEs), perfluorinated compounds, dioxin/furans and PCBs (Suominen et al., 2011). Therefore, the use of trash fish as fish feeds and as ingredients of fish meal for producing pellet feeds are the major sources of contaminates found in culture fish worldwide. It has been shown that predatory fish (*Dicentrarchus labrax*) fed with a diet containing 94% by plant protein (corn gluten meal, wheat gluten, extruded wheat, soybean meal, rapeseed metal) achieved a similar growth rate to those fed with high-quality fish meal (Kaushik et al., 2004). In Japan, Bake et al. (2009) found that recycled food waste could partially replace fish meal in fish feed. If the technique and industry of animal feed from food waste can be developed in Hong Kong, it could be one of the solutions towards the MSW problems in Hong Kong.

## 1.2 Food Safety Issues and Pollutants in Fish Products in Hong Kong

Fish consisted of a major part of Hong Kong's people diet. The demand of freshwater products in Hong Kong is mainly supplied by the import from PRD. During past thirty years, the industrial and agricultural activities have been increased rapidly in PRD accompanied with the rising population. These increases bring along with overuse of chemicals, hence, constituted serious adverse impacts on the environment. A number of food safety issues has been raised in recent years, notably the undesirable chemicals detected in imported fish. Malachite green which is harmful to humans and is probably carcinogenic (Culp S, 2004), was detected in turbots, eel, freshwater grouper, and mud carp (CFS, 2006d, a, c). Nitrofurans (antimicrobial agents for veterinary use) which may cause cancer in experimental animals were detected in silver carp and freshwater grouper (CFS, 2006c). Endosulfan, an organochlorine pesticide may cause chronic kidney damage, was found in live eels (CFS, 2006b).

In addition, environmental pollutants such as persistent organic pollutants (POPs) (e.g. OCPs and polycyclic aromatic hydrocarbon (PAHs)), metalloid/metals contained in fish may pose human health risks. Previous studies demonstrated rather high concentrations of different POPs in fish: PAHs (184 - 505 ng/g dry weight (dw)) in tilapia from Mai Po (Liang et al., 2007), PCBs (5.15 - 226 ng/g lipid weight) in different freshwater fish from fish ponds around the PRD (Nie et al., 2006), and OCPs (1.10-1018 ng/g wet weight (wt)) and PAHs (1.57-118 ng/g wt) in freshwater and marine fish species available in local markets in Hong Kong (Cheung et al., 2007). It has been reported Arsenic (As) and heavy metals (Mercury (Hg), cadmium (Cd), chromium (Cr), lead (Pb), copper (Cu), nickel (Ni) and zinc (Zn)) concentrations in marine and freshwater fish available in Hong Kong markets (Cheung et al., 2008; Wang et al., 2013b). The results revealed that a few fish species had average concentrations greater than the China National Standard Management Department (GB2762, 2005), potential non-cancer risk via diets of 36% of adults and 51% of children exceeded the reference dose adopted in the United State Environmental Protection Agency (USEPA) was used to estimate the non-cancer risk of MeHg for adults and children (USEPA, 2012a).

It is commonly observed that fish could accumulate metalloid/metals and POPs from their living environment and foods (Ip et al., 2005; Ciardullo et al., 2008; Qiu et al., 2011). In addition, there are clear indications showing Hg and OCPs can be enriched through the aquatic food chains via biomagnification, leading to organisms at higher trophic levels containing extremely high concentrations of contaminants (Campbell et al., 2005; Meng et al., 2009; Cheng et al., 2011).

It has been observed that concentrations of OCPs and PAHs in human milk and plasma were significantly correlated with the frequency of fish consumption in both Hong Kong and Guangzhou populations (Wong et al., 2002a; Wang et al., 2010a; Wang et al., 2013a). The OCPs have been included in the original "dirty dozen" under the Stockholm Convention on POPs (UNEP, 2005). Some OCPs, such as DDT and its metabolites, are detrimental to reproductive and nervous systems, although it has not been proven that DDTs are linked with cancers in humans (Soto et al., 1995; Li et al., 2008). PAHs are known animal carcinogens (IARC, 1987). Due to their carcinogenic properties and acute toxicity, 16 congeners of PAHs have been listed as priority control pollutants by the USEPA (USEPA, 2012a). The potential health effects of metalloid/metals contained in fish have become considerable concerns in Hong Kong. Dickman et al. (1998) reported high concentrations of Hg in the hair of subfertile males in Hong Kong, and Ip et al. (2004) revealed that the elevated Hg in blood and hair were significantly correlated the frequency of fish consumption. Ko (2004) showed that severe skin disorders and autism in children were linked with their high concentrations of Hg, Cd (coastal cities such as Hong Kong and Shanghai), and As and Pb (inland cities such as Beijing) detected in hair were possibility reflected the dietary differences between the coastal and inland populations. Qin et al. (2010) further observed that patients with uterine leiomyomas tended to have higher concentrations of PAHs, DDTs and Hg in the visceral adipose tissues which were significantly correlated with patients' seafood diet, body mass index (BMI) and age.

## 1.3 Hong Kong Inland Fisheries

Inland fishery in Hong Kong was prosperous in 1960s, with a rapid expansion of pond area between 1960s and 1980s (Lau et al., 2003). However, due to the change of economic base of Hong Kong, development of new towns in the New Territories, as well as the import of low-priced freshwater fish from the mainland further rendered the local fishery less profitable and drove some fishermen away from the industry, resulting in the decline of inland fishery (Lam, 1999). The pond area (1920 ha.) decreased by 40% (1130 ha.) from 1984 to 2004, while the number of inland fish farmers decreased from 1690 in 1991 to 637 in 2004. Simultaneously, the annual production of pond fish decreased from 6,500 tonnes (HK$ 104 million) in 1984 to 1,977 tonnes (HK$ 33 million) in 2004 (by 56.3 and 29.5% of the quantity and monetary value of total fish production, respectively) (Chan, 2005). Most fish ponds are now confined in the north-western part of the New Territories.

## 1.4 Fish Culture

Polyculture is the practice of culturing more than one species of aquatic organisms in the same pond, through the association of fish species of different food habits (e.g. filter feeders, herbivores and bottom feeders) for the effective use of available fish foods in the pond, where wastes produced by one species may serve as food items for other species (FAO, 2001). The concept of polyculture of fish is based on total utilization of different trophic and spatial niches of a pond, in order to obtain maximum fish production per unit area (FAO, 2001).

Different species combination in polyculture system also effectively contributes to improving the pond environment. Algal bloom is common in most manure fed ponds. By rearing bighead carp or silver carp in appropriate density, certain algal bloom can be controlled. Grass carp on the other hand keeps the macrophyte abundance under control due to its vegetarian feeding habit. The partially digested excreta will become the feed for the bottom feeders mud carp or tilapia. The bottom feeders (mud carp) could help resuspension of bottom nutrients to water while stirring the bottom mud in search of food. Such an exercise of bottom feeders also aerates the bottom sediment (Ruddle and Zhung, 1988; Chen et al., 2002). The mixture of fish gives better utilization of available natural food produced in a pond. The compatible fish species having complimentary feeding habits are stocked so that all the ecological niches of a pond ecosystem are effectively utilized (Chen et al., 2002). Polyculture is the traditional aquaculture farming model in the PRD region, where the pond water is drained after harvest to maintain water quality and reduce the incidence of fish diseases (Chen et al., 2002). The pond mud is also removed and used as fertilizer for crops growing on the dykes of fish ponds (Wong et al. 2004). It began in China more than 1000 years ago. Polyculture is the main fish culture system in Asia, the continent generating over 90% of the world aquaculture production.

However, due to the rapid socio-economic changes, the traditional fish farming system has been changed from polyculture to monoculture. Chemical fertilizers (for enriching pond water) and high protein feeds are used instead of waste materials (e.g. cocoons after extraction of silk), and very often monoculture of high priced fish (e.g. mandarin fish and largemouth bass) is practiced. This coupled with the deterioration of water quality due to the contaminations by nutrient and pesticides. Pond mud that used to be excavated and utilized as fertilizer for crops growing on the dykes of fish ponds is no longer used as fertilizer (Lau et al., 2003). Pond water was drained and pond sediment was removed in the past, but these are no longer practiced, and pond sediment is now served as a sink of different pollutants (Wong et al., 2004). All these are casting doubts on the fish quality and the health of consumers.

## 1.5 Integrated Fish Farming System

Integrated fish farming is a diversified and coordinated way of farming, accompanied with the production of agricultural items in the fish farms with fish as the main product (FAO, 2001). Grass and aquatic plants as fish feeds are used in integrated fish systems, and the model can be found in many parts of China, e.g. Changjiang and Pearl River Deltas (FAO, 2001). A more balanced pond ecosystem is maintained through polyculture of different fish with different feeding habits, which can enhance the utilization of nutrients and cropping of plankton to regulate water quality (Wong et al., 2004). Grass species such as rye grass (*Lolium perenne*) and Napier grasses (*Pennisetum purpureum*) serving as low-cost supplemental feeds for fish can be easily produced on the farm; with freshwater fish species such as bighead (*Aristichthys nobilis*), grass carp (*Ctenopharyngodon idellus*) and mud carp (*Cirrhina molitorella*) directly or indirectly feeding on these grasses. The production cost can be halved for grass-fed fish, when compared to cereal grain-fed fish, in terms of per kg of fish produced (FAO, 2001).

Cultivating low-trophic level fish (such as grass carp) can utilize solar energy better; as production of phytoplankton depends on sunlight and the available soluble nutrients in water. With higher availability of sunlight, fertilization of fish ponds using manure can therefore promote the growth of zooplankton and phytoplankton and benthic invertebrates for filter filters (bighead) and bottom feeders (such as mud carp). Furthermore, low-trophic level fish (herbivores) are less susceptible to the accumulation of toxic chemicals. Our past studies showed that black bass, a carnivore, had higher levels of OCPs, PAHs and Hg in its flesh than those of herbivores (grass carps), omnivores (tilapia, common carp) and filter feeders (bighead carp) (Zhou and Wong, 2000; Cheung et al., 2007; Cheng et al., 2011). A study in Brazil also showed high Cr, Pb and Zn concentrations in carnivorous *Oligosarcus hepsetus* than omnivorous *Geophagus brasiliensis* and detritivorous *Hypostomus luetkeni*, confirming that carnivorous species are prone to incorporate more heavy metals than fish in low-trophic levels (Terra et al., 2008).

However, due to the rapid socio-economic changes, the traditional integrated fish farming system has been changed from polyculture to monoculture. Pond mud that used to be excavated and utilized as fertilizer growing on the dykes of fish ponds is used no longer as fertilizer (Lau et al., 2003). Pond water was drained and pond sediment was removed in the past, but these are no longer practiced, and pond sediment is now served as a sink of different pollutants (Wong et al., 2004). Furthermore, livestock waste is no longer readily available for use as pond fertilizer due to the decrease in the rearing of livestock.

With the implementation of good aquaculture practice and utilization of food wastes, which can serve as fish feeds, fish production could be greatly enhanced. Reactivation of nearby farms is also necessary so that pond mud could be excavated and used as fertilizer. By redeveloping inland fisheries and farming, there would be an outlet for local food wastes, and at the same time producing safe and quality fish for local consumption, cutting down our ecological foot print.

## 1.6 Fish Pond Habitat Conservation

The conservation of fish pond habitats in north western New Territories contributes significantly to the ecological function of the ponds. Actively managed fish ponds provide water, food and shelter for aquatic and terrestrial animals, and provide breeding grounds for birds and other wildlife. The Mai Po Nature Reserve (MPNR) (Figure 1.2) are best known as a wintering site for up to 68.000 waterbirds, and other 30.000 shorebirds which use the sites as staging post during spring and autumn migration (Young, 2004). Over the 380 species of birds that inhabit the reserve, and 35 are of global conservation concern including the Saunders's Gull (*Chroicocephalus saundersi*) and the Black-faced Spoonbill (*Platalea minor*) (WWF, 2006). There are 1,200 ha commercial fish pond in MPNR, those traditionally operated fish ponds are as an example of the wise use of wetland and are of high ecological value (Young, 2004). Waterbirds can rest and feed on the fish and shrimps in the ponds and insets along the pond bunds (Young and Chan, 1997; Young, 1998), therefore the ponds was provide an important feeding habitat for waterbirds. More than 40,000 of the total occurrence for 45 bird species have been observed foraging on the exposed soft mud floor of five fish ponds located within and around the Mai Po Inner Deep Bay Ramsar Site during the 9-14 days of draining in 2001 (Lau et al., 2003)

## 1.7 Objectives of the Present Study

The present study is aimed at investigating the possibility of using food waste for cultivating lower trophic fish which are relatively free of contamination, by examining the levels of some typical environmental pollutants such as metalloid (As) /metals (Hg, Cd, Cr, Pb, Cu, Ni and Zn), and major POPs (OCPs, PAHs,) in water, sediment, suspended particulate matter (SPM), and fish muscle. The bioaccumulation and biomagnifications in food chains, their bioaccessbility and associated health risk assessments of these pollutants were also investigated.

The specific objectives of this research were:

(1) To analyze trophic relationships and health risk assessments of heavy metals in the freshwater fish ponds ecosystem of Pearl River Delta, China;

(2) To evaluate the environmental effects of using food wastes as fish feed, focusing on the concentrations and variations of metalloid/metals, OCPs and PAHs in the freshwater fish ponds in Sha Tau Kok;

(3) To analyze potential bioaccumulation and biomagnification of metalloid/metals, OCPs and PAHs in freshwater fish, using food wastes as fish feeds; and

(4) To evaluate the bioaccessibility of metalloid/metals, OCPs and PAHs via fish consumption using an *in vitro* gastrointestinal digestion model.

## 1.7 Framework

The conceptual framework of the current study is outlined in Fig 1.3 which highlights the main themes of this study and the interrelationships among different chapters.

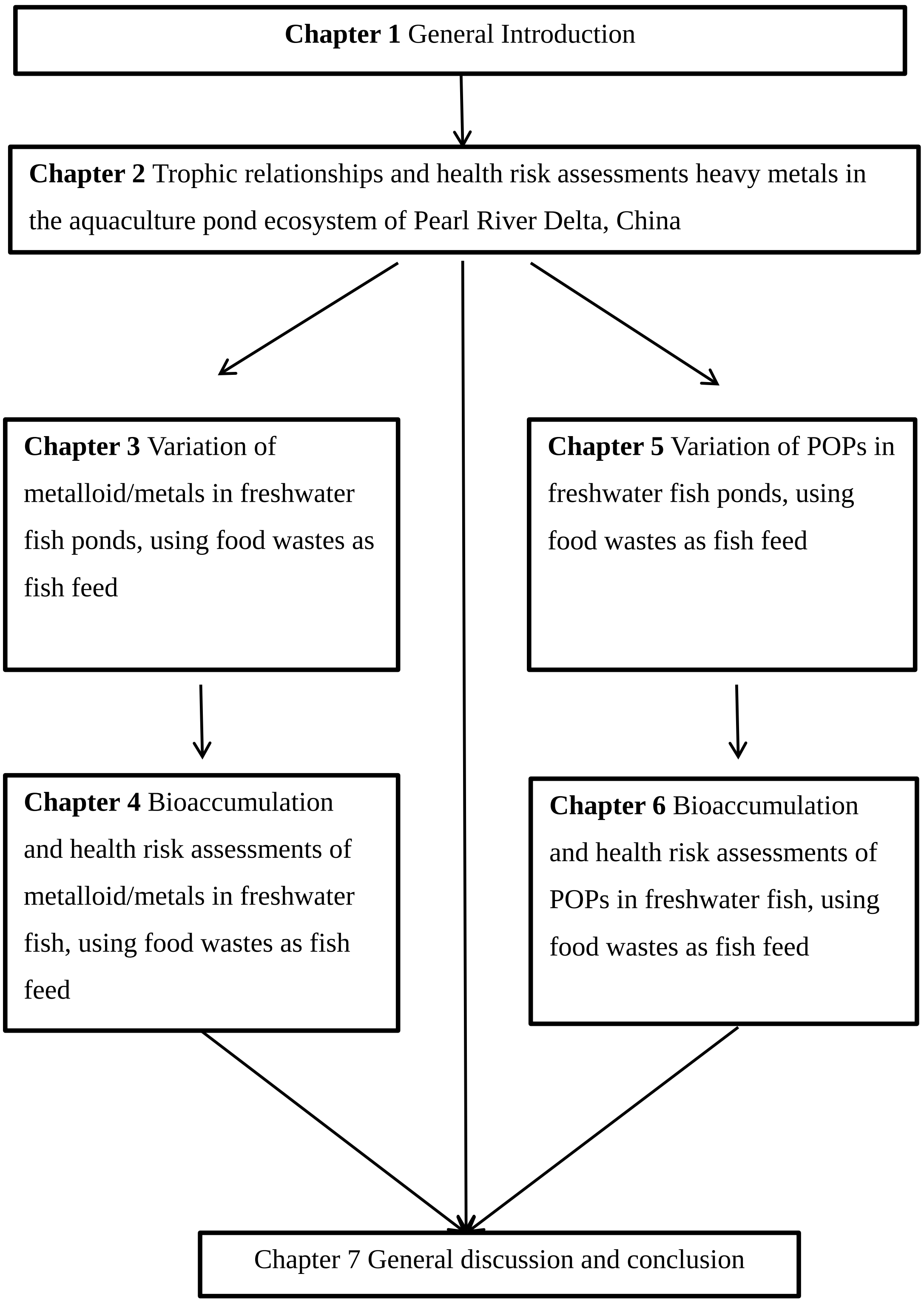



**Figure 1.4** Conceptual framework of the present study

## 1.8 Significance of the Present Study

1) This is the first attempt to use food waste to replace fish meal as protein source in fish feeds and adopt the traditional fish farming practice to produce safe and quality fish, and create a better habitat for migratory birds and wildlife.
2) This study provides important information about the feasibility of recycling the residual energy contained in food waste for fish culture, and reductions of the waste disposal pressure of existing landfills in Hong Kong.
3) This study indicates the possibility of reducing concentrations of environmental pollutants (metalloid/metals, PAHs and OCPs) in fish, by rearing low trophic level fish (grass carp, bighead carp and mud carp) using appropriate contents of food waste (cereals, fruits and vegetables).
4) The shorter food chain (omnivorous food chain: plankton, grass carp, bighead and mud carp) would enable lower bioaccumulation and biomagnification of environmental pollutants; leading to lower concentrations of these pollutants in the cultured fish, possing less health risks to consumers.
5) It is hoped that the information derived from the present study will be useful for reactivating our inland fisheries in Hong Kong.

# CHAPTER 2 TROPHIC RELATIONSHIPS AND HEALTH RISK ASSESSMENTS OF HEAVY METALS IN THE AQUACULTURE POND ECOSYSTEM OF PEARL RIVER DELTA, CHINA

## 2.1 Introduction

The Pearl River Delta (PRD) is an important area for agricultural, commercial, and industrial development of China. The Pearl River Delta has undergone rapid economic development with significant population increase during the last three decades. Environmental contamination by metalloid/metals and petroleum hydrocarbons, caused by anthropogenic activities, has been reported in recent studies (Cheung et al., 2008). In PRD region, most of the fish farmers are still using the traditional way of filling up fish ponds using river water (Ruddle and Zhung, 1988). Through atmospheric deposition, sewage outfalls, urban storm water and agricultural and industrial runoff, metalloid/metals may enter fish ponds. In addition, the fish feeds have been blamed as the major sources of some metalloid/metals to aquaculture (Lacerda et al., 2006; Lacerda et al., 2011). Sediments accumulate higher levels of metalloid/metals than water, causing serious problems due to their toxicity and propensity to bioaccumulate (Chen et al., 2000). If toxic metals are accumulated in fish tissues exceeding maximum permitted concentrations, it will pose a human health risk.

The anthropogenic metal contribution in sediment can be estimated from enrichment relative to uncontaminated reference materials or widely accepted background levels. Enrichment factor (EF) and geoaccumulation index ($I_{geo}$) are two pollution indicators wildly used to assess sediment quality (Loska et al., 1997; Diaz-de Alba et al., 2011). EFs are used as an indicator to reflect the degree of environmental contamination by comparing with background values representative for uncontaminated sample materials (Presley et al., 1992). $I_{geo}$ determines the status of contamination by comparing current metal contents with preindustrial levels (Müller, 1981). The concentration accepted as background value is multiplied each time by the constant 1.5 in order to take into account natural fluctuations of a given substance in the environment as well as very small anthropogenic influences (Loska et al., 1997). EF reflects the degree of environmental contamination by comparison with background values representative for uncontaminated sample materials. Iron or aluminium is commonly used as a normalization element to reduce the variations produced by heterogeneous sediment (Loska et al., 1997). The concentration ratio of $^{15}N/^{14}N$, expressed relative to a standard (i.e., $\delta^{15}N$), has been shown to increase with increasing trophic level because of the preferential excretion of lighter nitrogen isotopes (Deniro and Epstein, 1981; Minagawa and Wada, 1984). This technique has been used as a tool to determine the trophic relationships and to estimate the biomagnifications of organic and inorganic contaminants in aquatic ecosystems (Campbell et al., 2005; Cui et al., 2011).

Most studies on metalloid/metals contamination in aquaculture environments in the PRD are focused on offshore aquaculture (Qiu et al., 2011) comparatively, there is a lack of information concerning fresh water fish pond environments (Cheung et al., 2008). In addition, most studies investigated the quality and risk assessment of sediment or fish tissue samples, and metalloid/metals transport from sediment to organisms (bioaccumulation) (Qiu et al., 2011). In actually, however, aquatic organisms can also accumulate metalloid/metals from their feeds and with trophic transfer and the potential for biomagnification. This information is very useful for fisheries management in freshwater fish pond environment. This is the first study to use stable isotopes of nitrogen to investigate the biomagnification rates of heavy metals in various species of fish at different trophic levels in aquaculture pond ecosystems in the Pearl River Delta, China. With increasing public awareness concerning the safety of foods originating from mainland China, the major objectives of the present study were to (1) investigate the heavy metals contents contained in the freshwater aquatic products; (2) the heavy metals bioaccumulation and biomagnification in aquatic food chains of freshwater fish ponds in the PRD (the main source for consumption in Hong Kong); and (3) evaluate the potential health risks of exposure to the Hong Kong residents via dietary intake of these products.

## 2.2 Materials and Methods

### 2.2.1 Sampling

Fish and corresponding sediment samples were collected from 18 fish ponds

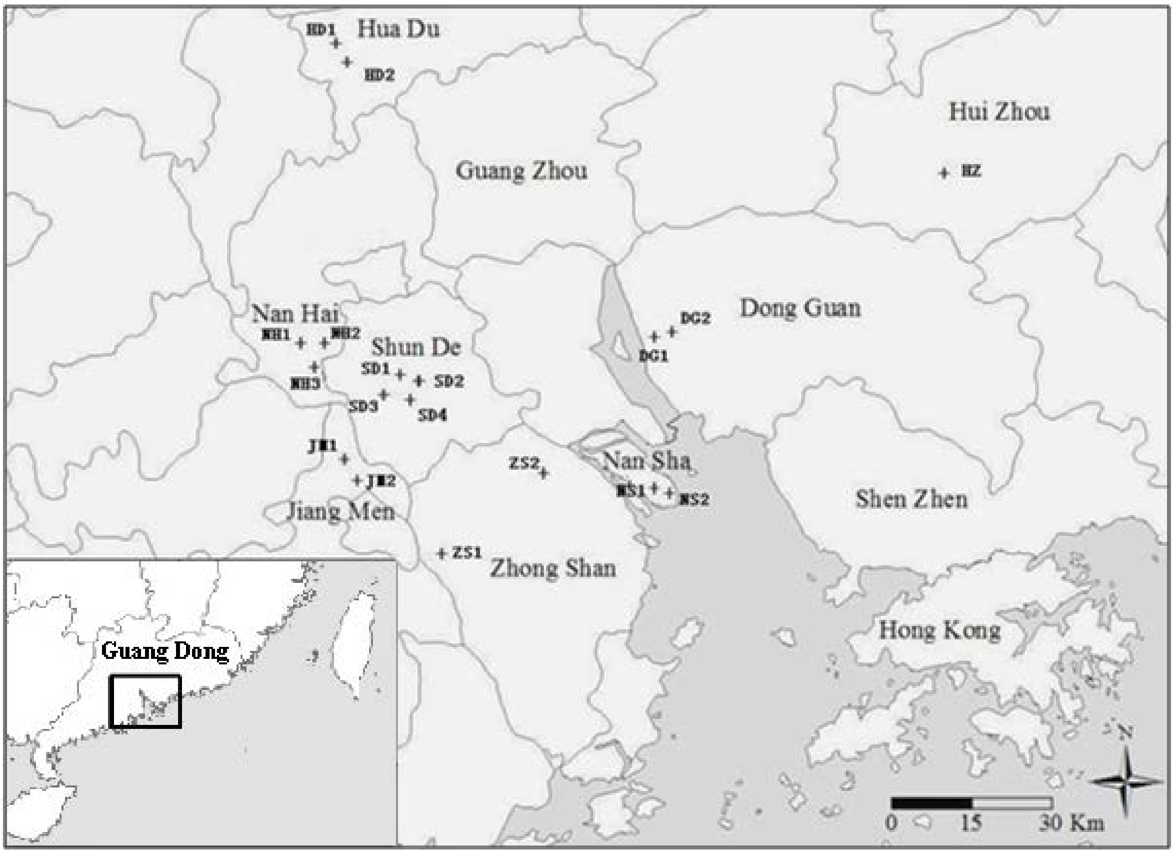



**Figure 2.1.** Map of the sampling locations in PRD, China

Note: Huadu= HD1 and HD2; Nanhai= NH1, NH2 and NH3; Shunde= SD1, SD2, SD3 and SD4; Jiangmen= JM1 and JM2; Zhongshan= ZS1 and ZS2; Nansha= NS1 and NS2; Dongguan= DG1 and DG2; Huizhou= HZ around the Pearl River Delta (PRD) area including Huadu, Nanhai, Shunde, Zhongshan, Jiangmen, Nansha, Dongguan, and Huizhou (Figure 2.1). Three random sediment samples (0-10cm) were collected within each pond (< 100 $m^2$ to > 10 ha) using a stainless steel grappler, yielding 54 sediment samples in total (3 samples × 18 ponds). Six fish species including northern snakehead (*Channa argus)* (n=25), mandrarin fish *(Siniperca chuatsi)* (n=27), largemouth bass (*Micropterus salmoides*) (n=25), bighead carp (*Aristichthys nobilis*) (n =15) and grass carp (*Ctenopharyngodon idellus)* (n=42) were collected from the fish ponds using a nylon net. Commercial fish feed pellets and live forage fish (mud carp [*Cirrhina molitorella*]) commonly used as fish feeds for feeding commercial fish were purchased in each sampling site from fish farmers. Sediment and fish samples were wrapped in aluminium foil and placed in polyethylene bags during transportation and stored at -20 ℃ in the laboratory for subsequent analyses.

Zooplankton were also sampled (at approximately 0.5-10 m depth) from each fish pond, using a non-metallic plankton net (202μm) for multiple vertical tows and stored in 100 ml acid-treated Teflon vials during transportation (Chen et al., 2000).

### 2.2.2 Analytical Methods

All the samples (fish and sediments) were freeze-dried and ground into powder. The sediment samples were homogenized by passing through a stainless steel 0.154 μm sieve. Sediment samples and fish samples were digested according to the USEPA

**Table 2.1** Summary of the BCR sequential extraction protocol

| Step | Fraction | Extraction Condition | Component extracted |
|---|---|---|---|
| F1 | Acid soluble | 20ml 0.11mol/L $CH_3COOH$, shaking at 22±5℃ for 16h | Exchangeable and bound to carbonates |
| F2 | Reducible | 20ml 0.5mol/L $NH_2OH \cdot HCl$ at Ph 1.5, shaking at 22±5℃ for 16h | Bound to Fe/Mn oxides |
| F3 | Oxidizable | Add 5ml 8.8mol/L $H_2O_2$ (adjust to pH 2.0 with $HNO_3$), digesting at room temperature for 1 h with occasional manual shaking, then cover the tube and heat at 85±2℃ for 1h, and the remove the cover and reduce the volume to a few milliliters/cover the tube and heat at 85±2℃ for 1 h, add another 5ml 8.8mol/L $H_2O_2$ (adjust to pH 2.0 with $HNO_3$) water bath 1 h at 85±2℃, and then remove the cover and reduce the volume to near dryness. Add 25ml 1 mol/L $NH_4OAc$ (adjust to pH 2.0 with $HNO_3$), shaking at 22±5℃ for 16h. | Bound to organic matter and sulfides |
| F4 | Residual | 8ml the mixture of concentrated $HNO_3$ and $HClO_4$ (1:3), same as the total digestion | Metal within lithogenic minerals |

standard method (3052) (USEPA, 1996a). Briefly, 2 g of sediment was placed in high-pressure Teflon containers, with 12 ml of mixture of acid (hydrochloric acid: nitric acid, 3:1 ratio [v/v]) (aqua regia), whereas, 0.15-0.5 g of biota samples or fish feed pellets, added with 10 ml nitric acid in high-pressure Teflon containers and placed in fume cupboard for predigestion for about 6-8 h. Subsequently, all the samples were digested by MARS Microwave Reactions System (CEM, USA). After digestion, extracts were filtered with Whatman No.42 filter papers and diluted to a volume of 25 ml with Milli-Q water. The filtrates were then analyzed for metals by Inductively Coupled Plasma-Mass Spectrometry (Perkin-Elmer, Elan 9000, Norwalk, CT). For determining the geochemical fraction of metals in sediment samples, the European Community Bureau of Reference (BCR) sequential extraction procedure was adopted in this study (Table 2.1). The metal (Cd, Pb, Zn, Cr, Cu, Ni and Mn) contents in extracts were also analyzed by Inductively Coupled Plasma-Mass Spectrometry (Perkin-Elmer, Elan 9000, Norwalk, CT). In addition, subsample of sediments were used for the analysis of total organic carbon (TOC), using thermal partitioning at 550 ℃ (USEPA, 1997).

### 2.2.3 Quality Control

All of the samples were tested in triplicate**.** Analytical blank and reference materials were included in every sequence**.** Four certified reference materials (CRM): NIST 8704 (Buffalo River sediment), NIST 1944 (New York /New Jersey Waterway sediment) and NIST 1566b (Oyster tissue) were obtained from National Institute Standards and Technology, (NIST, USA) and TORT-2 (Lobster hepatopancreas) was obtained from National Research Council of Canada). The recoveries for total metals ranged from 88-107% (Cr, Pb, Cr, Cu, Ni, Zn, Fe and Mn), whereas, the recoveries for BCR sequential extraction, the total concentration (Acid soluble fraction + Reducible fraction + Oxidizable fraction + Residual fraction) of BCR sequential extraction ranged from 85-113% (Cr, Pb, Cr, Cu, Ni, Zn, Fe and Mn).

### 2.2.4 Assessment of Sediment Quality

Anthropogenic impact on sediment is commonly accessed by calculating the enrichment factor (EF), based on the equation formula (Eq.1) (Loska et al., 1997). In this study, iron was used as reference element, because its content in this study sediment is predominantly (97%) associated with the parent material matrix of the sediment (Diaz-de Alba et al., 2011).

$$EF = (T_x \times Fe_b) / (T_b \times Fe_x) \qquad (1)$$

where $T_x$ is metal concentration in sediment samples, $T_b$ is metal concentration in background reference, $Fe_x$ is iron concentration in sediment samples and $Fe_b$ is iron concentration in background reference.

The geoaccumulation index ($I_{geo}$) determines pollution levels by comparing current metal contents with preindustrial levels (Müller, 1981). The geoaccumulation index was calculated using Eq. (2):

$$I_{geo} = \log_2 (C_n / (1.5 \times B_n)) \qquad (2)$$

**Table 2.2** Contamination categories base on the enrichment factor, geoaccumulation index and risk assessment code (Müller, 1981; Sutherland, 2000; Loska et al., 2003)

| Enrichment factor | | Geoaccumulation index | | Risk assessment code | |
|---|---|---|---|---|---|
| Value | Categorisation | Value | Pollution status | Metals in carbonate and exchangeable fraction (%) | Risk |
| ＜2 | Deficiency to minimal enrichment | ≤0 | Unpolluted | ＜1 | No risk |
| 2-5 | Moderate enrichment | 0-1 | Unpolluted to moderate | 1-10 | Low risk |
| 5-20 | Significant enrichment | 1-2 | Moderate | 11-30 | Medium risk |
| 20-40 | Very high enrichment | 2-3 | Moderate to strong | 31-50 | High risk |
| ＞40 | Extremely enrichment | 3-4 | Strong | ＞50 | Very high risk |
| | | 4-5 | Strong to extremely | | |
| | | 5＜ | Extreme | | |

where $C_n$ is the concentration of the metals content in sediment samples and $B_n$ is the background content in the Earth's crust (Hamilton, 2000). Criteria for these pollution indictors are given in Table 2.2.

### 2.2.5 Biota-Sediment Accumulation Factor (BSAF)

Biota-sediment accumulation factor can be obtained by Eq. (3) (Harrad and Smith, 1997):

$$BSAF = Ct / Cs \quad (3)$$

where Ct is metal concentration in the tissues and Cs Metal concentration in sediment.

### 2.2.6 Stable Isotope Analysis

The biota samples were analyzed for stable isotopes at Institute of Soil Science (Nanjing, China), Chinese Academy of Sciences. Stable isotope values were expressed as

$$\delta^{15}N = ( R_{sample} / R_{standard} - 1) \times 1000 \quad (4)$$

where $R_{sample}$ is Corresponding ratios of $^{15}N/^{14}N$ and $R_{standard}$ is atmospheric $N_2$ (air). Replicate measurements of internal laboratory standards (albumen) showed measurement errors of ±0.3‰ for stable nitrogen isotope measurement.

### 2.2.7 Trophic Level and Biomagnification Calculations

Trophic levels of biota samples were calculated using Eq (5) (Campbell et al., 2005).

$TL_{consumer} = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{zooplankton}) / 3.4$ (5)

where $TL_{consumer}$ *is* consumer trophic level and $\delta^{15}N$ of zooplankton was assumed to be 3, and 3.4 was the isotopic enrichment factor.

The trophic magnification factors (TMFs) were based on relationships between trophic level and the metal concentration using the following simple linear regression (Campbell et al., 2005):

$\text{Log [metal concentration]} = A + B \times TL$ (6)

where A is intercept and B is slope. The coefficient B was used to evaluate trophic magnification factor.

$TMF = 10^{B}$ (7)

Biomagnification factors (BMF) were evaluated by (Fisk et al., 2001):

$BMF = [Predator] / ([Prey] \times (TL_{predator} - TL_{prey}))$ (8)

where [Predator] is metal concentration in the predator species and [Prey] is metal concentration in the prey species.

### 2.2.8 Risk Assessment

According to the Human Health Evaluation Manual (USEPA, 1989b), human health risks are quantitatively assessed in terms of non-cancer and cancer risk. This study was aimed at quantifying the non-cancer risk exerted on Hong Kong citizens by consuming metal contaminated fish from PRD. Hence, the major exposure pathway in the study was ingestion consumption of freshwater fish.The risk assessment followed the guidelines recommended by USEPA (2012a). For non-carcinogenic effects, the estimated daily intake was compared with the recommended reference doses (RfD) (0.01 mg $kg^{-1}$ $day^{-1}$ for Cd, 0.004 mg $kg^{-1}$ $day^{-1}$ for Pb, 0.3 mg $kg^{-1}$ $day^{-1}$ for Zn, 1.5 mg $kg^{-1}$ $day^{-1}$ for Cr, 0.04 mg $kg^{-1}$ $day^{-1}$ for Cu, and 0.02 mg $kg^{-1}$ $day^{-1}$ for Ni and 0.14 mg $kg^{-1}$ $day^{-1}$ for Mn ) (USEPA, 2012a) as stated in Eq. (9):

$$\text{Hazard Ratio (HR)}=EDI / RfD= (C\times DR\times EF\times ED)/ (BW\times AT\times RfD) \quad (9)$$

Where EDI is estimated daily intake, RfD is reference dose (μg $kg^{-1}$ $day^{-1}$), C is metal concentration (mg $kg^{-1}$, ww), DR is daily consumption rate (kg $person^{-1}$ $day^{-1}$), BW is body weight (kg), EF is exposure frequency (365 day $year^{-1}$), ED is duration of exposure (year) and *AT* is average exposure time (365 days × ED year).

US EPA has defined exposure duration (ED) of at least 6 years or above for choric non-cancer risk assessment (USEPA, 1989b). Hence, the ED adopted in this study was 6 years. For conservation estimation, the receptors (adults and children) were assumed to eat fish every day. Therefore, the EF was 365 day $year^{-1}$ and the AT was 2190 days (365 days× 6 years). Body weight of 58.6 kg for adults (Wang et al., 2005) and 21.8 kg for children (Leung et al., 2000) were chosen to suit the local situation and fish DR of 93 g $d^{-1}$ for adults and 50 g $d^{-1}$ for children were also used specifically for Hong Kong citizens (Leung et al., 2000).

The HR exceeding 1 indicates that there is potential risk to human health, and HR ≤ indicated no adverse health effects. HRs can be added to generate a Hazard index (HI) in order to estimate the risk of mix contaminates (USEPA, 1989b). The guideline also stated that "any single chemical with an exposure level greater than the toxicity value will cause the Hazard Index to exceed unity, for multiple chemical exposures the Hazard Index can also exceed unity even if no single chemical exposure exceeds its RfD".

$$HI = \sum HRi \qquad (10)$$

where *i* is different metals.

### 2.2.9 Data Analyses

The data analyses were performed using SPSS 19.0 for Windows. Normality was confirmed by the Kolmogorov-Smirnov test. Data of metals concentrations were analyzed using two independent t-tests, Wilcoxon rank sum test, one-way ANOVA and Kruskal-Wallis test as the requirement.

## 2.3 Results and Discussion

### 2.3.1 Sediment

#### 2.3.1.1 Metalloid/Metals Concentrations in Sediment Samples

Concentrations expressed on a dry weight (dw) basis for the metals analyzed in the aquaculture pond sediments collected from PRD are shown in Table 2.3. There were no significant differences ($p>0.05$) in Cr, Zn, Cr and Cu concentrations in sediments among the aquaculture ponds in PRD. The highest Pb concentration in sediments was found in Shunde (73.7±10.1 mg/kg dw). Sediments from Huadu

**Table 2.3** Mean concentration (mg/kg dw) of heavy metals in fish pond sediments collected from the Pearl River Delta

| Area | Site | Cd | Pb | Zn | Cr | Cu | Ni | Fe (%) | Mn | TOC (%) | Fish |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Huadu | HD1 | 1.43±0.95 | 54.4±9.70 | 115±33.0 | 62.5±12.7 | 32.5±6.25 | 16.9±3.37 | 1.59±0.001 | 138±4.61 | 7.04±1.21 | Grass carp |
| | HD2 | 0.63±0.14 | 71.2±4.59 | 116±13.3 | 103±10.2 | 31.1±5.70 | 17.1±2.74 | 1.48±0.002 | 109±10.1 | 3.41±0.88 | Grass carp |
| Nanhai | NH1 | 0.38±0.06 | 40.1±1.53 | 141±3.17 | 84.5±10.2 | 52.9±2.10 | 35.5±3.00 | 1.62±0.001 | 352±10.6 | 2.42±0.22 | Largemouth bass |
| | NH2 | 0.58±0.14 | 37.3±0.73 | 158±24.6 | 53.1±4.49 | 39.0±8.24 | 42.0±4.51 | 1.53±0.001 | 405±8.60 | 2.97±0.39 | Grass carp & Bighead carp |
| | NH3 | 0.81±0.17 | 37.1±1.29 | 121±5.63 | 74.7±6.87 | 32.5±0.41 | 32.4±3.84 | 1.85±0.001 | 372±4.00 | 1.32±0.11 | Northern snakehead |
| Shunde | SD1 | 1.11±0.17 | 63.1±14.3 | 124±29.2 | 65.0±9.68 | 105±10.3 | 37.1±3.23 | 1.83±0.002 | 845±207 | 3.36±0.61 | Largemouth bass |
| | SD2 | 1.11±0.13 | 71.8±7.75 | 225±31.6 | 110±17.5 | 102±10.4 | 47.1±5.41 | 2.05±0.002 | 627±61.2 | 4.57±0.17 | Northern snakehead |
| | SD3 | 1.01±0.22 | 87.4±31.2 | 186±69.8 | 142±67.2 | 81.6±35.7 | 54.2±17.4 | 2.53±0.007 | 677±117 | 2.86±0.44 | Largemouth bass |
| | SD4 | 2.57±1.10 | 72.5±7.68 | 134±14.4 | 73.7±10.5 | 56.2±5.00 | 41.0±3.13 | 1.87±0.001 | 594±16.1 | 3.29±0.61 | Northern snakehead |
| Jiangmen | JM1 | 0.55±0.13 | 59.5±27.6 | 170±12.1 | 69.5±17.5 | 64.8±8.59 | 91.1±5.84 | 1.71±0.005 | 603±25.3 | 3.47±0.44 | Mandarin fish |
| | JM2 | 0.87±0.15 | 47.3±10.5 | 125±39.2 | 62.7±14.6 | 62.1±42.5 | 43.1±20.0 | 2.34±0.010 | 712±69.3 | 3.52±0.11 | Mandarin fish |
| Zhongshan | ZS1 | 0.38±0.04 | 52.4±5.61 | 163±19.9 | 123±15.5 | 71.3±4.49 | 48.2±3.85 | 2.72±0.001 | 821±97.2 | 3.41±0.11 | Mandarin fish |
| | ZS2 | 2.48±1.70 | 59.6±10.4 | 242±58.5 | 78.0±13.9 | 100±12.9 | 62.4±14.7 | 2.86±0.003 | 784±58.0 | 4.84±0.66 | Northern snakehead |
| Nansha | NS1 | 0.41±0.09 | 70.0±11.5 | 177±4.58 | 80.9±3.70 | 53.9±2.65 | 52.4±0.66 | 2.52±0.002 | 865±49.3 | 3.58±0.22 | Grass carp & Bighead carp |
| | NS2 | 0.43±0.08 | 54.3±3.72 | 164±4.14 | 75.0±8.75 | 50.5±2.63 | 50.4±3.60 | 1.69±0.002 | 860±41.2 | 2.81±0.28 | Grass carp & Bighead carp |
| Dongguan | DG1 | 1.34±0.34 | 59.2±3.25 | 158±3.63 | 69.6±2.82 | 53.8±0.81 | 27.7±0.97 | 1.26±0.001 | 1116±39.7 | 5.83±1.08 | Grass carp & Bighead carp |
| | DG2 | 0.43±0.06 | 52.8±30.8 | 176±87.5 | 61.0±39.0 | 34.8±15.6 | 20.8±14.4 | 1.38±0.009 | 906±37.2 | 7.37±0.88 | Grass carp |
| Huizhou | HZ | 0.61±0.35 | 50.3±15.8 | 179±58.5 | 86.4±51.0 | 90.4±3.72 | 51.5±23.1 | 1.20±0.006 | 310±56.0 | 1.49±0.36 | Mandarin fish |
| ERL guideline[a] | | 1.2 | 47 | 150 | 81 | 34 | 21 | NA[c] | NA | NA | |
| ERM guideline[d] | | 9.6 | 218 | 410 | 370 | 270 | 52 | NA | NA | NA | |

[a] ERL (Effects Range-Low) guideline values indicate concentrations below which adverse effects on biota are rarely observed (Long et al., 1995).

[b] ERM (Effects Range-Median) guideline values indicate concentrations above which adverse effects on biota are frequently observed (Long et al., 1995).

[c] NA : not available.

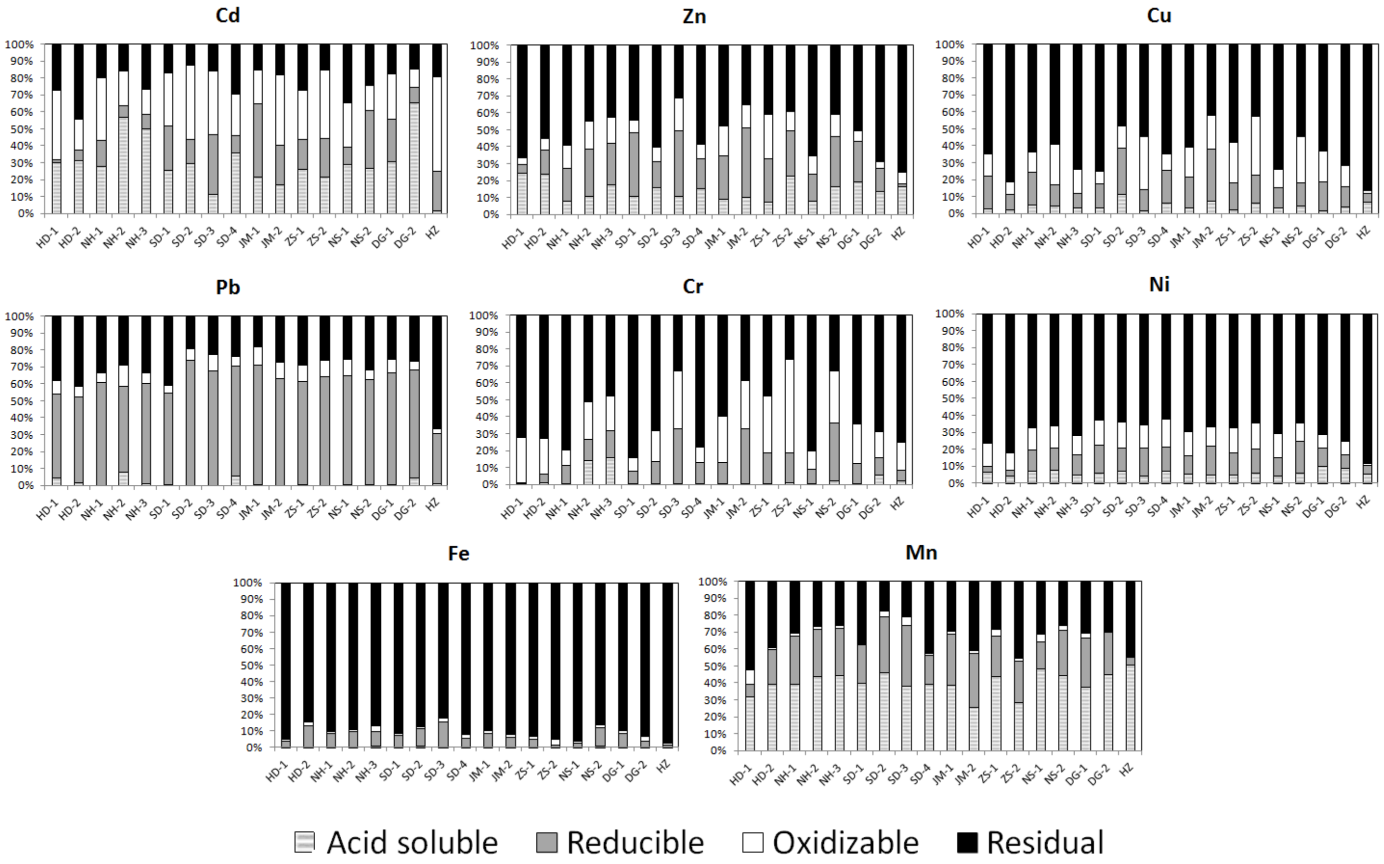



**Figure 2.2** The distribution of Cd, Zn, Cu, Pb, Cr, Ni, Fe and Mn in different geochemical phases of sediments collected from freshwater fish ponds in Pearl River Delta.

(17.1±10.1 mg/kg dw), Nanhai (36.6±4.9 mg/kg dw) and Dongguan (24.3±4.9 mg/kg dw) had lower concentrations of Ni ($p<0.05$), Pb, Cd and Cr levels in the most of sediments from Zhongshan, Huadu and Dongguan freshwater fish ponds were similar to those reported in our previous study with sediment samples from PRD (Cheung et al., 2008). The highest total metal concentrations of sediment in aquaculture pond were observed in Shunde and Zhongshan, which could be explained by the highly developed manufacturing industry at these two cities. All the metal concentrations of sediment in aquaculture ponds did not exceed the Effects Range-Median (ERM) guideline values (52 mg/kg dw) (Long et al., 1995), except for the Ni concentration in sediments of Jiangmen and Zhongshan which exceeded the guideline values, implying that biota might be regularly and occasionally exposed to toxic effects.

#### 2.3.1.2 Geochemical Fraction of Metalloid/Metals

The distribution of metalloid/metals in sediment samples (in percentage) following the BCR procedure is shown in Figure 2.2. The specific chemical forms or methods of binding of metalloid/metals decide their ecotoxicity and mobility in the environment (Quevauviller, 1998). It was observed that Cd was the most mobile metal mainly extractable by weak acids (range 1.6%-65.5%), implying Cd has a higher potential mobility and environmental risk in freshwater pond sediments of PRD. Cadmium has been reported as one of the most easily removed and labile metals (Marmolejo-Rodriguez et al., 2007), and the present results demonstrated this fact. Manganese was also found to be highly extractable by weak acid (25%-51%), as it has a close association with carbonates (Marmolejo-Rodriguez et al., 2007). This

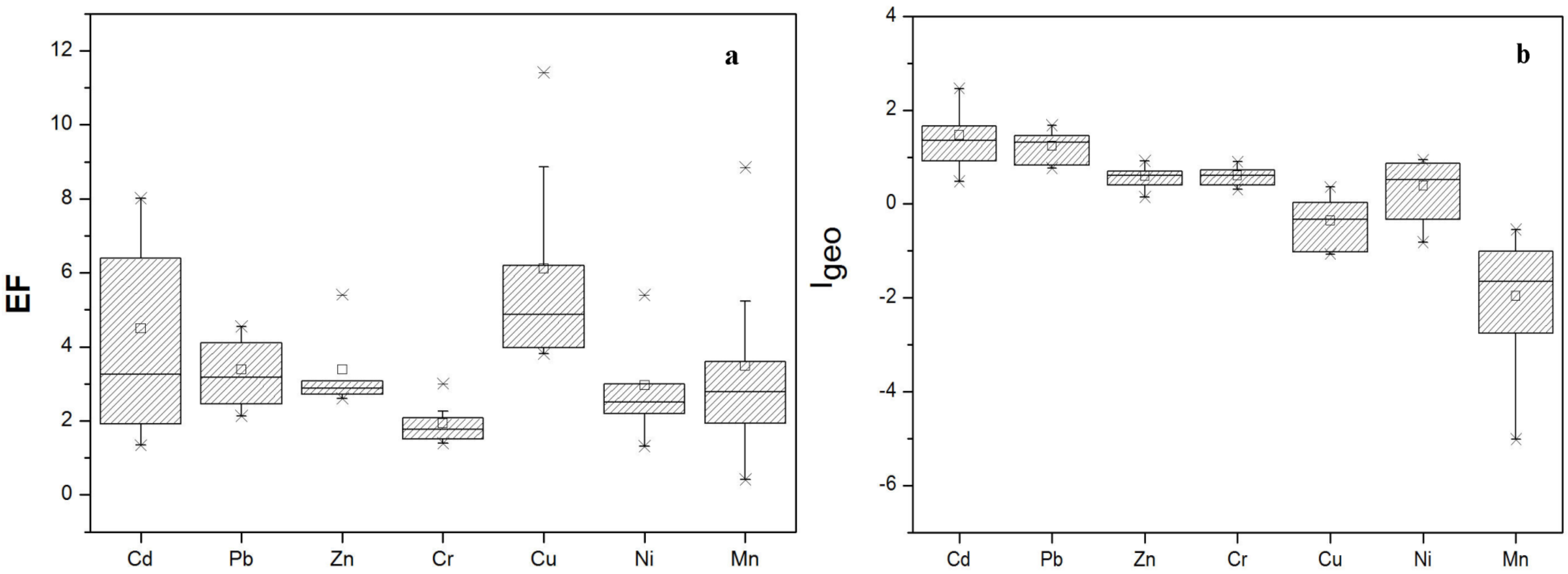



**Figure 2.3** The enrichment factors (EF) (a) and geoaccumulation index (Igeo) (b) in sediments for arsenic analysis of all the sampling sites (n=54) in freshwater fish Ponds of the Pearl River Delta. Each box represents interquartile range (25th and 75th percentile) of Igeo and EF.

explains the observed high percentages of Mn detected in the acid-soluble and carbonates fraction. The results showed that Pb had a higher proportion in the reducible fraction (29.9%-73.5%). The results showed that Pb had a higher proportion in the reducible fraction (29.9%-73.5%). The present results also showed that Fe and Mn oxides were important carriers of the metals in the sediments, which were in agreement with previous studies (Cuong and Obbard, 2006).

The highest contents in the oxidizable fraction were observed for Cd (14.9%-55.7%) and Cr (25%-51%). Higher concentrations were found in the residual fraction for Fe (81.8%-97.3%), Ni (62.1%-88.0%), and Cu (41.9%-81.3%), Cr (25.9%-83.7%) and Zn (31.0%-74.6%). These fractions were less bioavailable as they are strongly bound to silicates. Low contents of residual fraction were obtained for Mn and Cd, being more available (Diaz-de Alba et al., 2011). The freshwater fish ponds in PRD are strongly influenced by intensive human activities, and Cd, Pb and Zn are mainly derived from industry and municipal activities (e.g. electronics manufacturing industry and automobile exhaust) (Li et al., 2007).

**2.3.1.3 Assessment of Sediment Quality**

Figure 2.3 shows the EF and $I_{geo}$ for each metal based on the average metal concentrations in sediments from freshwater fish pond in PRD (Table 2.3) and using Fe content to normalize the data. Mean values of EF were principally between 2 and 5 except for Cu (Figure 2.3a), meaning that it was moderately enriched (Table 2.2) by metals in sediments from freshwater fish ponds in PRD and anthropogenic source was

**Table 2.4** Mean concentration (mg/kg ww) of heavy metals in aquaculture fish collected from the Pearl River Delta

| Common name | n | Cd | Pb | Zn | Cr | Cu | Ni | Mn | $\delta^{15}N$ | Water (%) | LIPID (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mandarin fish | 27 | 0.16±0.08 | 0.23±0.01 | 9.63±0.30 | 0.35±0.04 | 0.82±0.12 | 0.25±0.93 | 0.44±0.22 | 14.4±1.06 | 73.3±2.60 | 9.64±3.38 |
| Northern snakehead | 25 | 0.24±0.19 | 0.46±0.07 | 8.29±0.27 | 0.53±0.14 | 1.45±0.19 | 0.68±0.44 | 1.50±0.55 | 15.8±1.39 | 71.3±2.58 | 13.2±5.53 |
| Largemouth bass | 25 | 0.17±0.09 | 0.31±0.07 | 5.97±1.35 | 0.37±0.06 | 0.98±0.15 | 0.28±0.07 | 0.58±0.17 | 15.4±1.38 | 71.5±1.73 | 10.1±5.38 |
| Grass carp | 42 | 0.21±0.03 | 0.39±0.09 | 7.08±1.43 | 0.42±0.07 | 1.01±0.02 | 0.37±0.03 | 0.69±0.41 | 9.27±0.38 | 76.7±2.31 | 12.4±8.22 |
| Bighead carp | 15 | 0.08±0.01 | 0.12±0.02 | 8.20±1.50 | 0.27±0.03 | 0.58±0.05 | 0.15±0.03 | 0.88±0.58 | 14.1±1.26 | 76.1±1.60 | 6.32±3.02 |
| Zooplankton | 33 | 0.19±0.07 | 0.58±0.11 | 24.5±7.12 | 0.65±0.11 | 3.59±0.82 | 0.75±0.12 | 8.33±0.08 | 7.55±0.60 | 88.4±2.50 | 14.6±1.99 |
| Mud carp | 11 | 0.16±0.02 | 0.54±0.08 | 27.5±8.54 | 0.65±0.25 | 3.96±0.61 | 1.16±0.67 | 58.4±15.6 | 10.9±0.94 | 80.4±1.24 | 9.31±1.52 |
| Trash fish | 9 | 0.08±0.05 | 0.08±0.01 | 13.9±4.52 | 0.23±0.05 | 0.95±0.02 | 0.33±0.19 | 4.62±1.28 | 11.0±1.51 | 68.5±0.78 | 27.0±6.56 |
| Feed pellets | 12 | 2.08±0.23 | 2.24±0.82 | 102±23.0 | 1.58±0.19 | 24.2±5.35 | 4.10±1.08 | 157±20.3 | ND | 5.70±2.61 | 5.43±1.27 |

an important contributor (Loska et al., 1997). The highest EF values were found for Zn, Cu and Ni in the sediments from Huizhou. Those EF values all exceeded 5,indicating sediment in Huizhou was significantly enriched e.g. Cu and Ni. The higher EF values were found for Cd in Huadu, Zhongshan, Shunde and Dongguan, and most of fish farmers are still using the traditional way of filling up fish ponds by river water (Ruddle and Zhung, 1988), which contains high levels of Cd and due to the municipal and industrial wastewater discharges, and agricultural runoff. The average $I_{geo}$ of metals decreased in the order of 2 > Cd >Pd> 1 > Cr > Zn > Ni > 0 > Cu >Mn (Figure 2.3b). Using the criteria of pollution indicator in sediment based on $I_{geo}$ (Table 2.2) to assess metal pollution of pond sediment in PRD, Cu and Mn were unpolluted; Cr, Zn and Ni were unpolluted to moderately polluted; Cd and Pd were moderately polluted. Zn and Cr in sediment mainly originated from wastewater and exhaust deposition of metal processing, electroplating, and tanning industries in Pearl River Delta region (Li et al., 2007). Automobile exhaust, coal burning from power generation plants and industrial activities (such as Pb ore deposit smelting) in the region may be the major source of Pb in the sediments resulting from atmospheric inputs (Wong et al., 2002b).

### 2.3.2 Fish

#### 2.3.2.1 Metal Concentrations in Fish

The concentrations of Cd, Pb, Cr, Zn, Cu, Ni and Mn in different freshwater fish species collected from different ponds are shown in Table 2.4. Northern snakehead had higher concentrations of all metals (except Zn), than other fish species (*p<0.05*). The lowest concentrations of Cd (0.08±0.01 mg/kg ww), Pb (0.12±0.02 mg/kg ww), Cu (0.58±0.05 mg/kg ww) and Ni (0.15±0.03 mg/kg ww) were detected in bighead carp (*p<0.05*). The fish species collected from Zhongshan, Shunde and Huadu had higher Cd and Pb concentrations than from other areas. This is due to the fact that higher EF and $I_{geo}$ values were found for Cd in Huadu, Zhongshan and Shunde, metalloid/metals dissolved in water may be adsorbed onto particulates in the sediment, which may accumulate in aquatic organisms via absorption of water and ingestion of particulates from the water column (Chen et al., 2000; Casado-Martinez et al., 2010). The fish feed samples included zooplankton for bighead, mud carp for mandarin fish, trash fish for largemouth bass and pellet feeds for northern snakehead. The pellet feeds contained the highest concentrations of metals (Table 2.4), which were one or two order of magnitude higher than all fish samples, as the pellet feeds had the lowest water content (5.70%). Trash fish were small fish or fragment fish tissues collected from capture fisheries, with the lowest concentrations of all metals among all fish feed samples (Table 2.4). The freshwater fish are mainly fed with trash fish or pellet feeds, with fishmeal as a major protein adds into the pellet feeds, and most fishmeal is derived from trash fish (Wong et al., 2006; Cheung et al., 2008). The results indicate the fish feeds may be an important source of metalloid/metals to farmed fish, and the phenomenon was also be found in previous studies (Zhou and Wong, 2000; Lacerda et al., 2006). The Cd concentrations of the freshwater fish species approached or exceeded the Chinese maximum levels of contaminants in food (0.1 mg/kg ww)

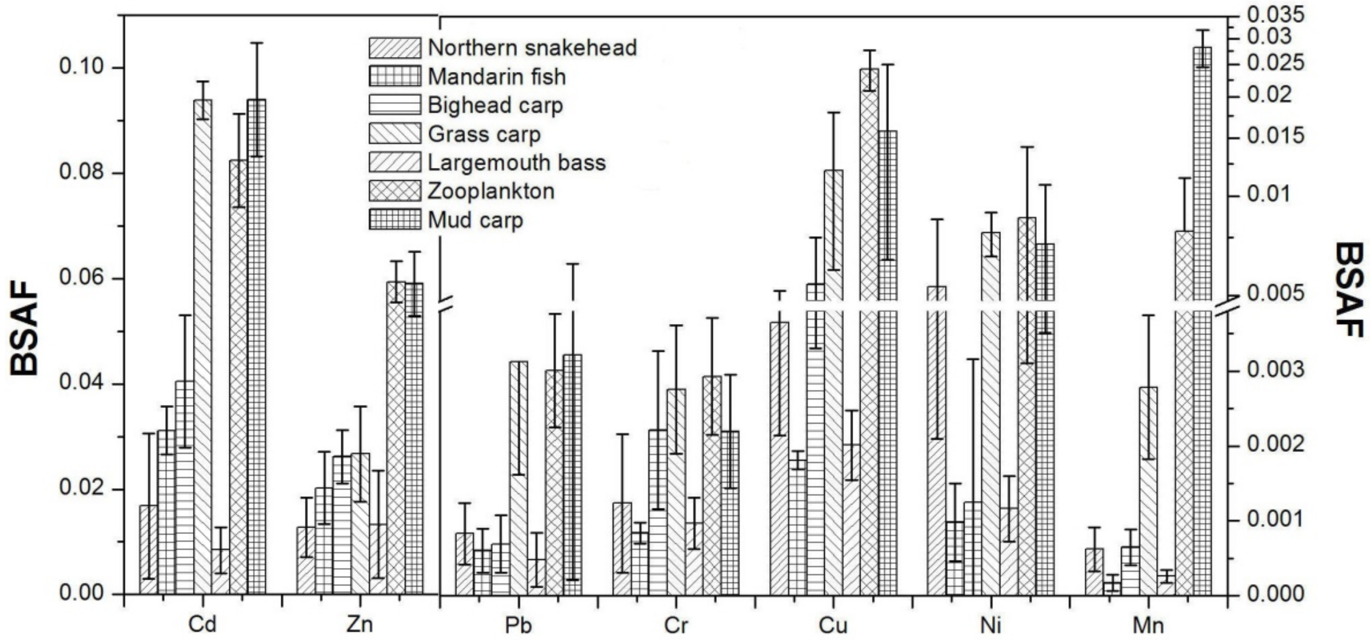



**Figure 2.4** Biota-sediment accumulation factor (BSAF) of Cd, Zn, Pb, Cr, Cu, Ni and Mn for fish species collected from the fish ponds in Pearl River Delta, China.

**Table 2.5** Predator-Prey biomagnifications factor (BMF) and trophic magnification factor (TMF) for heavy metals in the fish pond ecosystems in Pearl River Delta

| | Cd | Pb | Zn | Cr | Cu | Ni | Mn |
|---|---|---|---|---|---|---|---|
| **BMF** | | | | | | | |
| Mandarin fish-mud carp | 1.04 | 0.42 | 0.35 | 0.53 | 0.20 | 0.21 | 0.01 |
| Northern snakehead-feed pellets | 0.21 | 1.02 | 0.08 | 0.56 | 0.06 | 0.20 | 0.01 |
| Largemouth bass-trash fish | 2.26 | 4.08 | 0.47 | 1.73 | 1.13 | 0.96 | 0.14 |
| Bighead carp-zooplankton | 0.26 | 0.22 | 0.82 | 0.46 | 0.41 | 0.29 | 0.90 |
| Mud carp-zooplankton | 0.83 | 0.98 | 1.19 | 1.06 | 1.17 | 1.63 | 7.43 |
| **TMF** | | | | | | | |
| Predatory food chains | 0.86 | 0.67 | 0.79 | 0.79 | 0.65 | 0.73 | 0.60 |
| Omnivorous food chains | 0.83 | 0.69 | 0.78 | 0.80 | 0.68 | 0.73 | 0.70 |

(GB2762, 2005). The metalloid/metal concentrations detected in all the freshwater fish (except largemouth bass) of this study matched those detected in the freshwater fish available in Hong Kong market, while the Cd, Cr, and Pb concentrations in mandarin fish, grass carp and bighead carp were similar to those previously reported (Cheung et al., 2008).

#### 2.3.2.2 Biota-Sediment Accumulation Factor (BSAF)

A considerable transfer of these metals from sediment to fish may be an exposure pathway to humans. Biota-sediment accumulation factor (BSAF) is a reliable index to evaluate the accumulation profiles of these environmental pollutants (Qiu et al., 2011). Figure 2.4 shows the BSAF for metals in individual fish species and zooplankton. The BSAF levels of Zn and Cd in the freshwater fish species were significantly higher ($p<0.05$) than other metals. Cd (30.04%±15.19), Mn (40.40%±6.56) and Zn (14.56%±5.54) had a higher percentage in acid soluble phase of the pond sediments, and therefore they can be more easily dissolved in water and adsorbed on particulates for fish ingestion (Chen et al., 2000). This could also explain why the BSAF levels of zooplankton and bottom fish (mud carp) were higher than other fish species. The bioavailability of metals is another important factor that affect BSAF levels (Li and Davis, 2008). The total BSAF levels of carnivorous fish (mandarin fish (0.06), largemouth bass (0.03) and northern snakehead (0.04)) were much lower than those in omnivorous fish (bighead carp (0.08) and mud carp (0.21) and zooplankton (0.19).

#### 2.3.2.3 Biomagnifications (BMF)

The terms of biomagnification factor (BMF) and trophic magnification factors (TMF) can be used to express the ability of a contaminant to biomagnify.

Biomagnification factors were based on predator-prey relationships and corrected to unity for trophic level differences. The calculation of BMF also assumes that prey is completely consumed by predators. Largemouth bass had the strongest ability to accumulate Cd, Pb and Cr from its feeds (BMF>1) than other fish species (Table 2.5). The BMF value of mud carp-zooplankton (Zn, Cr, Cu and Mn), mandarin fish-mud carp (Cd), and northern snakehead-feed pellets (Pb) all exceeded 1 (Table 2.5), indicating Zn, Cr, Cu, Mn, Cd and Pb were biomagnified in those fish species through predator-prey relationships, respectively.

The trophic magnification factor (TMF) represents an average rate of contamination increase per trophic level in the food chain and assumes the uptake from the diet is the main exposure route (Campbell et al., 2005). A TMF greater than 1 indicates biomagnification of the metal in the food chain, while a value less than 1 suggests decreasing concentration with increasing trophic levels through the food chain. In this study, two typical food chains of freshwater fish pond in the PRD were selected for investigation: (1) Omnivorous food chain consisted of zooplankton, grass carp, and bighead carp; (2) Predatory food chain consisted of zooplankton, mud carp and mandarin fish. Mandarin fish, Snakehead and largemouth bass are predatory fish which are cultured based monoculture model under high density. Snakehead has been

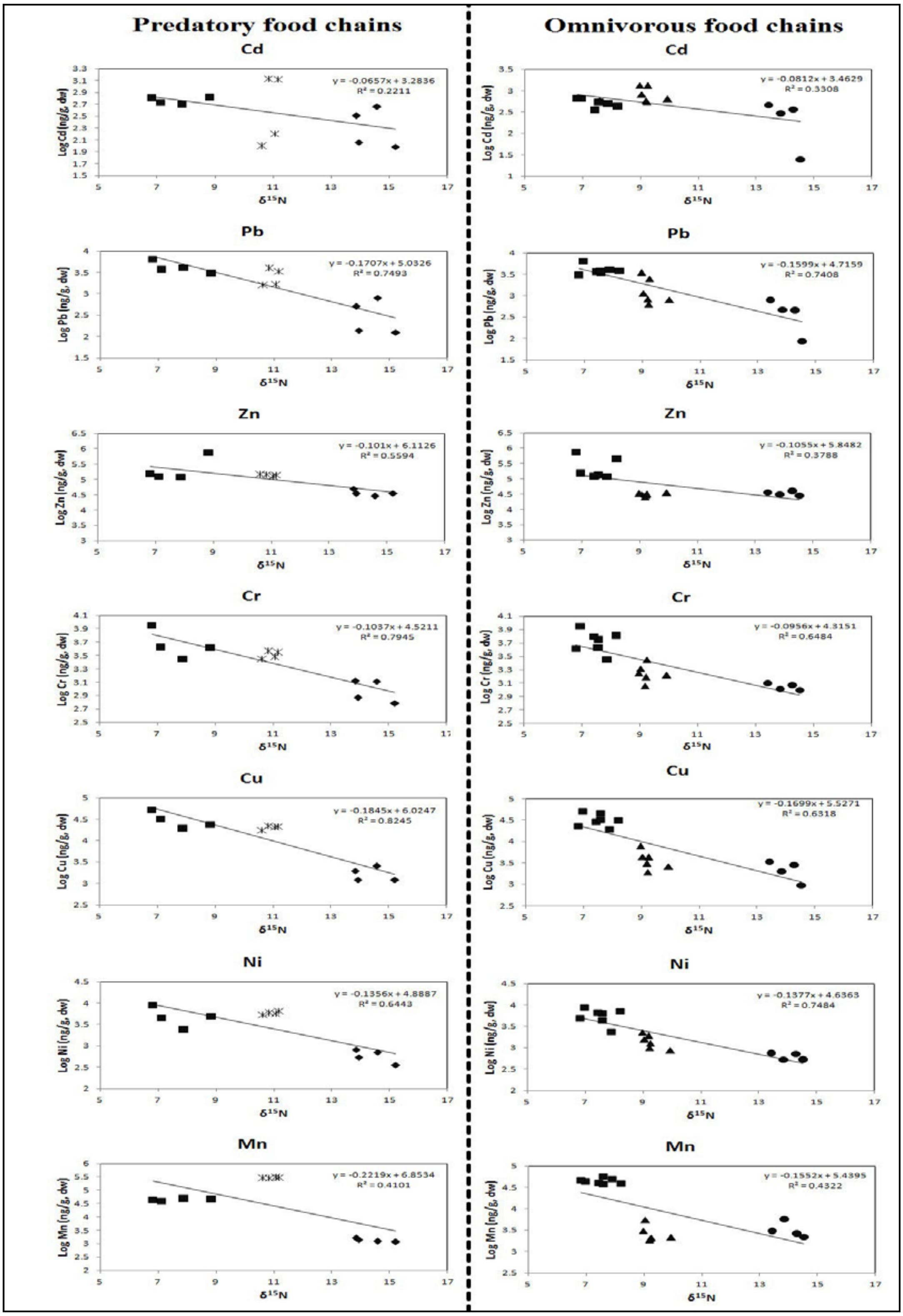



**Figure 2.5.** Regression relationships between, respectively, logarithm of trace element concentration and $\delta^{15}$N for omnivorous and predatory food chains in zooplankton and fish species from the freshwater fish ponds in Pearl River Delta, China. See text for details regarding the types of samples analyzed and species codes. (■) Zooplankton, (一) Mud carp, (▲) Grass carp, (●) Bighead carp, (◆) Mandarin fish.

domesticated to mainly eating fish feed pellets, largemouth bass is cultured by feeding trash fish, mandarin fish only eats life fish and which are usually cultured in ponds nearby. Therefore, only the mandarin fish food chain can be a typical predatory food chain found naturally. The TMF values of the two types of food chains ranged from 0.65 for Cu to 0.86 for Cd (Table 2.5), and there was no significant difference between the two types of food chains, indicating trophic level-dependent accumulation of Cd, Cu, Cr, Pb, Ni Cu and Mn were not found in organisms collected from the freshwater ponds in PRD.

The slope of the regression of $\delta^{15}N$ values and log-transformed metal concentrations, as biomagnification power, a slope greater than zero indicates that metals are accumulated in the food chain, while less than zero suggests the elimination of the metals from the food chain or an interrupted trophic transfer (Campbell et al., 2005). All seven of the studied metals showed negative relationships between $\delta^{15}N$ values and log-transformed metal concentrations ($p<0.05$) (Figure 2.5). Indicating that the seven metals were generally not biomagnified or biodiluted through the food chains in the PRD. Biodilution of Cd, Cu, Cr, Pb, Ni Cu and Mn in freshwater species was observed in Mekong Delta (Ikemoto et al., 2008a), Vietnam and Yellow River Delta, China (Cui et al., 2011). Nfon et al. (2009) reported biodilution of Ni, Zn, Cd and Pb and no biomagnification of Mn, Cr and Cu in Baltic Sea, North Europe, the same trend for Cd, Ni and Pb was also observed in a pelagic Arctic marine food web (Campbell et al., 2005).

**Table 2.6** Estimated hazard ratio (HR) for individual metals caused by the consumption of aquaculture freshwater fish in Pearl River Delta for inhabitants in Hong Kong

| | Cd | Pb | Zn | Cr | Cu | Ni | Mn |
|---|---|---|---|---|---|---|---|
| **Adult** | | | | | | | |
| Northern snakehead | 0.39 | 0.18 | 0.04 | 0.0006 | 0.05 | 0.05 | 0.02 |
| Mandarin fish | 0.26 | 0.09 | 0.05 | 0.0004 | 0.03 | 0.02 | 0.01 |
| Largemouth bass | 0.27 | 0.12 | 0.03 | 0.0004 | 0.04 | 0.02 | 0.01 |
| Bighead carp | 0.12 | 0.05 | 0.04 | 0.0003 | 0.02 | 0.01 | 0.01 |
| Grass carp | 0.33 | 0.15 | 0.05 | 0.0004 | 0.04 | 0.03 | 0.01 |
| **Children** | | | | | | | |
| Northern snakehead | 0.56 | 0.26 | 0.06 | 0.0008 | 0.08 | 0.08 | 0.02 |
| Mandarin fish | 0.37 | 0.13 | 0.07 | 0.0005 | 0.05 | 0.03 | 0.01 |
| Largemouth bass | 0.39 | 0.18 | 0.05 | 0.0006 | 0.06 | 0.03 | 0.01 |
| Bighead carp | 0.17 | 0.07 | 0.06 | 0.0004 | 0.03 | 0.02 | 0.01 |
| Grass carp | 0.48 | 0.22 | 0.05 | 0.0006 | 0.06 | 0.04 | 0.01 |

The dynamics and activities of most metals in organisms are dependent on transport proteins and binding site competition (Phipps et al., 2002). Generally, metallothionein and metallothionein-like proteins controlled essential element (Ni, Zn, Mn and Cu) concentrations in invertebrates and fish (Phipps et al., 2002). These proteins regulate the uptake, accumulation and excretion rates of metalloid/metals in biota, so it would be difficult for biomagnification to occur in food chains. However, biomagnification of essential elements such as Zn has been observed in invertebrate food webs (Campbell et al., 2005). The efficient accumulations of metalloid/metals or the lack of the necessary regulatory and detoxification mechanisms of higher vertebrates (mammals) could explain this phenomenon (Phipps et al., 2002; Reeves and Chaney, 2004). Some non-essential elements (e.g. Cd) can be bound to metallothionein, which is chemically similar to essential elements (e.g. Zn). There will be interactions with essential elements, binding site competition on metallothioneim, and therefore there will be an increase in uptake of both dietary essential elements and toxic elements (Reeves and Chaney, 2004). Furthermore, many physical and biological factors such as geography, sex, age, health status and body conditions would also influence metal deposition in animal tissues (Dehn et al., 2006). The induction of metallothionein and thus binding potential of some metalloid/metals is increased in larva and in pregnant individuals to store essential elements, thus giving the opportunity for other non-essential elements (e.g., Cd) to compete for binding sites (Phipps et al., 2002; Cui et al., 2011).

#### 2.3.2.4 Health Risk Assessment

The hazard ratio (HR) of individual metals through freshwater fish consumption for the inhabitants (adults and children) in Hong Kong is listed in Table 2.6. There are no HR values over 1 through consumption of all the fish species listed. This indicates that health risks associated with metal exposure for adults and children are insignificant if the inhabitants only ingest individual metals contained in the freshwater fish. Hazard index (HI) was used for estimating the risk of mix contaminates (Figure 2.6). HI values of all fish species were smaller than 1 for adult and children, indicating there is also no health risk for the inhabitants in Hong Kong by ingestion multiple metals contained in freshwater fish.

## 2.4 Conclusions

The metalloid/metal concentrations in sediment and freshwater fish from freshwater pond in PRD were investigated in this study. The highest total metal concentrations of sediment in aquaculture ponds were observed in Shunde and Zhongshan. The Cd concentration of the freshwater fish species approached or exceeded the Chinese maximum levels of contaminants in food (0.1 mg/kg ww) (GB2762, 2005). Different geochemical phases of pond sediment using four-step sequential extraction technique were studied and the results indicated that Cr, Cd, Pb and Ni were the most available metals. BSAF showed that omnivorous fishes and zooplankton accumulated more metalloid/metals from sediment than carnivorous fish. This is because the metalloid/metals that are dissolved in water may be adsorbed onto particulates in sediment, which may be accumulated in aquatic organisms via absorption of the water and ingestion of the particulates from the water column. Biomangnification of the selected metalloid/metals was not significant for the components of the pond food chains analyzed in this study. The results show that metalloid/metals contaminated fish feeds rather than biomagnification was the most important factor in managing metalloid/metals pollution in aquaculture pond ecosystems. Hazard index (HI) values of all fish species were smaller than 1 for adults and children, indicating there is no health risks for the inhabitants in Hong Kong by ingestion of multiple metals from the freshwater fish.

# CHAPTER 3 VARIATIONS OF METALLOID/METALS IN FRESHWATER FISH PONDS, USING FOOD WASTES AS FISH FEEDS

## 3.1 Introduction

Metalloid/metals are natural components of the earth's crust and generally occur in low concentration. Great quantities of metalloid/metals are discharged into the environment as contaminants each year by anthropogenic activities. These elements may enter agriculture areas (e.g.: crop and fish culture areas) through atmospheric deposition, sewage outfalls, and agricultural and industrial runoff, and then pose human health risks if the elements accumulated in bio-tissues exceeded maximum permitted concentrations. Elevated concentrations of arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg) and lead (Pb) were found in freshwater fish from different fish ponds scattered around the Pearl River Delta (PRD), including Mai Po, Hong Kong (Zhou and Wong, 2000; Cheng et al., 2013a). Cheung et al. (2008) analyzed metalloid/metal concentrations in 10 common marine and 10 common freshwater fish available in Hong Kong markets, and revealed that bighead carp, snakehead and grey mullet had average concentrations greater than the China standards for maximum levels of contaminants in foods for Cd (0.1 mg/kg ww) and Pb (0.5 mg/kg ww) established by the China National Standard Management Department (2005). Metalloid/metals contained in fish in associated with human health have also raised considerable public concerns in Hong Kong. Ko (2004) showed that severe skin disorders and autism in children were linked with their high concentrations of

Hg, Cd (coastal cities such as Hong Kong and Shanghai), As and Pb (inland cities such as Beijing) detected in hair which may reflect the dietary difference of the two populations with the costal population, consuming more fish.

Food waste is a global phenomenon that impacts the environment and society. The global food waste production is about 1.3 billion tonnes per year, which is about 33% of all edible food produced for human consumption (Gustavsson et al., 2011). In developed countries and areas, food wastage is very serious, e.g. per capita food wasted by consumers in sub-Saharan Africa and South/Southeast is about 10-15 times lower than in North America and Europe (Gustavsson et al., 2011). In the USA, almost 14% of the total municipal solid waste (MSW) stream was food waste (more than 34 million tonnes), and less than 3% of which was recovered and recycled in 2010 (USEPA, 2012b). In 2011, food waste comprised of one-third (about 330,000 tonnes) of the MSW loads at landfills (about 900,000 tonnes) (EPD, 2011). In Hong Kong, the remaining capacities of the three existing landfills will be exhausted by 2018 (EPD, 2011). Food processing and transportation also consume large quantities of freshwater and fossil fuels. In addition to producing wastewater, as food rots in landfill, it releases significant quantities of methane and carbon dioxide, leading to impacts on air quality and climate change (Hall et al., 2009).

The fish ponds in Mai Po Nature Reserve, northwestern Hong Kong serve as an important ecological function for the migratory birds for the north. The actively managed fish ponds provide water, food and shelter for aquatic and terrestrial animals, and provide breeding grounds for birds and other wildlife (Young and Chan, 1997; Young, 1998). These fish ponds provide an important feeding habitat for waterbirds especially during the traditional practice of draining pond water regularly (Young, 2004). Due to the decline of pond culture activities in Hong Kong, the conservation value of the area has gradually lost and the unmanaged fish ponds may become a sink for various pollutants, in addition to deterring usage of ponds for wildlife (Wong et al., 2004).

Fish meal is a nutrient riched with high protein supplement feed ingredient (commercial fish feeds) commonly used in aquaculture (Kaushik et al., 2004). However, most fish meal is derived from trash fish which are small fish or fragmented fish tissues collected from capture fisheries, with a low economic value. Our previous studies indicated the fish feeds may be an important source of trace elements entering cultured fish (Zhou and Wong, 2000; Cheung et al., 2008). Due to the declining fish stocks worldwide in recent years, alternative sources of protein for manufacturing fish feeds would be an important potential solution in aquaculture industry. It has been shown that European seabass (*Dicentrarchus labrax*) and sunshine bass (*Morone chrysops* × *M. saxatilis*) fed with a diet containing 94-96 % plant protein achieved a similar growth rate to those fed with high-quality fish meal (the values of daily growth index above 1.3%/day) (Kaushik et al., 2004; Rawles et al., 2011). Bake et al. (2009) also revealed that recycled food waste could partially replace fish meal in fish feeds.

It is hypothesized that the use of food waste fish feeds for culturing low trophic level fish (i.e. grass carp, bighead carp and mud carp) would ensure that the fish are relatively free of environmental pollutants. The major objectives of the present study were to (1) investigate the concentrations of metalloid/metals [Mercury (Hg), Arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), copper (Cu), nickel (Ni) and zinc (Zn)] in food waste fish feeds and the major food waste ingredients for making fish feeds; and (2) investigate the concentrations and variations of metalloid/metals in the pond water, suspended particulate matter (SPM) and sediment.

## 3.2 Materials and Methods

### 3.2.1 Experimental Design

Field experiments (rearing commercial fish using food wastes as feeds) were conducted in Sha Tau Kok Organic Farm in Hong Kong, China. The site is located near the Mai Po Nature Reserve and is relatively free of environmental pollution. There were three ponds (20 m×10 m) used in this experiment, filling up with spring water (depth: 4 m). Every three months, 30% water of the ponds were refreshed with spring water (pH: 6.84, dissolve oxygen: 3.99 mg/l, temperature: 21.8℃) for maintaining the water quality.

Three low trophic level species fish (1000 fish fry) (Bighead carp (*Aristichtys nobilis*) (10-12 cm), grass carp (*Ctenopharyngodon idellus*) (13-16 cm), and mud carp (*Cirrhina molitorella*) (4-6 cm) (all imported from mainland China)) were placed in experimental ponds, at the ratio of 1:3:1 (Chen et al., 2002). Grass carp mainly consumes macrophytes, and also commercial feed pellets and food waste feed pellets. Bighead carp (filter feeder) and mud carp (detritus feeder) commonly used to maintain the pond water quality in polyculture ponds (Wong et al., 2004).

The food wastes used in present study included food processing waste and partially post-consumption waste collected from local hotels and restaurants. They were classified into four major categories: vegetables and fruits, cereals, meat products, and bones. Fruit waste contained mainly peels with some flesh of various fruits, about 25% of pineapple,

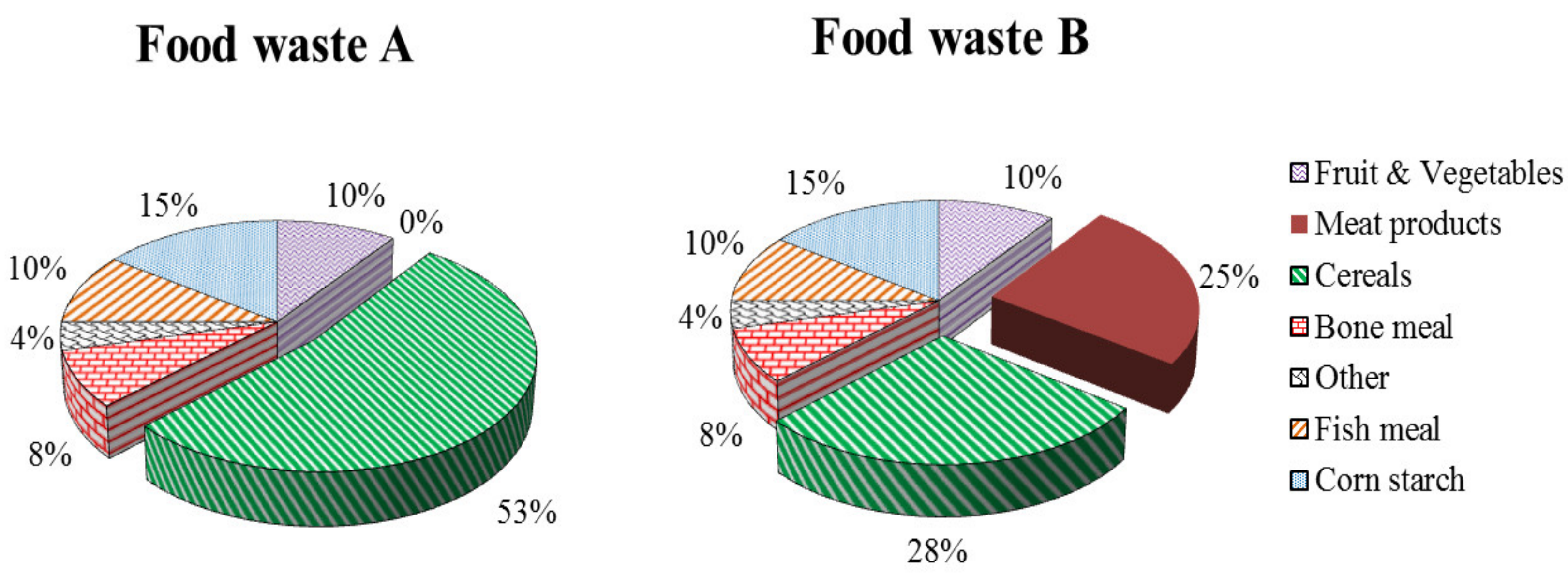



**Figure 3.1** Food waste fish feed formulation

25% watermelon, 15% Cantaloupe, and 35% others (e.g. strawberry, banana, apple, etc.). Meat waste included 60 to 70% of beef, pork and chicken, and 30 to 40% of fish such as salmon and grouper. Vegetable contained various types of leafy vegetables, such as lettuce and spinach. Cereals usually included rice bran, soy bean meal, rice grain and spaghetti. All the food wastes were collected every day from the hotels and transferred to a local food waste feed pellets factory (Kowloon Biotechnology Company Limited located in Pak Lai, NT) for further processing. They were diced into small pieces, with excessive water was squeezed out by machines. After drying at 80℃ for 6 h, they were ground into powder to form different food waste products. Different ratios of food waste products (Figure 3.1 and Table 3.1) were mixed with other raw materials, such as fish meals, corn starch for pelleting fish feeds. In general, food waste up to 75% in the pellet was needed to maintain feed quality. The major ingredients of FW A were cereal food wastes e.g. rice bran, soy bean meal, rice grain and spaghetti, which would be ideal protein sources for herbivores and omnivores (e.g. grass carp and grey mullet). On the other hand, some meat products were replaced with parts of cereals in FW B. The commercial feed Jinfeng®, (613 formulated feed with ~30 % protein) contained mainly wheat middling, flour, bean pulp, rapeseed meal, fish meal, bean oil and fish oil. This common fish feed used in aquaculture in Pearl River Delta and Hong Kong, was used as a control. All fish were fed twice per day at a fixed feeding rate of 4 % body weight per day for 12 months (October 2011- December 2012).

**3.2.2. Sampling**

During October 2011- December 2012, water, sediment and plankton samples were collected from the experimental ponds (Sampling frequency: bi-monthly during the first

**Table 3.1** Food wastes collected from hotels and restaurants

| Categories | Food | Amount (%) | Non-cooked or cooked |
|---|---|---|---|
| Fruit peel and vegetables | pineapple | 20 | Non-cooked |
| | watermelon | 20 | Non-cooked |
| | cantaloupe | 15 | Non-cooked |
| | strawberry, banana, apple | 30 | Non-cooked |
| | leaf vegetables | 15 | Non-cooked |
| Meat | beef, pork and chicken | 60-70 | Cooked |
| | salmon | 35 | Non-cooked |
| | groupers | 5 | Cooked |
| Cereals | rice bran | 50 | Non-cooked |
| | soy bean meal | 20 | Cooked |
| | rice grain | 20 | Cooked |
| | spaghetti | 10 | Non-cooked |
| Bone | beef, pork and chicken | 60-70 | Cooked |
| | salmon | 30 | Non-cooked |
| | groupers | 10 | Cooked |

**Table 3.2** The general information of seven sampling sites in Pearl River Delta (PRD) and Hong Kong, China.

| | Area | Sit | | Age | Culture model | Fish species | Feeding mode | Food items |
|---|---|---|---|---|---|---|---|---|
| PRD | Guangzhou | GZ | N23° 04′ 02.24′′, E113° 13′ 13.82″ | >15 | Polyculture | Bighead carp | Filter feeder | Zooplankton |
| | | | | | | Grass carp | Herbivorous | Grass and other submerged higher plants |
| | Shunde | SD | N22° 54′ 57.85′′, E113° 09′ 35.50″ | 2 | Polyculture | Grass carp | Herbivorous | |
| | | | | | | Bighead carp | Filter feeder | Zooplankton |
| | Gaoyao | GY | N23° 01′ 47.28′′, E112° 33′ 41.70″ | 2 | Monoculture | Largemouth bass | Carnivorous | Small fish, fingerling and trash fish |
| Hong Kong | Mai Po | MP-1 | N22° 29′ 49.95′′, E114° 03′ 33.41′′ | >10 | Abandoned | Tilapia | Omnivorous | Plant tissue, small fish,shrimp, detritus and sediment |
| | | MP-2 | N22° 29′ 46.97′′, E114° 03′ 38.14′′ | >10 | Abandoned | Tilapia | Omnivorous | |
| | Pok Wai | PW-1 | N22° 27′ 57.37′′, E114° 02′ 37.29′′ | 5-6 | Abandoned | Tilapia | Omnivorous | |
| | | PW-2 | N22° 27′ 57.30′′, E114° 02′ 37.29′′ | 5-6 | Abandoned | Tilapia | Omnivorous | |

half year and tri-monthly during the second half year). To compare with our experiments conducted in Sha Tau Kok, two similar culture model pond (polyculture) reared bighead cap and grass carp located in Shunde and Zhongshan, one monoculture pond reared largemouth bass located in Gaoyao, four abandon ponds located in Mai Po and Pok Wai were selected for sampling (Table 3.2). Sediment samples were wrapped in aluminum foil, frozen in zip-lock bags at -20 °C and transported to the laboratory until analyses.

Water and zooplankton were sampled at approximately 0.5-1.0 m depth from fish ponds of each sampling site. Water samples were collected from each site in precleaned amber glasses bottles and acidified immediately with 4 M HCl to pH <1 and stored at 4°C. Water samples were transported to the laboratory and filtered with glass fiber filters (Whatman GF/F with 0.45 μm an effective pore size; precombusted at 450°C for 5h) to separate the dissolved and particulate fraction for the analysis of total organic carbon (TOC using a Shimadzu TOC-V Series analyzer). The GF/F filters were placed in precleaned glass dishes and wrapped with aluminum foil, and stored at −20°C until extraction. Zooplankton samples were collected using non-metallic plankton net (202 μm) for multiple vertical tows, stored in 100 ml acid-treated Teflon vials (Chen et al., 2000) and transported to the laboratory.

The samples (fish feed pellets and sediments) were freeze-dried and ground into powder. In addition, subsamples of sediments were used for the analysis of total organic carbon (TOC), using thermal partitioning at 550 ℃ (USEPA, 1997).

### 3.2.3. Analytical Methods

Measurements of trace elements (As, Cd, Pb, Cr, Cu, Ni and Zn) in water, sediment, fish feeds pellets and fish followed the procedures described previously (Cheng et al., 2013a). The sediment samples were homogenized by passing through a stainless steel 0.154 μm sieves. Sediment samples (0.2 g) and fish feed pellets (0.5 g) were used to determine the concentrations of As, Cd, Pb, Cr, Cu, Ni and Zn by Inductively Coupled Plasma-Mass Spectrometry (Perkin-Elmer, Elan 9000, Norwalk, CT).

Total Hg (THg) concentration was determined by DMA-80 Direct Mercury Analyzer (MILESTONE), following USEPA method 7473 (USEPA, 2007). The detection limit for this method was about 0.03 ng of THg, corresponding to 0.3 ng/ g for a typical 0.1 g sample. For methylmercury (MeHg) analysis, sediment (0.3g) and SPM (0.3) samples were extracted by $HNO_3$/ $CuSO_4$/ dichloromethane and back extracted to water (Liang et al., 2004). MeHg was determined using aqueous ethylation, purge, trap, and gas chromatography – cold vapor atomic fluorescence spectrometry (GC-CVAFS) detection (Books Rand, MERX), following USEPA method 1630 (USEPA, 2001). 45 mL of water sample (preserved with 0.4% HCl) was added with 0.5 ml of 9 mol/L $H_2SO_4$, 10 mL Milli-Q water and 0.2 mL buffer. After distillation, MeHg was determined by GC-CVAFS. The detection limit for this method was about 0.5 pg of MeHg, which corresponding to 0.005 ng/ g for a typical 0.1 g sample.

### 3.2.4. Quality Control

All the samples were tested in triplicate. Analytical blank and reference materials were included in every batch of extraction. Four certified reference materials (CRM): NIST 8704 (Buffalo River sediment), NIST 1944 (New York /New Jersey Waterway sediment) and NIST 1566b (Oyster tissue) were obtained from National Institute Standards and Technology, (NIST, USA) and TORT-2 (Lobster hepatopancreas) was obtained from National Research Council of Canada). The recoveries for total metals ranged from 90-

104% (As, Cr, Pb, Cr, Cu, Ni and Zn). The recovery rates of the THg and MeHg of the standard reference material were within the certificate values. The recovery rates for THg and MeHg were 101 ± 2.27% and 99.3 ± 5.34%, respectively. The coefficient of variation (SD/mean) for the duplicate samples was <10%.

### 3.2.5 Statistical Analyses

All the statistical tests were performed using SPSS 19.0 for Windows. Normality was confirmed by the Kolmogorov-Smirnov test. Data of metalloid/metal concentrations were analyzed using two independent t-tests, Wilconxon rank sum test, one-way ANOVA and Kruskal-Wallis test.

## 3.3 Results and Discussion

### 3.3.1 Fish Feeds

Figure 3.1 shows the food waste accounted for about 75% in the pellets. The major protein sources of FW A were cereals food waste (rice bran, soy bean meal, rice grain and spaghetti), which could be easily digested by herbivores and omnivores (e.g. grass carp and grey mullet). Meat wastes were used to replace parts of cereals in FW B, and the major ingredients of FW B were similar to the control feed which contained mainly of fish meal, wheat middling, flour, and rapeseed meal. The protein contents of three types of fish feed pellets were not significantly different ($p>0.05$), indicating food waste may be used as an alternative source of protein for fish culture. Metalloid/metal concentrations in fish feeds used in the experiment are shown in Table 3.2. There was no significant difference ($p>0.05$) in Cd and Pb concentrations in the three type fish feed pellets. The lowest concentrations of As, Pb, Cr, Cu, Ni and Zn were found in the control feed and the

Table 3.3 Metalloid/metal concentrations (mg/kg dw ± SD) in fish feed which using in the experiment.

| | As | Cd | Pb | Cr | Cu | Ni | Zn | THg | MeHg |
|---|---|---|---|---|---|---|---|---|---|
| **Metalloid/metal concentrations of the experiment feeds** | | | | | | | | | |
| Control | 0.93±0.27 | 0.22±0.07 | 0.23±0.05 | 1.64±0.36 | 16.9±0.47 | 4.28±0.86 | 109±18.7 | 19.2±8.21 | 7.80±0.50 |
| Food waste A | 4.22±1.64 | 0.27±0.05 | 0.55±0.23 | 5.10±3.58 | 40.1±5.69 | 12.1±5.69 | 352±25.5 | 18.5±1.53 | 7.29±5.47 |
| Food waste B | 5.71±2.83 | 0.46±0.29 | 0.35±0.14 | 7.10±2.08 | 82.4±21.6 | 16.7±9.07 | 606±141 | 16.4±1.66 | 5.85±4.37 |
| **Metalloid/metal concentrations in major food waste ingredients for making fish feed pellets (FW A and FW B)** | | | | | | | | | |
| (1) Fish meal | 8.23±0.23 | 0.52±0.02 | 0.03±0.01 | 1.81±0.12 | 3.89±0.08 | 8.37±0.02 | 127±5.00 | 149±80.9 | 15.4±2.55 |
| (2) Fruit and Vegetables+ Cereals | 2.40±0.04 | 0.08±0.01 | 0.78±0.25 | 5.60±0.08 | 11.9±3.35 | 11.0±3.04 | 77.6±9.69 | 10.4±2.10 | 1.23±1.42 |
| (3) Fruit and Vegetables+ Bone meal | 2.58±0.10 | 0.08±0.02 | 0.33±0.04 | 5.60±0.47 | 6.90±0.82 | 15.7±0.64 | 60.5±4.10 | 47.4±15.7 | 4.11±0.99 |
| (4) Meat products | 1.83±0.11 | 0.05±0.01 | 0.07±0.04 | 2.68±0.05 | 4.13±0.15 | 8.33±1.12 | 41.3±2.19 | 78.5±48.3 | 5.42±1.68 |

Note: FW A: food waste A pellet, FW B: food waste B pellet, Control: Commercial pellet.

highest concentrations of Cu and Zn were found in FW B (*$p<0.05$*). The analyses of trace elements in various food waste ingredients for making fish pellets (FW A and FW B) showed that vegetables, cereals and bone meal were major sources of trace element (except Hg) contamination for FW A and FW B. Fish meal was also contributed significant sources of As (8.23 ±0.23 mg/kg dry weight (dw)), Cd (0.52 ±0.02 mg/kg dw) and Zn (127±5.00mg/kg dw) in the food waste fish feed pellets. Due to the burgeoning industrial development with loose regulation on pollution control, more and more pollutants have been released to the surrounding coastal area, and marine pollution has become a serious problem. Most fish meal is derived from trash fish which are small fish or fragmented fish tissue which are commonly contaminated. There was no significant difference ($p>0.05$) in concentrations of Hg (THg and MeHg) in FW A and FW B, when compared with the control diet (Table 1). The analyses of THg and MeHg in various food waste ingredients (FW A and FW B) showed that fish meal was the major source of Hg (THg and MeHg: 149 ± 80.9 and 15.4 ± 2.5 ng/g, respectively.) detected in making FW A and FW B.

### 3.3.2 Environmental Samples

The metalloid/metal concentrations in water and sediment of fish ponds collected from the studied ponds did not exceed the Chinese Water Quality Standard for fisheries (GB11607, 1989) and the Effect Range-Median guideline values (Long et al., 1995) (determination of percent incidence of adverse biological effects) (Tables 3.3 and 3.4), respectively, indicating the pond sediment and pond water were safe for fish cultivation. Table 3.3 shows that, concentrations of As and Cd in pond water were increased during the experimental period ($p<0.05$). On the contrary, metalloid/metal concentrations

Table 3.4 Concentrations of metalloid/metals in water (µg/l ± SD) of sampling sites in Hong Kong and Pearl River Delta (PRD)

[a] Chinese water quality standard for fisheries (GB11607, 1989)

| Area | Site | As | Cd | Pb | Cr | Cu | Ni | Zn | THg (ng/l) | MeHg (ng/l) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment sites** | | | | | | | | | | |
| 1st half year | Control | 2.27±1.15 | 0.05±0.01 | 0.60±0.04 | 0.71±0.13 | 1.28±0.33 | 0.87±0.27 | 8.67±6.43 | 34.4±25.7 | 0.25±0.41 |
| | FW A | 2.22±1.07 | 0.03±0.01 | 0.54±0.41 | 0.56±0.08 | 1.75±0.96 | 1.16±0.86 | 23.2±9.12 | 29.2±6.72 | 0.10±0.15 |
| | FW B | 2.28±1.24 | 0.04±0.02 | 0.42±0.45 | 0.46±0.20 | 2.30±1.90 | 1.10±0.54 | 12.5±6.89 | 27.8±8.50 | 0.07±0.06 |
| 2nd half year | Control | 4.27±0.13 | 0.14±0.02 | 1.18±1.02 | 0.88±0.19 | 3.87±3.02 | 2.18±0.10 | 23.3±4.27 | 32.4±23.1 | 0.03±0.02 |
| | FW A | 10.5±6.40 | 0.09±0.03 | 1.16±0.11 | 0.94±0.10 | 1.66±0.65 | 1.30±0.52 | 20.0±2.44 | 24.2±9.74 | 0.01±0.01 |
| | FW B | 6.64±0.78 | 0.12±0.04 | 1.55±0.31 | 0.89±0.33 | 1.17±0.67 | 1.76±0.08 | 24.2±5.16 | 16.3±3.32 | 0.02±0.01 |
| **Control sites** | | | | | | | | | | |
| Shunde | SD | 4.44±0.89 | 0.20±0.04 | 6.64±0.49 | 6.59±0.61 | 6.11±0.47 | 5.13±0.37 | 57.0±3.12 | 84.0±9.85 | 0.27±0.02 |
| Gaoyao | GY | 8.99±2.82 | 0.11±0.01 | 4.16±0.44 | 3.66±0.99 | 4.77±0.55 | 2.72±0.32 | 19.3±2.10 | 42.2±32.9 | 0.29±0.03 |
| Guangzhou | GZ | 13.1±3.16 | 1.36±1.07 | 10.0±4.87 | 10.6±2.90 | 7.44±2.41 | 3.32±0.91 | 80.2±21.8 | 36.5±5.91 | 1.71±1.04 |
| Mei Po | MP-1 | 5.06±0.93 | 0.05±0.06 | 0.09±0.06 | 0.95±0.55 | 2.91±0.52 | 9.45±1.46 | 3.37±0.71 | 68.0±20.9 | 0.02±0.01 |
| | MP-2 | 3.57±0.30 | 0.02±0.01 | 0.05±0.01 | 0.58±0.10 | 0.73±0.08 | 5.21±0.58 | 6.01±0.07 | 36.3±2.38 | 0.07±0.05 |
| Pok Wai | PW-1 | 76.8±16.5 | 0.27±0.40 | 0.35±0.14 | 4.23±1.34 | 17.7±7.11 | 19.7±3.50 | 1.92±0.50 | 40.7±6.32 | 0.28±0.20 |
| | PW-2 | 75.7±14.7 | 0.05±0.01 | 0.06±0.01 | 2.19±0.35 | 21.3±4.88 | 20.5±4.63 | 2.08±0.36 | 27.2±3.63 | 0.17±0.08 |
| Water quality standard[a] | | $0.05\times10^3$ | $0.005\times10^3$ | $0.05\times10^3$ | $0.1\times10^3$ | $0.01\times10^3$ | $0.05\times10^3$ | $0.1\times10^3$ | 500 | -[b] |

[b] - : not available.

PW: Pok wai, MP: Mai Po, GZ: Guangzhou, SD: Shunde, GY: Gaoyao, HK: Hong Kong, FW A: food waste A pellet, FW B: food waste B pellet, Control: Commercial pellet.

Table 3.5 Concentrations of metalloid/metals in sediment (mg/dw ± SD) of sampling sites in Hong Kong and Pearl River Delta (PRD)

| Area | Site | As | Cd | Pb | Cr | Cu | Ni | Zn | THg (ng/dw) | MeHg (ng/dw) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment sites** | | | | | | | | | | |
| 1st half year | Control | 7.45±2.84 | 0.06±0.04 | 23.1±4.17 | 8.76±1.68 | 6.52±1.74 | 3.05±1.70 | 29.3±16.8 | 43.3±17.7 | 0.34±0.13 |
| | FW A | 5.48±0.61 | 0.03±0.01 | 23.7±3.37 | 6.30±1.42 | 5.50±2.22 | 2.50±0.97 | 26.4±11.8 | 31.7±15.8 | 0.10±0.07 |
| | FW B | 5.96±1.13 | 0.02±0.01 | 20.0±4.45 | 5.05±0.96 | 4.87±1.32 | 1.95±1.08 | 20.9±10.5 | 34.6±16.4 | 0.09±0.10 |
| 2nd half year | Control | 5.35±4.30 | 0.04±0.02 | 14.7±3.86 | 6.22±3.35 | 11.3±6.67 | 2.23±0.22 | 33.7±20.8 | 46.2±11.9 | 0.09±0.07 |
| | FW A | 5.42±2.89 | 0.05±0.03 | 17.2±11.8 | 8.17±1.29 | 23.6±5.92 | 3.34±1.01 | 37.9±3.92 | 36.1±9.71 | 0.12±0.08 |
| | FW B | 8.62±4.97 | 0.03±0.03 | 11.8±7.32 | 5.27±0.41 | 18.0±2.48 | 4.33±2.16 | 18.5±1.73 | 30.8±9.65 | 0.08±0.04 |
| **Control sites** | | | | | | | | | | |
| Shunde | SD | 6.01±0.93 | 0.14±0.03 | 12.5±1.46 | 24.7±16.8 | 9.06±1.82 | 12.1±2.02 | 69.1±16.4 | 42.0±10.5 | 0.11±0.05 |
| Gaoyao | GY | 8.16±0.82 | 0.07±0.02 | 47.0±5.01 | 48.9±32.6 | 10.5±0.80 | 9.03±0.59 | 69.6±9.10 | 46.8±5.95 | 0.21±0.25 |
| Guangzhou | GZ | 22.3±3.87 | 1.09±0.22 | 96.7±21.3 | 58.9±14.6 | 128±42.9 | 28.5±2.12 | 271±72.5 | 5436±3372 | 16.1±4.18 |
| Mei Po | MP-1 | 19.8±1.74 | 0.21±0.02 | 53.0±4.74 | 12.4±6.41 | 87.3±9.07 | 11.4±1.21 | 58.9±6.26 | 71.5±0.08 | 0.14±0.04 |
| | MP-2 | 19.6±2.06 | 0.17±0.04 | 67.7±4.57 | 24.9±7.75 | 29.0±0.75 | 19.6±2.01 | 66.6±5.22 | 108±8.09 | 0.20±0.02 |
| Pok Wai | PW-1 | 10.2±0.89 | 0.11±0.01 | 50.7±4.70 | 9.41±3.21 | 10.7±0.88 | 9.29±0.71 | 33.4±3.02 | 63.8±4.71 | 0.01±0.01 |
| | PW-2 | 17.7±3.69 | 0.19±0.02 | 54.7±3.62 | 14.4±9.59 | 15.2±1.57 | 9.15±0.67 | 36.4±1.63 | 49.2±0.83 | 0.02±0.01 |
| ERL guideline[a] | | 8.2 | 1.2 | 47 | 81 | 34 | 21 | 150 | 150 | -[c] |
| ERM guideline[d] | | 70 | 9.6 | 218 | 370 | 270 | 52 | 410 | 710 | - |

[a] ERL (Effects Range-Low) guideline values indicate concentrations below which adverse effects on biota are rarely observed (Long et al., 1995).

[b] ERM (Effects Range-Median) guideline values indicate concentrations above which adverse effects on biota are frequently observed (Long et al., 1995).

[c] - : not available.
PW: Pok wai, MP: Mai Po, GZ: Guangzhou, SD: Shunde, GY: Gaoyao, HK: Hong Kong, FW A: food waste A pellet, FW B: food waste B pellet, Control: Commercial pellet.

Table 3.6 Concentrations of metalloid/metals in suspended particulate matter (SPM) (mg/dw ± SD) of sampling sites in Hong Kong and Pearl River Delta (PRD).

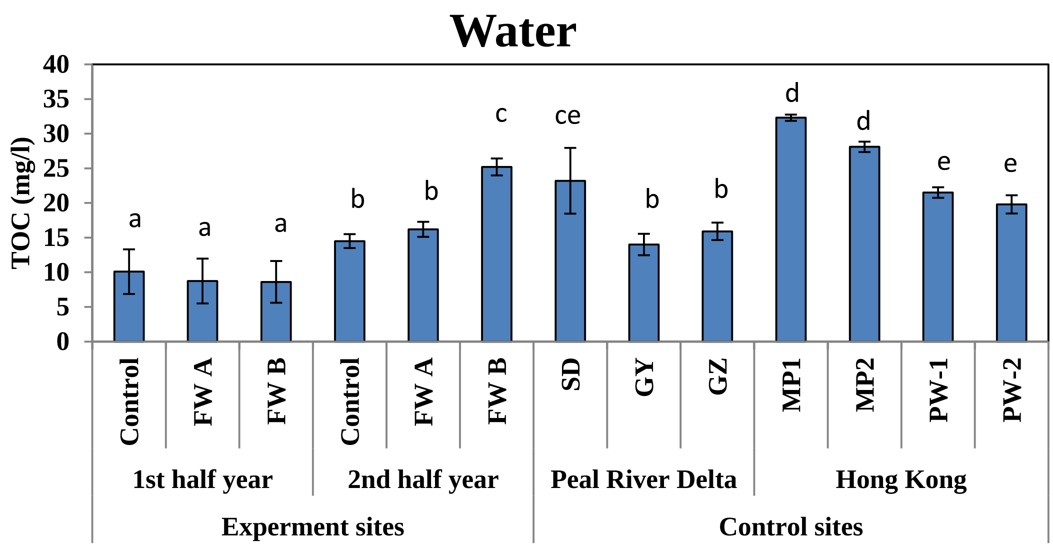
| Area | Site | As | Cd | Pb | Cr | Cu | Ni | Zn | THg (ng/dw) | MeHg (ng/dw) |
|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment sites** | | | | | | | | | | |
| | Control | 2.92±1.84 | 0.03±0.01 | 5.08±3.21 | 11.5±9.58 | 1.21±0.51 | 1.65±0.71 | 7.72±2.32 | 10.8±5.45 | 0.11±0.16 |
| 1st half year | FW A | 1.45±1.27 | 0.01±0.01 | 2.25±1.21 | 7.03±4.02 | 0.88±0.42 | 0.88±0.54 | 7.88±4.38 | 13.7±10.9 | 0.05±0.04 |
| | FW B | 2.23±2.11 | 0.01±0.01 | 3.11±2.84 | 7.25±4.60 | 1.22±0.55 | 1.14±0.74 | 9.31±6.76 | 13.2±11.9 | 0.05±0.08 |
| | Control | 1.29±0.37 | 1.66±0.30 | 10.9±9.54 | 18.0±6.26 | 29.6±12.2 | 3.10±2.26 | 35.7±16.0 | 23.0±4.01 | 0.80±0.37 |
| 2nd half year | FW A | 1.36±1.15 | 5.26±2.43 | 9.91±8.23 | 23.1±5.48 | 15.2±6.67 | 5.00±1.16 | 46.6±4.61 | 13.7±4.31 | 0.27±0.09 |
| | FW B | 1.86±0.04 | 4.56±4.34 | 11.3±3.80 | 23.3±7.43 | 13.4±11.6 | 4.68±1.40 | 49.0±3.23 | 25.9±6.45 | 0.45±0.14 |
| **Control sites** | | | | | | | | | | |
| Shunde | SD | 1.96±0.88 | 0.03±0.03 | 2.27±0.99 | 1.91±0.83 | 3.03±1.21 | 1.60±0.62 | 14.2±6.19 | 19.3±2.27 | 0.06±0.01 |
| Gaoyao | GY | 1.35±0.17 | 0.10±0.02 | 4.64±0.31 | 1.78±1.54 | 2.04±0.44 | 0.99±0.07 | 13.7±2.15 | 11.0±4.02 | 0.02±0.01 |
| Guangzhou | GZ | 0.10±0.29 | 0.08±0.03 | 1.11±1.06 | 1.09±0.15 | 1.69±0.39 | 0.49±0.19 | 10.3±1.92 | 222±22.1 | 0.47±0.06 |
| Mei Po | MP-1 | 0.36±0.21 | 0.02±0.01 | 0.49±0.31 | 1.36±0.79 | 1.18±0.41 | 0.84±0.50 | 4.34±2.99 | 17.7±2.34 | 0.01±0.01 |
| | MP-2 | 1.55±0.09 | 0.03±0.04 | 2.19±0.26 | 1.51±0.13 | 1.65±0.67 | 0.80±0.07 | 8.64±3.70 | 15.1±12.4 | 0.02±0.01 |
| Pok Wai | PW-1 | 2.28±0.35 | 0.04±0.02 | 0.85±0.09 | 1.63±0.29 | 2.00±0.76 | 0.61±0.12 | 12.9±3.59 | 29.0±12.8 | 0.05±0.05 |
| | PW-2 | 2.49±0.23 | 0.02±0.01 | 1.01±0.08 | 1.23±0.02 | 1.81±0.50 | 0.56±0.03 | 36.2±16.1 | 31.0±18.0 | 0.12±0.07 |

Note: PW: Pok wai, MP: Mai Po, GZ: Guangzhou, SD: Shunde, GY: Gaoyao, HK: Hong Kong, FW A: food waste A pellet, FW B: food waste B pellet, Control: Commercial pellet.

(except Cu) in sediment of the three ponds did not change significantly ($p>0.05$) during the experimental period. Table 3.5 shows that, concentrations of Cd, Cr, Cu, Zn and MeHg in pond SPM were increased during the experimental period ($p<0.05$). There were no significant differences ($p>0.05$) in metalloid/metal concentrations of water, SPM and sediment among the 3 ponds.

In general, metalloid/metal concentrations of pond water and sediment of the present experimental ponds were similar or lower than those of other fish ponds scattered around the PRD (control sites) (Tables 3.3 and 3.4). The Pok Wai (Hong Kong) site had the highest As, Cu and Ni in water ($p<0.05$), and the Guangzhou site had the highest metalloid/metal concentrations in sediment among all sampling sites ($p<0.05$). This may be due to the highly developed manufacturing industries located near these ponds, and the fish ponds had never been drained and sediment removed during the past 5-12 years (Pok Wai, 5-6 years; Guangzhou, >15 years). Therefore, trace elements would enter into the pond water, and then accumulated in sediment through atmospheric deposition or surface runoff. The fish ponds in Gaoyao and Shunde were new ponds with sediment removed recently (Table 3.1), and located far away from the urban center and manufacturing industry area. These two ponds had lower metalloid/metal levels in all environmental samples.

Metalloid/metal concentrations in water and sediment samples of the 3 fish ponds were similar to or even lower than those in freshwater fish ponds around the PRD region (obtained by previous and present studies), suggesting that the experimental site was relatively free of trace element contaminations, and would be suitable for farming fish. Metalloid/metal concentrations in sediment of abandoned fish ponds were similar or



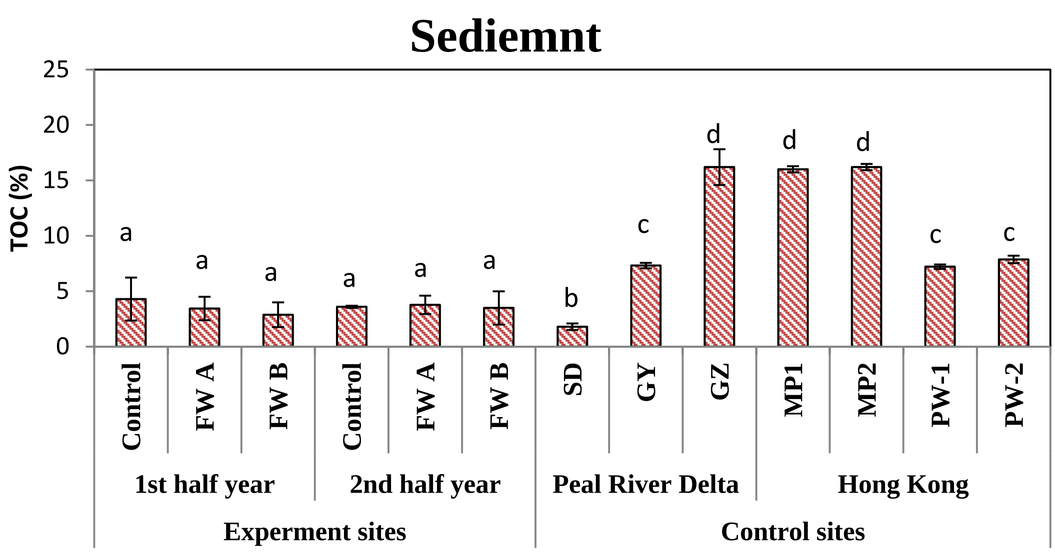




**Figure 3.2** Total organic carbons in water and sediment of sampling sites in Hong Kong and Pearl River Delta (PRD). Different letters (a, b, c, d) indicate significant difference between groups: water and sediment.

PW: Pok wai, MP: Mai Po, GZ: Guangzhou, SD: Shunde, GY: Gaoyao, HK: Hong Kong, FW A: food waste A pellet, FW B: food waste B pellet, Control: Commercial pellet.

higher than in all farmed ponds (except in Guangzhou), suggesting traditional farming (a diversified and coordinated way of farming or producing agricultural products in the fish farms with fish as the main product) would improve the environmental quality of fish ponds.

In the present study, the abandoned fish ponds in Hong Kong contained the highest TOC levels in water (mean 25.4 mg/l, range 19.9-32.2 mg/l) and sediment samples (mean 11.3%, range 7.21-16.2%), followed by farmed ponds in PRD (water: mean 17.7 mg/l, range 14.0-23.2 mg/l; sediment: mean 8.42%, range 1.79-16.2%) and Sha Tau Kok (water: mean: 10.8 mg/l, range 14.5-25.2; sediment: mean 4.33%, range 3.49-3.59), respectively ($p<0.05$) (Figure 3.2). The concentrations of TOC in pond water in Sha Tau Kok were increased during the experimental period ($p<0.05$), but TOC in the pond sediment remained more or less the same ($p>0.05$). In this study, positive correlations ($p<0.05$) between TOC and metalloid/metal concentrations in sediment were observed (except Cr, THg and MeHg ($p>0.05$)). Organic carbon is an important factor in the transport of both organic and inorganic Hg from sediment to water, Hg bioavailability to methylating bacteria and bioaccumulation by benthic organisms and plankton (Muhaya et al., 1997; Lambertsson and Nilsson, 2006). In addition, Pikaray et al. (2005) reported that sediment organic matter could serve as adsorbents for As, and the observed higher concentrations of As with increasing organic matter contents were possibly caused by the formation of organo-As complexes. The positive correlations of sediment organic matter with As was also reported by Wang et al. (2010b), suggesting that organic matter plays a significant role in controlling As transport in the sediment.

## 3.4 Conclusions

The present results showed that metalloid/metal concentrations in pond water and sediment samples of the 3 experimental fish ponds were similar to or even lower than those in freshwater fish ponds around the PRD region suggesting that the experimental site was relatively free of trace element contaminations, and would be suitable for farming fish. Due to the fish ponds are drained and sediment removed after each harvest, avoiding accumulation of metalloid/metals in sediments, and therefore this traditional fish farming practice can improve the environmental quality of fish ponds and can also provide a better habitat for birds and other wildlife.

# CHAPTER 4 BIOACCUMULATION AND HEALTH RISK ASSESSMENT OF METALLOID/METALS IN FRESHWATER FISH, USING FOOD WASTES AS FISH FEEDS

## 4.1 Introduction

During the last three decades, the industrial and agricultural activities have rapidly increased in the Pearl River Delta (PRD). These together with soaring population and abuse of chemicals have resulted in serious impacts on the environment. Freshwater fish products for Hong Kong consumption mainly depend on the import from the PRD. Our previous studies demonstrated rather high concentrations of different POPs and heavy metals in fish reared in fish ponds around the PRD (Zhou and Wong, 2000; Nie et al., 2006), and purchased in local markets in Hong Kong (Cheung et al., 2007; Cheung et al., 2008). It was also observed that mercury (Hg) concentrations in fish tissue were correlated to Hg levels in their ambient environment notably sediment (Zhou and Wong, 2000). Sediments could accumulate higher concentrations of metalloid/metals from the sources (e.g. coal power plants) through atmospheric deposition, urban storm water, agricultural and industrial runoff entering into fish ponds (Ruddle and Zhung, 1988). Sulfate-reducing bacteria can convert the inorganic Hg in sediments into MeHg, releasing through the chemical flux into the water, which can be adsorbed by particulate matter, and then accumulated by fish (Raposo et al., 2008). The poor quality fish feeds seemed to be the major source for metalloid/metals accumulated in fish (Cheng et al., 2011; Lacerda et al., 2011; Cheng et al., 2013a). It is commonly observed that metalloid/metals can be bioaccumulated or biomagnified through the food chains and ultimately exert human health risks (USEPA, 2000a), and therefore food safety and quality have become serious issues of recent public concern.

In most previous studies, concentrations of metalloid/metals were used for estimating the human health risks via consumption of fish (Cheung et al., 2008; Shao et al., 2011). Nevertheless, using metalloid/metals concentration to conduct risk assessments usually overestimate the actual health risk because these pollutants were extracted in total form from the fish flesh, but not in bioavailable form. Evaluating the health risks using bioavailable pollutant concentrations is commonly regarded as a more accurate method, because only the bioavailable portion of the pollutants, will ultimately reach our bloodstream and exert adverse effects on our body (Brown et al., 1999). The use of animal models usually brings along with ethical concerns due to the involvement of animal experiments. Therefore, assessing bioaccessible fractions of pollutants (metalloid/metals) would be a suitable alternative in portraying the reality by using *in vitro* digestion models for conducting risk assessment (Moreda-Pineiro et al., 2011).

The fish ponds in Mai Po Nature Reserve (MPNR), north western of Hong Kong are regarded as an important site for migratory waterbirds (Young and Chan, 1997; Young, 1998). They provide an important feeding habitat for waterbirds especially during the traditional practice of draining pond water regularly (Young, 2004). Due to the decline of pond fish culture in Hong Kong, the conservation value of fish ponds has gradually disappeared and the inactively managed fish ponds may become a sink for various pollutants (Wong et al., 2004).

It is hypothesized that food waste can replace part of the fish meal used in fish feeds to produce quality fish. The major objectives of the present study were (1) to analyze bioaccumulations and biomagnifications of metalloid/metals (Hg, As, Cd, Cr, Pb, Cu, Ni and Zn) in the food chains; (2) using *in vitro* digestion method for analyzing the bioaccessibility of these pollutants contained in fish muscle; and (3) assess potential health risks based on digestible these pollutants concentrations in fish muscle.

## 4.2 Materials and Methods

### 4.2.1 Experimental Design

The same experimental design stated in Chapter 3 (Section 3.2.1) was adopted.

### 4.2.2 Sampling

In April and December 2012, fish samples were collected from each pond. Fish samples were collected from the fish ponds using a nylon net, with fish lengths and weights recorded (Table 4.1) To compare with our experiments conducted in Sha Tau Kok (Hong Kong), 4 abandon ponds in Hong Kong, and 3 farmed ponds located in the Guangdong province were selected for sampling (Table 3.1). Fish samples were wrapped in aluminum foil, frozen in zip-lock bags at -20 °C and transported to the laboratory until analyses.

### 4.2.3 Analytical Methods

Fish was freeze-dried and ground into powder. Measurements of metalloid/metals (As, Cd, Pb, Cr, Cu, Ni and Zn) in fish followed the procedures described in Section 2.2.2 at Chapter 2.

Total Hg (THg) concentration was determined by DMA-80 Direct Mercury Analyzer (MILESTONE), following USEPA method 7473 (USEPA, 2007). The detection limit for this method was about 0.03 ng of THg, corresponding to 0.3 ng/g for a typical 0.1 g sample. For MeHg analysis, 0.1 to 0.15 g (dry weight) of biota sample was digested in 3 ml 25% KOH/MeOH solution for 4 h at 65℃, and then brought to 37ml with methanol. MeHg was determined using aqueous ethylation, purge, trap, and gas chromatography – cold vapor atomic fluorescence spectrometry (GC-CVAFS) detection (Books Rand, MERX), following USEPA method 1630 (USEPA, 2001). The detection limit for this method was about 0.5 pg of MeHg, which corresponding to 0.005 ng/g for a typical 0.1 g sample.

### 4.2.4 Quality Control

All the samples were tested in triplicate. Analytical blank and reference materials were included in every batch of extraction. Four certified reference materials (CRM): NIST 1566b (Oyster tissue) were obtained from National Institute Standards and Technology, (NIST, USA) and TORT-2 (Lobster hepatopancreas) was obtained from National Research Council of Canada). The recoveries for total metals ranged from 90-104% (As, Cr, Pb, Cr, Cu, Ni and Zn). The recovery rates of the THg and MeHg of the standard reference material were within the certificate values. The recovery rates for THg and

MeHg were 101 ± 2.27% and 99.3 ± 5.34%, respectively. The coefficient of variation (SD/mean) for the duplicate samples was <10%.

**4.2.5 Bioaccessbility of Metalloid/Metals**

The *in vitro* digestion test was performed according to the methods described by Moreda-Pineiro et al. (2011) and Xing et al. (2008) with slight modifications. The entire digestion process was performed in capped Teflon centrifuge tubes (50 ml) in the dark to simulate the anaerobic condition of the stomach. Briefly, 3 g of freeze-dried fish samples were first added into 30 ml of synthetic gastric juice (2.0 g/l pepsin in 0.15 M NaCl, acidified with HCl to pH 1.8) and shaken at 100 rpm for 2 h at 37 ℃. The mixture was then centrifuged (20 min, 37 ℃, 3000 rpm) and the supernatant filtered through a 0.45 mm glass fiber filter. Artificial intestinal juice (30 ml, 2.0 g/l pancreatin, 2.0 g/l amylase and 5 g/l bile salts, in 0.15 M NaCl, pH 6.8) was added. Then, the mixture was resuspended and shaken at 30 rpm for 6 h at 37 ℃. Finally, the tubes were centrifuged at 3000 rpm at 37 ℃ for 20 min to separate supernatant and solids and the supernatant was filtered through a 0.45 mm glass fiber filter. The bioaccessibility (%BA) of metalloid/metals was calculated by adding the percentages in stomach and intestinal phase (Oomen et al., 2002).

$$\%\text{BA}=\frac{\text{BA extracted pollutant (stomach phase + intestinal phase)}}{\text{pollutant concentration in muscle tissue}}\times 100\% \qquad (4.1)$$

### 4.2.6 Bioaccumulation Factor

Bioaccumulation factor (BAF) and biota-sediment accumulation factor (BAF) can be obtained by Eq. (4.2) (Streets et al., 2006) and (4.3) (Szefer et al., 1999):

$$BAF = C_t / C_w \tag{4.2}$$

$$BSAF = C_t / C_s \tag{4.3}$$

where $C_t$ is the pollutant concentration in the tissues , $C_w$ is the pollutant concentration in water, $C_s$ is the pollutant concentration in sediment.

### 4.2.7 Stable Isotope Analysis

The biota samples were analyzed for stable isotopes at the Institute of Soil Science (Nanjing, China), Chinese Academy of Sciences. Stable isotope values were expressed as

$$\delta^{15}N = ( R_{sample} / R_{standard} - 1) \times 1000 \tag{4.4}$$

where $R_{sample}$ is Corresponding ratios of $^{15}N/^{14}N$ and $R_{standard}$ is atmospheric $N_2$ (air) (Hobson et al., 1995). Replicate measurements of internal laboratory standards (albumen) showed measurement errors of ±0.3‰ for stable nitrogen isotope measurement.

### 4.2.8 Trophic Level and Biomagnification Calculations

Trophic levels of biota samples were calculated using Eq (4.5) (Fisk et al., 2001).

$$TL_{consumer} = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{zooplankton}) / 3.4 \tag{4.5}$$

where $TL_{consumer}$ *is* consumer trophic level and $\delta^{15}N$ of zooplankton was assumed to be 3, and 3.4 was the isotopic enrichment factor.

The trophic magnification factors (TMFs) were based on relationships between trophic level and the mercury concentration using the following simple linear regression (Fisk et al., 2001):

$$\text{Log [pollutant concentration concentration]} = A + B \times TL \quad (4.6)$$

$$TMF = 10^{B} \quad (4.7)$$

where A is the intercept and B is the slope, the level of statistical significance of the regression Eq. (4.6) was set at $p < 0.05$.

### 4.2.9 Health risk Assessment

US EPA Standard Equations for evaluating non-cancer exerted on humans via fish consumption were adopted in this study (USEPA, 1989b, 2000a). For non-carcinogenic effects, the estimated daily intake (EDI) was compared with the recommended reference doses (RfD) ($10^{-4}$ or $2.29\times10^{-4}$ mg/kg-day for MeHg, $3\times10^{-4}$ mg/kg-day for As, $10^{-2}$ mg/kg-day for Cd, $4\times10^{-3}$ mg/kg-day for Pb, 0.3 mg/kg-day for Zn, 1.5 mg/kg-day for Cr, $4\times10^{-2}$ mg/kg-day for Cu, and $2\times10^{-2}$ mg/kg-day for Ni) (JECFA, 2003; USEPA, 2012a) as stated in Eq. (4.8) and (5):

$$\text{EDI/= Metal concentration } (\mu g/g \text{ ww}) \times \text{consumption rate (g/day) / body weight (kg)} \quad (4.8)$$

$$\text{Hazard Ratio (HR)} = \text{EDI / RfD} \quad (4.9)$$

The HR ≤ 1, it indicates no adverse health effects, whereas HR > 1 indicates that there is potential risk to human health (USEPA, 1989b). HRs can be added to generate a Hazard index (HI) in order to estimate the risk of mix contaminates (USEPA, 1989b).

$$HI = \Sigma\ HRi \quad (4.10)$$

where i is different elements.

For the carcinogenic effects, the cancer risk (CR) was obtained by using the oral slope factor of arsenic ($OSF_{As}$) (1.5mg/ kg-day) (USEPA, 2012a) as stated in Eq (4.11).

$$CR = EDI \times OSF_{As} \tag{4.11}$$

#### 4.2.10 Statistical Analyses

All of the statistical tests were performed using SPSS 19.0 for Windows. Normality was confirmed by the Kolmogorov-Smirnov test. Data of metalloid/metal concentrations, TLs and bioaccessibility in gastric and intestinal condition were analyzed using two independent t-tests, Wilconxon rank sum test, one-way ANOVA and Kruskal-Wallis test.

## 4.3 Results and Discussion

#### 4.3.1 Metalloid/Metal Concentrations

According to Table 4.1, the values of length and weight grass carp in Sha Tau Kok fish ponds, fed with FW A and control diet were significantly higher than these fed with FW B ($P<0.05$). During the first half year to second half year of the experiment, As, Cu, Ni, and Zn concentrations in grass carp, bighead carp and mud carp in Sha Tau Kok fish ponds decreased ($p<0.05$), while Pb concentrations in grass carp fed with food waste pellets and Hg concentrations in the three fish species increased ($p<0.05$). In Sha Tau Kok, As and Cu concentrations in grass carp fed with FW A, and Pb concentrations in grass carp and As and Cu concentrations in bighead carp fed with all food waste fish feed pellets, were higher than in the relevant fish species which fed with the control diet,

Table 4.1 Metalloid/metal concentrations (mg/kg dw) in fish (fed with food waste fish feed pellets and commercial fish feed pellets) from Sha Tau Kok and control area.

| | | n | length cm | weight kg | As | Cd | Pb | Cr | Cu | Ni | Zn | THg (ng/g) | MeHg (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sha Tau Kok** | | | | | | | | | | | | | |
| Control | GC | 11 | 34.3±5.07 | 0.37±0.10 | 0.27±0.03 | 0.04±0.01 | 0.27±0.05 | 0.81±0.41 | 0.16±0.41 | 0.40±0.08 | 34.0±6.34 | 93.8±1.89 | 27.0±11.3 |
| | BH | 19 | 23.5±2.41 | 0.13±0.05 | 0.47±0.03 | 0.05±0.01 | 0.16±0.04 | 0.50±0.17 | 0.19±0.07 | 0.68±0.44 | 22.1±10.8 | 465±4.77 | 107±9.23 |
| | MD | 6 | 13.3±1.26 | 0.02±0.01 | 0.67±0.08 | 0.04±0.01 | 0.56±0.09 | 0.86±0.17 | 1.14±0.38 | 0.98±0.80 | 44.6±16.6 | 419±8.90 | 318±18.6 |
| FW A | GC | 9 | 31.3±1.53 | 0.42±0.12 | 0.36±0.01 | 0.03±0.01 | 1.05±0.06 | 0.64±0.08 | 1.15±0.08 | 0.28±0.13 | 33.1±9.71 | 389±20.73 | 92.9±12.0 |
| | BH | 25 | 21.2±1.48 | 0.10±0.02 | 0.56±0.05 | 0.03±0.01 | 0.21±0.07 | 0.64±0.15 | 0.69±0.15 | 0.76±0.18 | 28.4±6.60 | 631±20.8 | 105±37.5 |
| | MD | 6 | 16.0±0.33 | 0.02±0.01 | 0.78±0.09 | 0.05±0.03 | 0.28±0.22 | 0.69±0.10 | 2.11±1.28 | 1.18±0.71 | 31.3±15.3 | 129±19.6 | 104±12.4 |
| FW B | GC | 10 | 21.4±2.22 | 0.08±0.03 | 0.31±0.04 | 0.03±0.01 | 0.89±0.12 | 0.68±0.16 | 0.49±0.16 | 0.33±0.15 | 20.8±4.87 | 69.0±12.2 | 30.0±6.63 |
| | BH | 21 | 23.5±0.71 | 0.11±0.01 | 0.52±0.03 | 0.04±0.01 | 0.12±0.06 | 0.53±0.12 | 0.60±0.12 | 0.48±0.26 | 24.3±9.42 | 429±134 | 103±32.0 |
| | MD | 6 | 13.0±4.24 | 0.02±0.01 | 0.72±0.19 | 0.04±0.02 | 0.37±0.07 | 0.51±0.05 | 2.13±1.21 | 0.75±0.44 | 34.5±13.0 | 238±30.4 | 126±16.9 |
| **Control area** | | | | | | | | | | | | | |
| Guangzhou | GC | 5 | 20.7±1.10 | 0.85±0.11 | 0.80±0.49 | 0.03±0.01 | 0.08±0.05 | 0.29±0.04 | 0.62±0.12 | 3.28±1.37 | 37.8±5.80 | 191±35.2 | 45.0±9.67 |
| Shunde | GC | 3 | 47.6±3.30 | 1.47±0.35 | 0.36±0.17 | 0.09±0.07 | 0.12±0.02 | 0.29±0.05 | 0.52±0.12 | 1.33±0.90 | 23.2±4.62 | 30.3±8.52 | 26.8±2.10 |
| | BH | 3 | 50.4±11.4 | 1.66±1.05 | 0.99±0.37 | 0.05±0.03 | 0.08±0.03 | 0.24±0.03 | 0.72±0.15 | 3.10±1.22 | 29.1±7.04 | 46.6±3.93 | 41.8±0.25 |
| Gaoyao | LB | 6 | 27.1±3.02 | 0.30±0.08 | 2.11±1.01 | 0.09±0.08 | 0.09±0.05 | 0.26±0.05 | 0.48±0.17 | 5.54±2.55 | 34.6±8.81 | 70.3±29.8 | 38.4±7.13 |
| Hong Kong | T | 8 | 27.9±9.41 | 0.18±0.36 | 8.25±0.97 | 0.06±0.01 | 1.98±0.34 | 0.83±0.06 | 3.78±1.65 | 8.87±0.42 | 89.2±16.8 | 30.2±3.22 | 23.2±2.43 |

Note: GC: grass carp; BH: bighead carp; MD: mud carp; LB: largemouth bass; T: tilapia; C: concentration; BA: bioaccessibility (%); FW: A food waste A, FW B: food waste B, Control: Commercial pellet.

respectively ($p<0.05$). No significant difference ($p>0.05$) was obtained for Cd, Cr, Ni and Zn concentrations in grass carp, bighead carp and mud carp in the three experiment fish ponds. In Sha Tau Kok, no significant difference ($p>0.05$) was obtained for MeHg and THg concentrations in Grass carp in the three fish ponds. However, bighead and mud carp fed with control feed pellets contained the highest MeHg and THg concentrations in muscle ($p<0.05$).

When compared with the control sites, it was observed that As (8.25±0.97 mg/kg dw), Pb (1.98±0.34 mg/kg dw) and Zn (67.4±24.1 mg/kg dw) concentrations in tilapia collected from abandoned fish ponds in Hong Kong were significantly higher than other fish samples ($p<0.05$). The abandoned ponds (Pok Wai site) had the highest As and Zn in water (Section 3.3.2 at Chapter 3), and the fish ponds had never been drained and sediment removed during the past 5-6 years. The concentrations of As and Ni in grass carp collected from Sha Tau Kok fish ponds were significantly lower than those in other species (except grass carp collected from Shuande) (Table 3) ($p<0.05$). Lead concentrations in grass carp and Cr concentrations in the three species fish in Sha Tau Kok fish ponds were higher than the fish samples collected from other fish ponds in PRD ($p<0.05$). The THg concentrations in muscle of grass carp (191± 35.2 ng/g ww) collected from Guangzhou was significantly higher than other fish samples ($p<0.05$), followed by largemouth bass (70.3± 29.8 ng/g ww). The concentrations of THg and MeHg in tilapia and grass carp were significantly lower ($p<0.05$) than those in other species (Table 4). The MeHg concentrations in grass carp fed with food waste fish feed pellets (FW A, MeHg 12.8 ± 3.27 ng/g ww and FW B, MeHg 11.6 ± 4.80 ng/g ww ) in Sha Tau Kok fish ponds was significantly lower than the fish from Guangdong province farmed ponds ($p<0.05$).

In the present study, Pb concentration in tilapia collected from abandoned fish ponds exceeded the Chinese maximum level Pb level in food (0.5 mg/kg ww) (GB2762, 2005). All other elements (the mean As concentration of inorganic As was estimated by using a value of 10% of total As in according with the United States Food and Drug Association (USFDA, 1993)) in other fish samples was below the maximum permissible levels of the local standard (CFS, 2007), and other international standards for metalloid/metal (USEPA, 2000; HC, 2007). This implied that all the fish species (grass carp, bighead carp and mud carp), fed with food waste fish pellets (FW A and FW B) and commercial fish feed pellets (control) in Sha Tau Kok fish ponds were safe for human consumption, in terms of the metalloid/metals tested.

**4.3.2 Bioaccessibility and Health Risk assessment**

The bioaccessibilities of As, Cd, Pb, Cr, Cu, Ni, Zn and MeHg in all fish samples varied between 10.9-43.7%, 5.41-48.1%, 3.80-27.1%, 13.2-73.3%, 10.2-64.6%, 4.82-53.4%, 11.8-76.6% and 30.7-65.6%, respectively (Figures 4.1 and 4.2). The mean bioaccessibility of As (30.0%), Cd (26.6%), Cu (25.2%), Ni (23.5%) and MeHg (45.6%) for the digested muscles of the investigated fish (fed with food waste pellets and commercial pellets) in Sha Tau Kok were not significantly different with the fish collected from freshwater fish ponds in PRD and Hong Kong. These values were comparable to that obtained in razor shell (*Ensis ensis*) (Cd, 21.1%), variegated scallop (*Chlamys varia*) (Cd, 12.7%), hake (*Merluccius merluccius*) (Cr, 36.7%) in Span

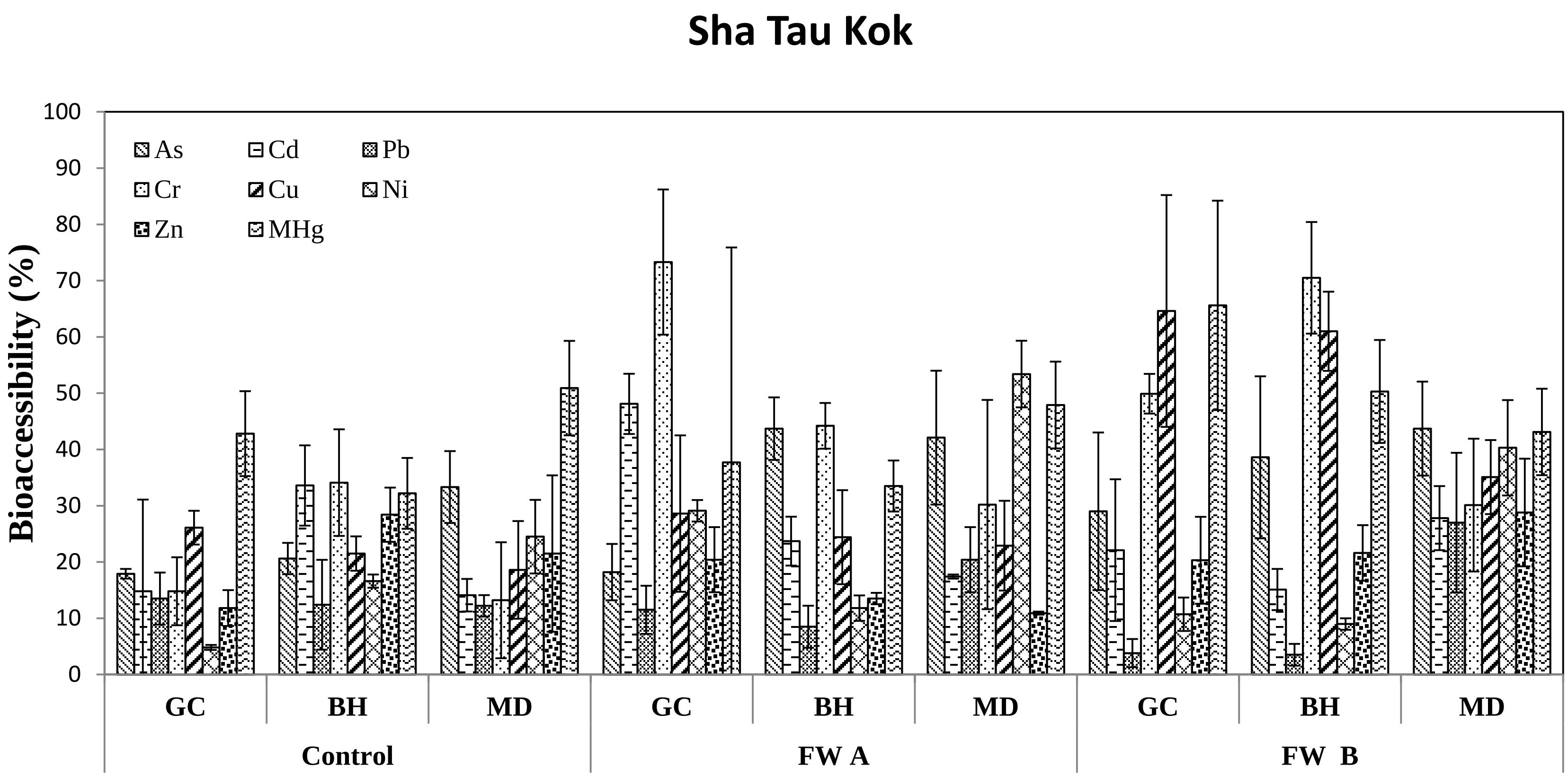



**Figure 4.1** Average bioaccessbility of metalloid/metals in fish muscles collected from Sha Tau Kok experimental fish ponds in gastrointestinal conditions.

Note: GC: grass carp; BH: bighead carp; MD: mud carp; LB: largemouth bass; T: tilapia; FW: A food waste A, FW B: food waste B, Control: Commercial pellet

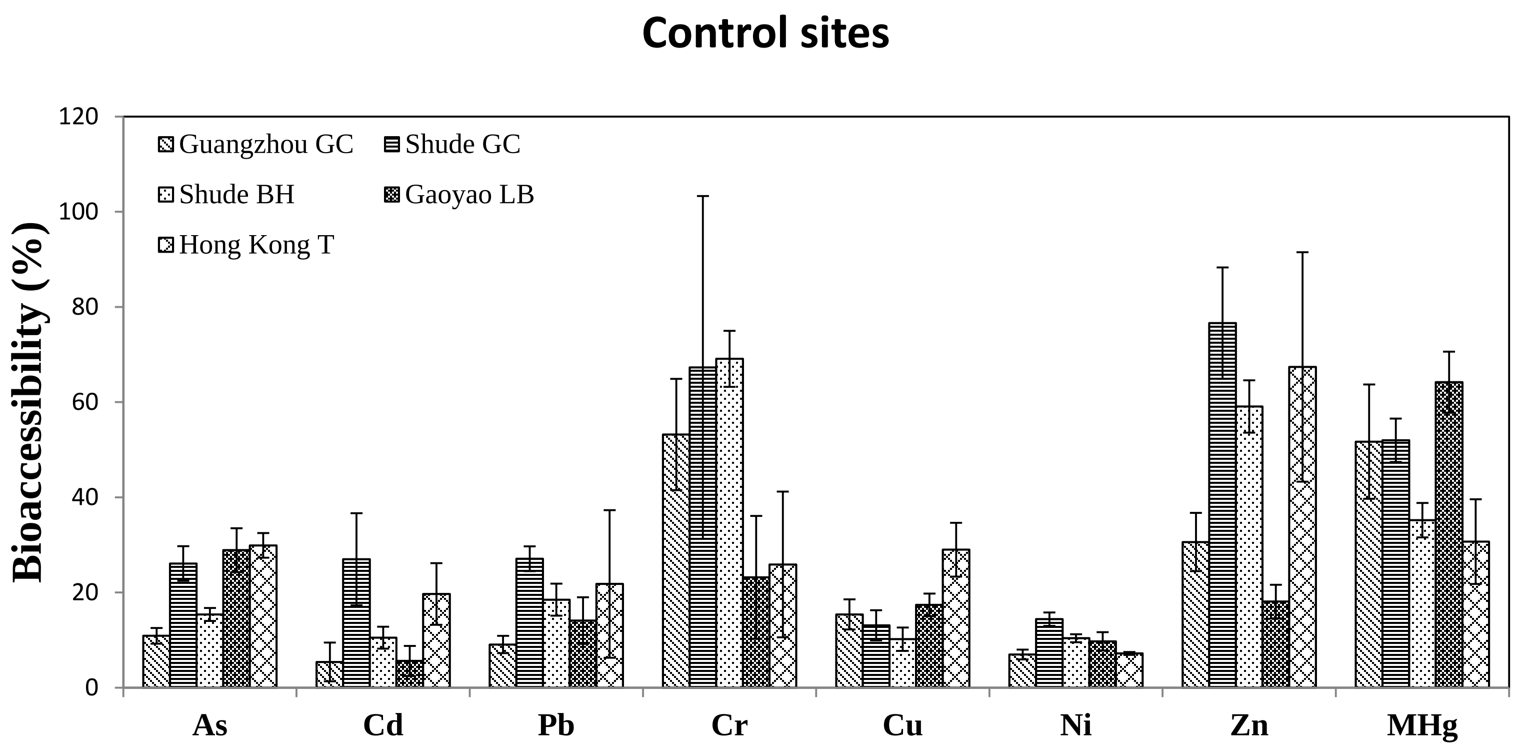



**Figure 4.2** Average bioaccessbility of metalloid/metals in fish muscles collected from control sites in gastrointestinal conditions.

Note: GC: grass carp; BH: bighead carp; MD: mud carp; LB: largemouth bass; T: tilapia; FW: A food waste A, FW B: food waste B, Control: Commercial pellet

(Moreda-Pineiro et al., 2012), but were considerably lower than the mean bioaccessibilities determined for freshwater crayfish (*procambarus clarkia*) (As, 69%) (Williams et al., 2009), and cod (*Gadus gadidae*) (Cr, 13.1%)(Moreda-Pineiro et al., 2012). These values were comparable to that obtained in razor shell (*Ensis ensis*) (Cd, 21.1%), variegated scallop (*Chlamys varia*) (Cd, 12.7%), hake (*Merluccius merluccius*) (Cr, 36.7%) in Span (Moreda-Pineiro et al., 2012), but were considerably lower than the mean bioaccessibilities determined for freshwater crayfish (*procambarus clarkia*) (As, 69%) (Williams et al., 2009), and cod (*Gadus gadidae*) (Cr, 13.1%)(Moreda-Pineiro et al., 2012).

The mean bioaccessibility of MeHg was comparable to that obtained in perch (*Perca fluviatilis*) (47%), herring (*Clupea harengus*) (61%), flounder (*Platichthys flesus*) (35%) of Baltic fish in Poland (Kwasniak et al., 2012) and arctic char (*Salvelinus alpinus*) in northern Canada (57%) (Laird et al., 2009). It was noted that these values were considerably higher than the mean bioaccessibility rates determined for tuna (*Thunnus spp*) 9%, European pilchard (*Sardina pilchardus*) 13% and black scabbardfish (*Aphanopus carbo*) 17% in Spanish (Cabanero et al., 2004, 2007).

Previous studies revealed that Hg, Cd, Cr, Ni, Cu and Zn bioaccessbility ratios exhibited a positive correlation with carbohydrate and dietary fibre contents, and a negative correlation with protein content (Cabanero et al., 2007; Moreda-Pineiro et al., 2012). Therefore, the fish species may be an important factor for the difference obtained in bioaccessibility.

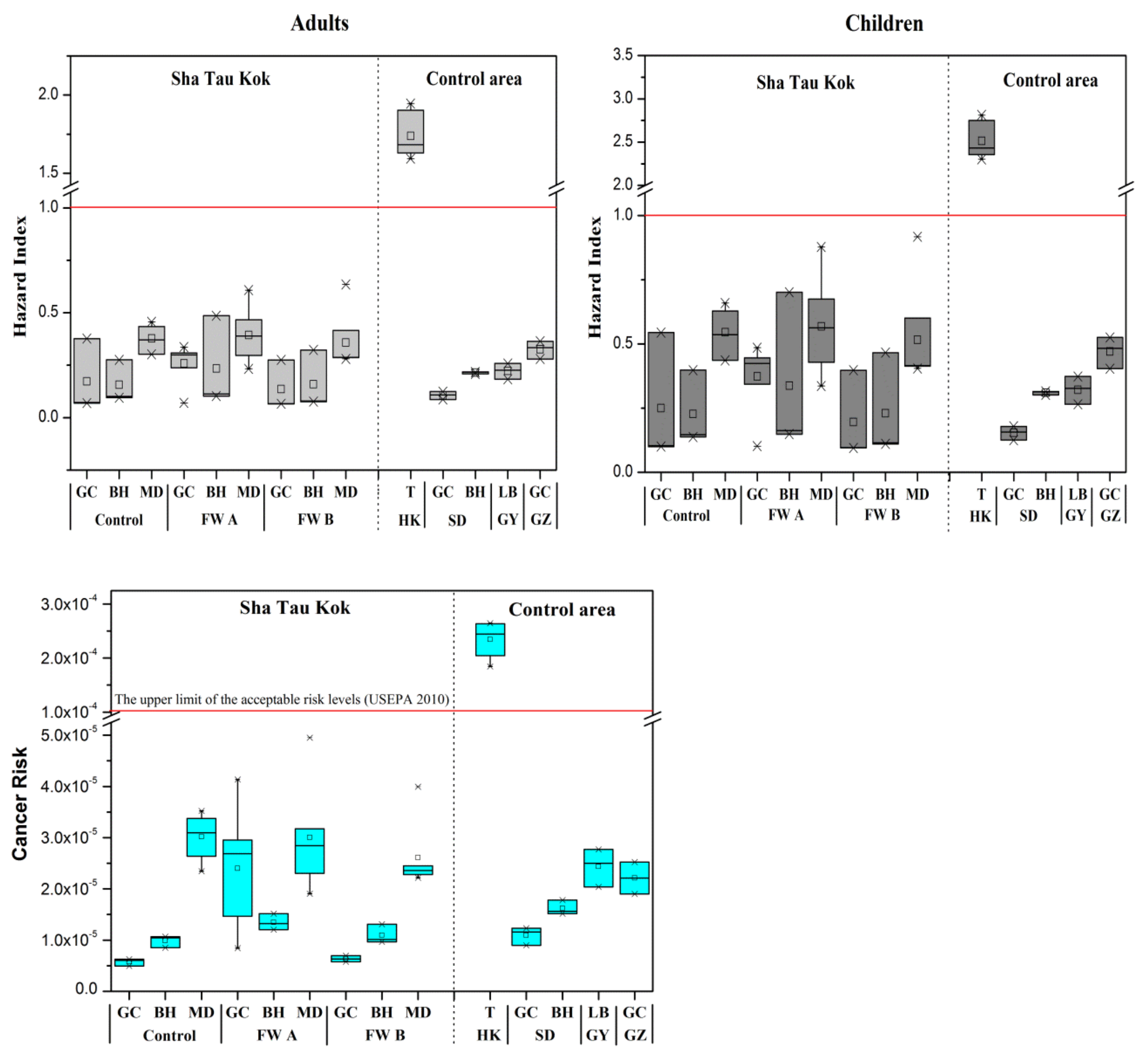



**Figure 4.3** Hazard indexes of metalloid/metals (expect MeHg) and cancer risk of inorganic As through freshwater fish by adults and children in Hong Kong. The consumption rates are 93 g $d^{-1}$ for adults and 50 g $d^{-1}$ for children, respectively. Each box represents interquartile range (25th and $75^{th}$ percentile) of hazard ratios and cancer risk of each fish. MD: mud carp, T: tilapia, LB: largemouth bass, BC: bighead carp, GC: grass carp, GZ: Guangzhou, SD: Shunde, GY: Gaoyao, HK: Hong Kong, FW: A food waste A, FW B: food waste B, Control: Commercial pellet.

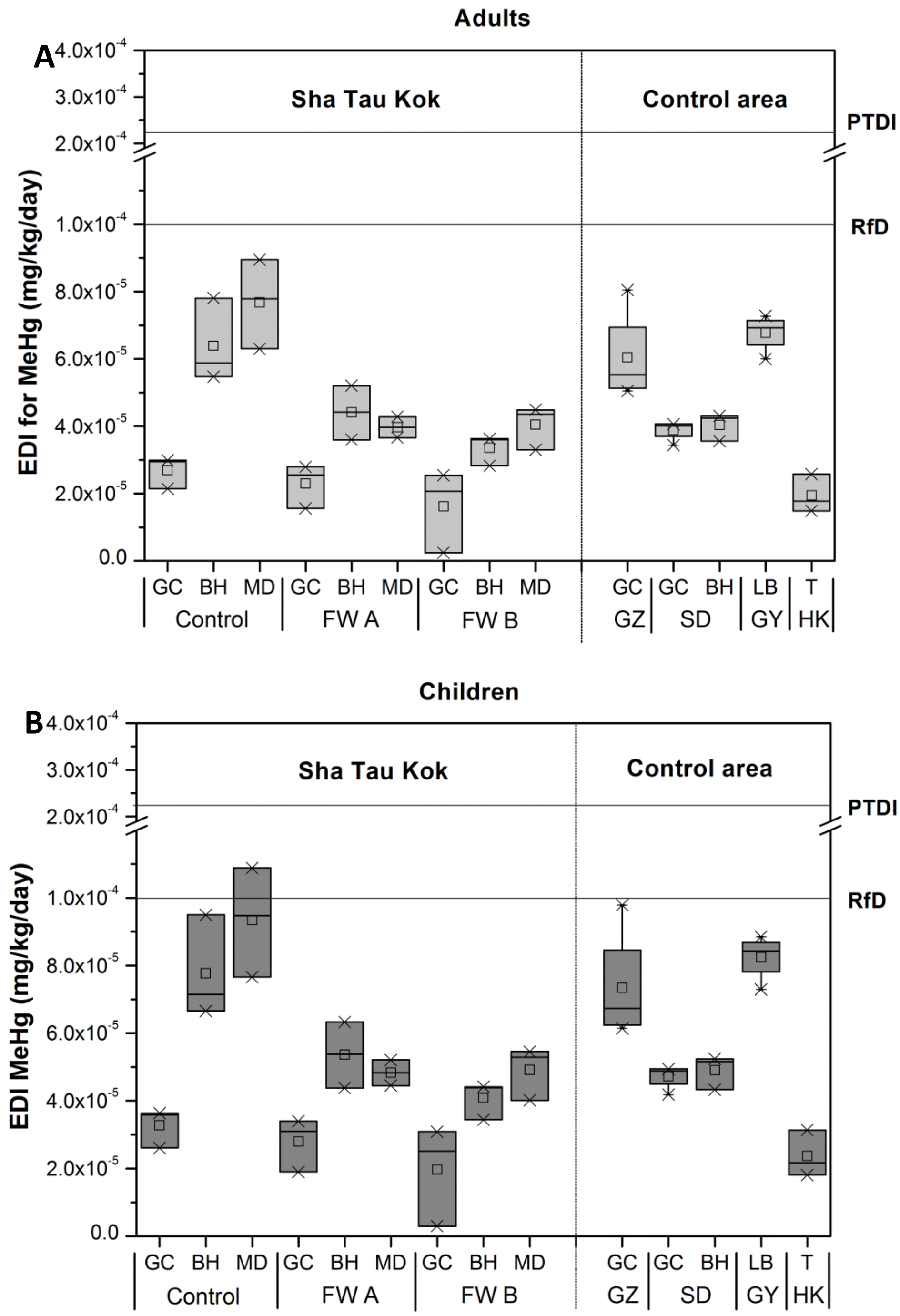



**Figure 4.4** Estimated daily intakes (EDI) of MeHg through fresh water fish by adults and children in Hong Kong. The consumption rates are 164.4 g/day for adults and 50 g/day for children, respectively. EDI = estimated daily intake; RfD= reference dose of (1.00E-04 mg/kg/day) (USEPA, 2012a); PTDI= provisional tolerable daily intake ( 2.29E-04 mg/kg/day) (JECFA, 2003). Each box represents interquartile range (25th and 75th percentile) of EDI of each fish. MD: mud carp, T: tilapia, LB: largemouth bass, BC: bighead carp, GC: grass carp, GZ: Guangzhou, SD: Shunde, GY: Gaoyao, HK: Hong Kong, FW: A food waste A, FW B: food waste B, Control: Commercial pellet.

In the present study, the major parameters used for the risk assessment were: BW of 58.6 kg used for adults (Wang et al., 2005) and 21.8 kg for preschool children (Leung et al., 2000), and daily consumption rate of fish in Hong Kong was estimated as 93 g/day for adults and 50 g/day for children (Leung et al., 2000). Figure 4.3 shows the hazard index (HI) of trace elements through freshwater fish consumption for Hong Kong residents. A HI higher than 1 implies that the EDI exceeds the RfD for the contaminant of interest which may exert potential harmful health effects. There were unlikely non-cancer risk for metalloid/metals (expect MeHg) exerted on adults and children via consumption of the three fish species fed with food waste pellets and commercial pellets in Sha Tau Kok. However, HI value of tilapia (1.73 for adults, 2.51 for children) was over 1 in all fish samples, suggesting that the residents may have a significant potential health risk by consuming tilapia collected from abandoned fish ponds in Hong Kong, with children having higher risks than adult. A risk above $10^{-6}$ value is considered by the USEPA (USEPA, 1989b) as an acceptable risk for cancer when estimating the lifetime excess CR of As. The CR values of fish were all above $10^{-6}$, and only tilapia ($2.34\times10^{-4}$) was higher than the upper limit of the acceptable risk levels ($10^{-4}$) (USEPA, 2012a), based on the 10% of organic As out of total As condition (USFDA, 1993).

According to Figure 4.4, there were unlikely non-cancer risk for MeHg exerted on adults and children via consumption of bighead carp, grass carp and mud carp (fed with food waste fish feed pellets and commercial fish feed pellets) in Sha Tau Kok, when using the PTDI suggested by Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2003). However, when the reference dose of 1.00E-04 (mg/kg/day) adopted in the USEPA used to estimate the non-cancer risk of MeHg for children (USEPA, 2012a), potential non-cancer risk via consumption of mud carp (33.3%) [fed with commercial fish feed pellets] were observed in children. Mud carp is an omnivore, living in the bottom of pond, mainly consumes water plants, insects, planktons, and organic detritus in the pond mud, as well as scraping organisms attached to underwater rocks and other objects. Therefore, mud carp can more easily accumulate Hg from sediment more easily than other fish.

In reality, due to different dietary habits of residents, the species and frequency of fish consumption varied tremendously, and many other factors such as cooking method, doneness level, and food processing, could all affect the final lifetime cancer risk for Hong Kong residents by ingesting these freshwater fish. However, the present results provided some useful information showing that consumption of grass carp, bighead and mud carp which fed food waste fish feed pellets was safe for the Hong Kong residents and more importantly, commercial fish pellets could be partially replaced by food waste for culturing fish.

### 4.3.3 Bioaccumulation and Biomagnification

In the present study, BAF or BSAF is defined as the observed ratio of biota tissue concentration to dissolved water or sediment concentration. It is assumed that aquatic organisms only accumulated trace elements from water and sediment for both BAF and BSAF (Lawrence and Mason, 2001; Streets et al., 2006). As shown in Table 4.2, log BAF was higher than log BSAF in all the samples, and the mean log BAF values in Sha Tau Kok were higher than that in control area. This is plausible considering that although sediment contained higher metalloid/metal concentrations than water, these elements accumulated in fish were mainly derived from the surrounding water. The

**Table 4.2** Bioaccumulation (BAF) and biota-sediment accumulation (BSAF) of freshwater fish (fed with food waste fish feed pellets and commercial fish feed pellets) from Sha Tau Kok and control sites

| | | log BAF | | | | | | | | | log BSAF | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | As | Cd | Pb | Cr | Cu | Ni | Zn | THg | MeHg | As | Cd | Pb | Cr | Cu | Ni | Zn | THg | MeHg |
| **Sha Tau Kok** | | | | | | | | | | | | | | | | | | | |
| Control | GC | 1.80 | 2.49 | 2.36 | 2.74 | 1.61 | 2.26 | 3.16 | 3.60 | 6.44 | -0.93 | 0.29 | -1.25 | -0.67 | -2.00 | -0.72 | 0.25 | 0.60 | 2.82 |
| | BH | 2.05 | 2.58 | 2.15 | 2.53 | 1.7 | 2.49 | 2.98 | 4.03 | 6.89 | -0.69 | 0.38 | -1.46 | -0.88 | -1.92 | -0.48 | 0.08 | 1.03 | 3.28 |
| | MD | 2.74 | 3.06 | 2.64 | 3.30 | 3.32 | 3.49 | 4.03 | 3.91 | 6.90 | -0.77 | 0.08 | -1.94 | -0.79 | -0.39 | -0.06 | 0.50 | 0.91 | 3.29 |
| FW A | GC | 1.67 | 2.45 | 2.76 | 1.36 | 2.62 | 2.28 | 2.93 | 3.90 | 6.46 | -1.59 | 0.34 | -0.87 | -0.91 | -1.82 | -1.34 | 0.02 | 0.92 | 2.95 |
| | BH | 1.89 | 2.49 | 1.92 | 1.24 | 2.71 | 2.43 | 3.03 | 4.05 | 6.92 | -1.37 | 0.38 | -1.71 | -1.02 | -1.73 | -1.18 | 0.09 | 1.07 | 3.41 |
| | MD | 2.70 | 3.26 | 2.88 | 3.03 | 3.05 | 3.34 | 3.68 | 4.00 | 6.78 | -0.71 | 0.32 | -1.79 | -1.02 | -0.28 | 0.09 | 0.45 | 1.02 | 3.27 |
| FW B | GC | 1.56 | 2.52 | 2.96 | 1.27 | 3.24 | 2.57 | 3.52 | 3.54 | 6.42 | -0.97 | 0.05 | -0.93 | -1.06 | -1.54 | -0.93 | -0.09 | 0.74 | 3.14 |
| | BH | 1.72 | 2.57 | 2.26 | 1.27 | 3.02 | 2.76 | 3.45 | 3.71 | 6.77 | -0.78 | 0.01 | -1.62 | -1.06 | -1.76 | -0.73 | -0.16 | 0.91 | 3.49 |
| | MD | 2.74 | 3.33 | 2.64 | 3.05 | 3.09 | 3.28 | 3.58 | 3.55 | 6.62 | -0.66 | 0.2 | -2.01 | -1.01 | -0.41 | -0.06 | 0.52 | 0.75 | 3.34 |
| **Control area** | | | | | | | | | | | | | | | | | | | |
| Guangzhou | GC | 2.26 | 1.35 | 0.89 | 1.43 | 1.92 | 3.00 | 2.67 | 4.39 | 5.10 | -1.44 | -1.56 | -3.10 | -2.31 | -2.32 | -0.94 | -0.86 | -0.78 | 1.12 |
| Shude | GC | 1.44 | 2.65 | 1.31 | 1.65 | 1.93 | 2.41 | 2.61 | 3.06 | 5.50 | -1.22 | -0.2 | -1.96 | -1.93 | -1.24 | -0.96 | -0.47 | 0.36 | 2.89 |
| | BH | 1.88 | 2.38 | 1.09 | 1.56 | 2.07 | 2.78 | 2.71 | 3.43 | 5.88 | -0.78 | -0.47 | -2.18 | -2.02 | -1.10 | -0.59 | -0.38 | 0.73 | 3.27 |
| Gaoyao | LB | 2.37 | 2.92 | 1.36 | 1.85 | 2.00 | 3.31 | 3.25 | 3.82 | 5.71 | -0.59 | 0.11 | -2.70 | -2.28 | -1.34 | -0.21 | -0.30 | 0.77 | 2.87 |
| Hong Kong | T | 2.03 | 2.58 | 3.99 | 2.41 | 2.29 | 2.62 | 4.61 | 2.68 | 4.31 | -0.24 | -0.39 | -1.42 | -1.16 | -0.54 | -0.04 | 0.36 | -0.51 | 2.82 |

Note: GC: grass carp; BH: bighead carp; MD: mud carp; LB: largemouth bass; T: tilapia; O: Omnivorous food chains; P: Predatory food chains, FW A: food waste A, FW B: food waste B, Control: Commercial pellet.

BSAF values of Cd and Zn in the fish species in Sha Tau Kok were significantly higher than other metals ($p<0.05$). This may be due to the fact that Cd and Zn possessing a higher percentage in acid soluble phase of the sediments, and they can be more easily dissolved in water and adsorbed on particulates for fish ingestion (Chen et al., 2000). In this study, the mean BAF for MeHg in omnivorous food chains (plankton, grass carp, bighead carp, mud carp and tilapia) is higher than in predatory food chains (plankton, largemouth bass) and the mean of BAF for THg in predatory food chains is similar or lower than omnivorous food chains. The same trend is also noted in BSAF. These indicated that omnivorous food chains possess a higher bioaccumulation ability of Hg than predatory food chains. Our previous studies observed a positive correlation ($p<0.05$) between TOC levels in sediment and the BSAF of Hg in the aquaculture pond ecosystem of the PRD (Zhou and Wong, 2000; Cheng et al., 2011). This phenomenon is also observed in the present study.

Due to the preferential excretion of lighter nitrogen isotopes, the concentration ratio of $^{15}N/^{14}N$, expressed relative to a standard (i.e., $\delta^{15}N$), has been shown to increase with increasing trophic levels (Deniro and Epstein, 1981; Minagawa and Wada, 1984). This technique has been used as a tool to determine the trophic relationships and to estimate the biomagnifications of organic and inorganic contaminants in aquatic ecosystems (Dehn et al., 2006; Yu et al., 2009; Zhang et al., 2012). In this study, two typical food chains of freshwater fish pond in Sha Tau Kok and PRD were selected for investigation: the omnivorous and predatory food chains. Five significant linear relationships were obtained between $\delta^{15}N$ and log metalloid/metal concentrations, and the slop of the regression as biomagnification power (Table 4.3), all the slops (expect MeHg) were

**Table 4.3** Statistics for the regression between the $\log_{10}$ trace element concentrations and $\delta^{15}N$ values of freshwater fish (fed with food waste fish feed pellets and commercial fish feed pellets) from Sha Tau Kok and control sites

| | | As | | Cd | | Pb | | Cr | | Cu | | Ni | | Zn | | THg | | MeHg | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Slop | $R^2$ | Slop | $R^2$ | Slop | $R^2$ | Slop | R2 | Slop | $R^2$ | Slop | $R^2$ | Slop | $R^2$ | Slop | R2 | Slop | R2 |
| **Sha Tau Kok** | | | | | | | | | | | | | | | | | | | |
| Control | O | -0.14 | 0.40 | -0.05 | 0.04 | -0.21 | 0.62 | -0.19 | 0.64 | -0.25 | 0.53 | -0.12 | 0.38 | -0.08 | 0.78 | 0.09 | 0.78 | 0.13 | 0.64 |
| FW A | O | -0.17 | 0.44 | -0.06 | 0.07 | -0.21 | 0.43 | -0.21 | 0.53 | -0.26 | 0.52 | -0.14 | 0.32 | -0.07 | 0.63 | 0.01 | 0.07 | 0.11 | 0.55 |
| FW B | O | -0.17 | 0.43 | -0.08 | 0.49 | -0.25 | 0.60 | -0.2 | 0.58 | -0.25 | 0.5 | -0.14 | 0.44 | -0.07 | 0.46 | 0.04 | 0.54 | 0.12 | 0.64 |
| **Control area** | | | | | | | | | | | | | | | | | | | |
| PRD | O | -0.07 | 0.20 | -0.14 | 0.67 | -0.3 | 0.59 | -0.13 | 0.6 | -0.18 | 0.47 | -0.02 | 0.05 | -0.09 | 0.44 | 0.11 | 0.73 | 0.11 | 0.73 |
| PRD | P | -0.06 | 0.88 | -0.13 | 0.98 | -0.24 | 0.50 | -0.10 | 0.95 | -0.18 | 0.98 | 0.01 | 0.08 | -0.07 | 0.98 | 0.10 | 0.85 | 0.12 | 0.97 |

Note: O: Omnivorous food chains; P: Predatory food chains, FW A: food waste A, FW B: food waste B, Control: Commercial pellet.

lower than zero suggesting the metalloid/metals were generally not biomagnified or biodiluted through the food chains in the Sha Tau Kok and control sites. The similar phenomenon was also observed in Yellow River Delta (Cui et al., 2011), Mekong Delta (Ikemoto et al., 2008b), Baltic Sea (Nfon et al., 2009) and Arctic marine food web (Campbell et al., 2005). This may be due to the dynamics and activates of most elements in organisms are dependent on transport proteins and binding site competition (Phipps et al., 2002). The transport proteins (e.g. metallothionein) regulate the uptake, accumulation and excretion rates of elements in biota, so it would be difficult for biomagnification to occur in food chains (Phipps et al., 2002).

On the contrary, five significant linear relationships were obtained between log THg and $\delta^{15}N$ and between log MeHg and $\delta^{15}N$, and the slope is smaller in magnitude in the present subtropical aquaculture pond ecosystem, when compared to those reported for temperate and arctic marine and freshwater ecosystems (Kidd et al., 2003; Campbell et al., 2005; Ikemoto et al., 2008b). These aquaculture ponds ecosystems are characterized by the lower species diversity and abundance with different food sources available for fish species, as well as the fish pond food chains are substantially shorter and simpler than those of other marine and fresh water ecosystems, and with the short culture periods (from 4 to 6 months). These would also lead to decreased accumulation of metalloid/metals through the food chain (Nfon et al., 2009; Cheng et al., 2013a). According to the present results, birds feeding on the fish reared in the fish ponds would not impose any health risks caused by the studied metalloid/metals.

## 4.4 Conclusions

Concentrations of metalloid/metals in the three different fish species cultured in Sha Tau Kok fish ponds were all below the Hong Kong, international (mainland China, Canada and USA) the maximum permissible levels in fish. The MeHg concentration in grass carp fed with food waste feed pellets of the present study was significantly lower than those fish collected from PRD fish ponds. The results of health risk assessment showed that consumption of grass carp, bighead and mud carp fed with food waste pellets was safe for Hong Kong residents. In addition, as there were no or lower biomagnifications, and low concentrations of the metalloid/metals contained in the fish ponds (both water and sediment) indicated the traditional fish farming using food wastes can provide a better fish pond habitat for birds and other wildlife.

# CHAPTER 5 VARIATIONS OF PAHs AND OCPs IN FRESHWATER FISH PONDS, USING FOOD WASTES AS FISH FEEDS

## 5.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) are well-documented class of contaminants found in water and sediments, and are an environmental concern as they are toxic, long-lived, and can travel long distances (Neff, 1979; UNEP, 2005). PAHs are a large group of compounds composed of two or more fused aromatic rings, with more than 100 congeners. They are byproducts of incomplete combustion of organic materials, which are usually deposited in soils and sediments of aquatic systems, through petroleum contamination, fallout from air pollution, and terrestrial runoff (Christensen et al., 1997; Yang, 2000). Due to their carcinogenic properties and acute toxicity, 16 congeners of PAHs have been listed as priority control pollutants by the USEPA (USEPA, 2012a). Organochlorine pesticides mainly include: dichlorodiphen- yltrichloroethane (DDT) and its metabolites dichlorobischlo-rophenylethane (DDD) and dichlorodiphenyldichloroethylene (DDE), hexachlorocyclohexanes ([HCHs]; sum of $\alpha$-, $\beta$-, $\gamma$-, and $\delta$-HCH). These compounds have extremely strong bonds between their chlorine and carbon components and are attracted to fat and highly insoluble in water. Therefore, they possess chronic toxicity, persistency, and bioaccumulative ability (Fisk et al., 2001; Hop et al., 2002), and are also endocrine disrupting chemicals (Soto et al., 1995; Li et al., 2008).

Sediments are considered ultimate sink for PAHs and OCPs in the aquatic environment. Therefore, it is commonly regarded as the primary detection target to monitor levels of different pollutants. Sediment samples collected from (323-14,812 ng/g dw) estuary and (1,168-21,329 ng/g dw) river areas revealed that PAH contamination around the PRD was moderately high when compared with other parts of the world (Mai et al., 2002; Luo et al., 2006). Elevated concentrations of OCPs were found in the sediment collected in Pearl River (Zhang et al., 2002), Daya Bay (Zhou et al., 2001), Pearl River Estuary and Macau Inner Harbor (Yu et al., 2008). The levels of OCPs in the sediment from PRD were relatively higher than that from heavily polluted locations, such as Rhône prodelta in the north-west Mediterranean region (124–657 ng/g dw) (Tolosa et al., 1995).

Organochlorine pesticides and PAHs could contaminate pond sediments through irrigation and atmospheric deposition over a long period, subsequently entering into food chains, accumulating in fish, and finally reaching humans. The conservation of fish pond habitats in northwestern New Territories contributed significantly to the ecological function of the ponds. Waterbirds can rest and feed on fish and shrimps thriving in the ponds and insects along the pond bunds (Young and Chan, 1997; Young, 1998), and therefore the ponds provide an important feeding habitat for waterbirds. Due to the decline of pond culture activities in Hong Kong, the ecological function of the area has gradually decreased and the unmanaged fish ponds may even become a sink for various pollutants, in addition to threatening wildlife health (Wong et al., 2004).

Fish meal is commonly used in aquaculture as a nutrient riched protein supplement ingredient (Kaushik et al., 2004). Most fish meal is derived from trash fish which are small fish or fragmented fish tissues collected from capture fisheries, with a low economic value. Pollutants can be accumulated in trash fish. It has been demonstrated that the use of trash fish as fish feeds was more polluted than using pellet feeds, based on the concentrations of POPs such as OCPs, bromated flame retardant (PBDEs) and PCBs (Guo et al., 2009b). In addition, our previous studies also showed that trash fish used in PRD contained higher levels of Hg than pellet feeds (Cheng et al., 2011; Liang et al., 2011). In European counties, fish meal derived from the trash fish for production of pellet feeds also contained high concentrations of bromated flame retardant (PBDEs), perfluorinated compounds, dioxin/furans and PCBs (Suominen et al., 2011). Therefore, the use of trash fish as fish feeds and as ingredients of fish meal for producing pellet feeds are the major sources of pollution worldwide.

It is hypothesized that food waste can replace part of the fish meal used in fish feeds to produce quality fish. In this study, food waste was used to replace part of the fish meal used in fish feeds to produce quality fish. The major objectives of the present study were to (1) investigate the concentrations of PAHs and OCPs in food waste fish feeds and the major food waste ingredients for making fish feeds; and (2) to monitor the concentrations and variations of PAHs and OCPs in the pond water, suspended particulate matter (SPM) and sediment. The information will help evaluate whether the traditional practice of draining pond water would improve the environmental quality of fish ponds using food wastes, and also provide a better fish pond habitat for wildlife.

## 5.2 Materials and Methods

### 5.2.1 Experiment Design

The same experimental design stated in Chapter 3 (section 3.2.1) was adopted.

### 5.2.2 Sampling of Water, SPM and Sediment

The same sampling procedures and frequency stated in Chapter 3 (section 3.2.2) were adopted.

Sediment samples were wrapped in aluminum foil, frozen in zip-lock bags at -20 °C and transported to the laboratory until analyses. Zooplankton samples were collected at approximately 0.5-1.0 m depth from fish ponds of each sampling site. They were collected using non-metallic plankton net (202 μm) for multiple vertical tows, stored in 100 ml acid-treated Teflon vials (Chen et al., 2000) and transported to the laboratory. Water samples were collected from each site in precleaned amber glasses bottles and acidified immediately with 4 M HCl to pH <1 and stored at 4 °C. They were then transported to the laboratory and filtered with glass fiber filters.

### 5.2.3 Sample Extraction

Samples of filtered water (1 L) were extracted using a solid-phase extraction (SPE) system from Supelco. The Extract-Clean™ SPE C18-HC cartridges (obtained from Alltech Corporation, USA) were pre-conditioned with 10 mL of dichloromethane (DCM) and 10 mL of methanol. Water samples were spiked with the internal standards prior to extraction and were passed through the cartridges at a flow rate of 5-10 mL/min under vacuum. After air-dried for at least 1 h, the target compounds were eluted with 15 mL DCM.

Fish feed pellets and sediments were freeze-dried and ground into powder. The sediment samples were homogenized by passing through a stainless steel 0.154 µm sieves. Samples of sediment (5-8 g) and fish feeds (3-5 g) were extracted for 18 h with a mixture of acetone, DCM, n-hexane (v: v: v 1:1:1, 120 ml) in a Soxhlet extractor, according to Standard Method 3540C (USEPA, 1996b). Sufficient acid washed copper powder was added to remove sulfur. Florisil column and silica gel clean-up were used for purification of the concentrated extract according to Standard Method 3620B (USEPA, 1996c). Deuterated PAHs internal standard (acenaphthene-$d^{10}$, phenanthrene-$d^{10}$, chrysene-$d^{12}$ and perylene-$d^{12}$) and OCP internal standard (2, 4, 5, 6-Tetrachloro-mxylene, TCmX) were added into all extracts to the concentration of 320 ng/g and 100 ng/g, respectively, prior to instrumental analysis for quantification.

### 5.2.4 Instrumental Analyses

Concentrations of PAHs were determined with a Hewlett-Packard (HP) 6890 N gas chromatograph (GC) coupled with a HP-5973 mass selective detector (MSD) and a 30 m × 0.25 mm × 0.25 µm DB-5 capillary column (J & W Scientific Co. Ltd., USA), using Standard Method 8270C (USEPA, 1996d). The PAH standards (AccuStandard, New Haven, CT) consisted of 16 priority pollutant PAHs: naphthalene (Nap), acenaphthylene (Acel), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (An), fluoranthene (FlA), pyrene (Py), benz(a)anthracene (BaA), chrysene (Chry), benzo(a)pyrene (BaP), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), indeno(1,2,3-cd)pyrene (IP), dibenz(a,h)anthracene (DahA) and benzo(g,h,i)perylene (BghiP). The peaks of benzo (b) fluoranthene (BbF) and benzo (k) fluoranthene (BkF) were extremely close and difficult to be distinguished; therefore, these two compounds were combined as one, namely B (b+k) F. The standard curve was obtained by using 0, 5, 10, 20, 50, 80, 100, 200 ng/g PAH standards. Concentrations based on individually resolved peaks were summed to obtain the total PAH concentrations (ΣPAH).

The oven temperature for OCPs was programmed from 60 ℃ (initial time, 1 min) to 290 ℃ at a rate of 4 ℃ /min, held for 10 min. The 20 targeted OCP compounds included DDT and metabolites (*o, p'*-DDD, *p, p'*-DDD, *o, p'*-DDE, *p, p'*-DDE, *o, p'*-DDT and *p, p'*-DDT), hexachlorocyclohexanes (HCHs, *α*-HCH, *β*-HCH, *γ*-HCH, and *δ*-HCH), CHLs (Heptachlor, trans-Chlordane, cis-Chlordane, trans-Nonachlor, and cis-Nonachlor), DRINs (Aldrin, Dieldrin, and Endrin), HCB (Hexachlorobenzene) and Mirex.

### 5.2.5 QA/QC

Using the NIST (USA) SRM 1941b as a reference, the recoveries of individual PAHs and OCPs ranged from 71.4% to 107.6% and from 76.2% to 115%, respectively. For each batch of 15 field samples, a method blank (solvent), a spiked blank (standards spiked into solvent), a sample duplicate, and a standard reference material (NIST SRM 1941b) sample were processed. The variation coefficient of PAH and OCP concentrations between duplicate samples was less than 8%. The limit of detection (LOD) using the present method was determined as the concentrations of analytes in a sample that gives rise to a peak with a signal-to-noise ratio (S/N) of 3, which ranged from 0.03 to 0.10 ng/g for PAHs and from 0.05 to 0.20 ng/g for OCPs.

### 5.2.6 Statistical Analyses

All the statistical tests were performed using SPSS 19.0 for Windows. Normality was confirmed by the Kolmogorov-Smirnov test. Data of trace element concentrations and

**Table 5.1** Concentrations (ng/g dw ± SD) of PAHs in fish feeds used in the experiment.

| | Nap | Acel | Ace | Fl | PhA | An | FlA | Py | BaA | Chry | BbkF | BaP | IP | DahA | BghiP | ∑**PAH** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PAHs concentration of the experiment feeds** | | | | | | | | | | | | | | | | |
| Commercial fish pellets | 3.83 | 0.11 | 0.86 | 1.10 | 7.56 | 1.52 | 1.00 | 1.43 | 0.62 | 0.06 | 0.30 | 0.54 | nd | nd | nd | 18.9±3.58 |
| Food waste A | 6.00 | 1.96 | 2.90 | 4.91 | 26.2 | 0.33 | 2.06 | 11.5 | 1.46 | 0.28 | 0.81 | 0.67 | nd | nd | nd | 59.1±10.8 |
| Food waste B | 6.13 | 1.34 | 1.69 | 3.35 | 20.3 | 0.31 | 1.96 | 8.50 | 1.18 | 0.21 | 1.51 | 3.27 | nd | nd | nd | 49.7±28.8 |
| **PAHs concentrations in major food waste ingredients for making fish feed pellets (FW A and FW B)** | | | | | | | | | | | | | | | | |
| Fish meal | 4.83 | 1.68 | 1.63 | 2.21 | 8.34 | 0.26 | 1.08 | 3.43 | 0.65 | 0.14 | 0.20 | 0.31 | nd | nd | nd | 24.8±7.51 |
| Fruit &Vegetables+Cereals | 6.78 | 1.21 | 2.77 | 7.38 | 31.3 | 9.38 | 3.02 | 18.3 | 2.76 | 0.27 | 0.45 | 0.53 | nd | nd | nd | 84.2±7.78 |
| Fruit & Vegetables+Bone meal | 9.23 | 1.43 | 6.08 | 15.5 | 57.3 | 9.31 | 3.27 | 29.4 | 3.38 | 0.95 | 0.31 | nd | nd | nd | nd | 136±23.9 |
| Meat products | 9.47 | 3.71 | 4.44 | 8.15 | 30.2 | 5.44 | 1.33 | 11.8 | 1.15 | 0.34 | nd | 0.45 | nd | nd | nd | 76.7±22.8 |

Note: FW A: food waste A pellet; FW B: food waste B pellet; Control: Commercial pellet; nd: under detection limit (0.03 ng/g).

**Table 5.2** Concentrations (ng/g dw ± SD) of OCPs in fish feeds used in the experiment.

| | *α*-HCH | *β*-HCH | *γ*-HCH | *δ*-HCH | **Σ HCHs** | *o, p'*-DDE | *p, p'*-DDE | *o, p'*-DDD | *o, p'*-DDT | *p, p'*-DDD | *p, p'*-DDT | **Σ DDTs** | **Σ OC** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **OCPs concentration of the experiment feeds** | | | | | | | | | | | | | |
| Commercial fish pellets | 0.63 | nd | 0.72 | nd | 1.35±0.82 | 0.09 | 0.13 | 4.87 | nd | nd | 0.84 | 5.94±1.86 | 7.29±2 |
| Food waste A | nd | nd | 0.39 | nd | 0.39±0.23 | 0.11 | 0.15 | 3.42 | nd | nd | 0.89 | 4.58±0.52 | 4.97±1 |
| Food waste B | 0.32 | nd | 0.59 | nd | 0.91±0.35 | 0.16 | 0.18 | 2.16 | nd | nd | 0.83 | 3.32±1.18 | 4.23±2 |
| **OCPs concentrations in major food waste ingredients for making fish feed pellets (FW A and FW B)** | | | | | | | | | nd | nd | | | |
| Fish meal | nd | nd | nd | nd | 0.00 | 0.11 | 0.13 | 2.49 | nd | nd | 0.77 | 3.51±0.49 | 3.51±0 |
| Fruit &Vegetables+Cereals | nd | nd | nd | nd | 0.00 | 0.37 | 0.23 | nd | nd | nd | 0.68 | 1.28±0.52 | 1.28±0 |
| Fruit & Vegetables+Bone meal | 0.67 | nd | 1.10 | nd | 1.77±0.68 | 0.30 | 0.30 | 8.06 | nd | nd | 0.83 | 9.49±0.45 | 11.3±2 |
| Meat products | nd | nd | nd | nd | 0.00 | 0.09 | 0.17 | 6.93 | nd | nd | 0.91 | 8.09±1.74 | 8.09±1 |

Note: FW A: food waste A pellet; FW B: food waste B pellet; Control: Commercial pellet; nd: under detection limit (0.05 ng/g).

bioaccessibility in gastric and intestinal conditions were analyzed using two independent t-test, Wilconxon rank sum test, one-way ANOVA and Kruskal-Wallis test.

## 5.3 Results and Discussion

### 5.3.1 Fish Feeds

Concentrations of ΣPAHs and ΣOCPs in fish feeds used in the experiment are shown in Tables 5.1 and 5.2. Control feeds (18.9±3.58 ng/g dw) contained the lowest concentration of ΣPAHs ($p<0.05$), among the three types of feed pellets. There was no significant difference ($p>0.05$) in concentrations of ΣPAHs between FW A (59.1±10.8 ng/g dw) and FW B (49.7±28.8 ng/g dw). The analyses of ΣPAHs in various food waste ingredients for making fish pellets showed that fruits, vegetables, bone meal, cereals and meat products were the major sources of ΣPAHs contamination for producing FW A and FW B.

According to Table 5.2, no significant difference ($p>0.05$) in ΣDDT, whereas significantly ($p<0.05$) lower ΣHCHs were found in FW A and FW B, when compared with the control diet. The *op*-DDT, *pp*-DDD, *β*-HCH and *δ*-HCH were not detected in the three types of fish feeds. The analyses of ΣOCPs in various food waste ingredients showed that fruits, vegetables and bone meal were the major source of ΣOCPs, and meat products was also contributed significant source of ΣDDTs for making fish pellets (FW A and FW B). Previous studies observed high concentrations of PAHs and OCPs in farm soils of PRD and Hong Kong (Chen et al., 2005; Cai et al., 2007; Man et al., 2011; Man et al., 2013), and different crops could accumulate these pollutants to different extent from the soils. In addition, livestocks fed with polluted feeds could also accumulate PAHs and OCPs.

### 5.3.2 PAHs in Environmental Samples

Tables 5.3 and 5.4 show that, there was no significant difference (*p>0.05*) in concentrations of ΣPAHs in water and SPM of fish ponds collected from the studies ponds between the 1st half year (October 2011 to April, 2012) and 2nd half year (May 2012 to December 2012). The results indicated that was no seasonal changes in total dissolved and particulate phase PAH concentrations during the experimental periods in Sha Tau Kok. There were no significant differences (*p>0.05*) in ΣPAHs in water and SPM among the 3 ponds. In general, ΣPAHs in pond water and SPM of the experimental ponds were similar or higher than those of other fish ponds scattered around the PRD (control sites). The concentrations of total PAHs in pond water (18.2–132 ng/l ) and SPM (0.60-13.2 ng/l) obtained in this study were similar to those obtained from Pearl River Estuary (SPM 2.60-39.1 ng/l, water 12.9-182.4 ng/l) (Luo et al., 2008) and Xijiang River (SPM 0.17-58.2 ng.l, water 21.7-138 ng/l ) (Deng et al., 2006), but lower than those detected in the Daya Bay (water +SPM: 691-6457 ng/l) (Zhou and Maskaoui, 2003) and Baiyangdian Lake (SPM 1011-6268 ng/l, water 88.92-907 ng/l) (Guo et al., 2011).

Table 5.5 shows that were no significant (*p>0.05*) difference among three ponds in ΣPAHs in sediment (mean 328.2 ng/g dw, 80.4-393 ng/g dw) during the 2nd half year experiment period, but ΣPAHs in sediment in 2nd half year were higher than that in the 1st half year (mean 119 ng/g dw, 83–190 ng/g dw) (*p>0.05*). The results suggested that, using more fish feeds to culture fish in the 2nd half year might increase PAHs

Table 5.3 Concentrations of ΣPAHs in pond water (ng/l ±SD) of sampling sites in Hong Kong and Pearl River Delta (PRD)

| Area | Site | Nap | Acel | Ace | Fl | PhA | An | FlA | Py | BaA | Chry | BbkF | BaP | IP | DahA | BghiP | ΣPAHs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment sites** | | | | | | | | | | | | | | | | | |
| 1st half year | Control | 27.3 | 0.10 | 2.28 | 2.14 | 18.4 | 7.51 | 0.52 | 1.20 | 0.94 | 0.52 | 0.71 | nd | nd | nd | nd | 61.6±16.6 |
| | FW A | 14.6 | 0.36 | 1.87 | 2.89 | 15.4 | 6.55 | 1.04 | 1.71 | 0.92 | 0.67 | 1.35 | nd | nd | nd | nd | 47.0±22.7 |
| | FW B | 13.0 | 0.13 | 1.92 | 3.18 | 16.9 | 6.96 | 0.69 | 1.57 | 0.94 | 0.35 | 3.19 | nd | nd | nd | nd | 48.0±21.1 |
| 2nd half year | Control | 15.1 | 0.81 | 2.54 | 3.11 | 11.3 | 2.64 | 0.47 | 2.42 | 10.80 | 18.05 | nd | nd | nd | nd | nd | 57.0±11.0 |
| | FW A | 5.87 | 0.68 | 3.06 | 6.68 | 17.5 | 8.91 | 0.41 | 1.89 | 5.10 | 11.59 | nd | nd | nd | nd | nd | 55.0±12.8 |
| | FW B | 21.4 | 0.87 | 3.64 | 3.81 | 14.4 | 3.83 | 1.39 | 2.26 | 9.11 | 15.62 | nd | nd | nd | nd | nd | 64.3±16.3 |
| **Control sites** | | | | | | | | | | | | | | | | | |
| Gaoyao | GY | 23.4 | 0.17 | 0.97 | 1.26 | 9.16 | 0.53 | 0.30 | 0.58 | 0.83 | 0.10 | nd | nd | nd | nd | nd | 37.3±1.08 |
| Guangzhou | GZ | 13.7 | 0.88 | 2.14 | 8.29 | 8.85 | 0.89 | 0.46 | 0.68 | 1.09 | 0.21 | 9.58 | 16.8 | nd | nd | nd | 63.6±4.35 |
| Shunde | SD | 22.6 | 0.23 | 0.95 | 1.26 | 9.16 | 0.56 | 0.23 | 0.76 | 0.99 | 0.26 | nd | nd | nd | nd | nd | 37.0±11.0 |
| Pok Wai | PW-1 | 17.2 | 1.10 | 2.18 | 5.31 | 6.95 | 0.48 | 0.51 | 0.72 | 0.85 | 0.19 | 6.59 | 12.3 | nd | nd | nd | 54.4±8.78 |
| | PW-2 | 28.3 | 0.42 | 1.61 | 6.17 | 26.9 | 2.48 | 1.29 | 1.62 | 1.28 | 0.32 | 3.43 | 6.68 | nd | nd | nd | 80.4±48.4 |
| Mei Po | MP-1 | 13.0 | 0.12 | 0.63 | 1.98 | 5.97 | 2.89 | 0.31 | 0.61 | 0.79 | 0.19 | 3.28 | 6.22 | nd | nd | nd | 36.0±12.3 |
| | MP-2 | 18.7 | 0.21 | 0.84 | 3.64 | 8.17 | 3.06 | 0.44 | 0.68 | 1.05 | 0.21 | 3.25 | 5.71 | nd | nd | nd | 45.9±3.40 |

Note : nd= under detection limit (0.03 ng/g); Control= commercial fish feed pellets, FW A and FW B = Food waste fish feed pellets A and B, respectively, naphthalene (Nap), acenaphthylene (Acel), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (An), fluoranthene (FlA), pyrene (Py), benz(a)anthracene (BaA), chrysene (Chry), benzo(a)- pyrene (BaP), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), indeno(1,2,3-cd)pyrene (IP), dibenz(a,h)anthracene (DahA), and benzo- (g,h,i)perylene (BghiP).The peaks of benzo(b)fluoranthene (BbF) and benzo- (k)fluoranthene (BkF) were extremely close and difficult to distinguish; therefore, these two compounds were combined as one, namely, B(bk)F.

Table 5.4 Concentrations of ΣPAHs in suspended particulate matter (SPM) (ng/l ±SD) of sampling sites in Hong Kong and Pearl River Delta (PRD)

| Area | Site | Nap | Acel | Ace | Fl | PhA | An | FlA | Py | BaA | Chry | BbkF | BaP | IP | DahA | BghiP | ΣPAHs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment sites** | | | | | | | | | | | | | | | | | |
| 1st half year | Control | 2.27 | 0.03 | 0.09 | 0.43 | 2.30 | 0.09 | 0.08 | 0.26 | 0.33 | 0.02 | 0.03 | 0.11 | nd | nd | nd | 6.05±4.54 |
| | FW A | 3.50 | 0.05 | 0.12 | 0.55 | 2.70 | 0.11 | 0.10 | 0.27 | 0.19 | 0.02 | 0.05 | 0.09 | nd | nd | nd | 7.73±5.69 |
| | FW B | 3.48 | 0.04 | 0.13 | 0.37 | 2.19 | 0.08 | 0.16 | 0.25 | 0.14 | 0.02 | 0.04 | 0.08 | nd | nd | nd | 6.98±3.08 |
| 2nd half year | Control | 2.16 | 0.05 | 0.20 | 0.16 | 2.44 | 0.07 | 0.12 | 0.04 | 0.33 | 0.02 | nd | nd | nd | nd | nd | 6.59±3.80 |
| | FW A | 2.13 | 0.09 | 0.29 | 0.12 | 2.14 | 0.10 | 0.09 | 0.08 | 0.08 | 0.09 | nd | 0.12 | nd | nd | nd | 6.42±4.37 |
| | FW B | 1.42 | 0.11 | 0.37 | 0.06 | 2.15 | 0.09 | nd | 0.16 | 0.07 | 0.01 | 1.58 | 0.02 | nd | nd | nd | 6.21±3.07 |
| **Control sites** | | | | | | | | | | | | | | | | | |
| Gaoyao | GY | 0.76 | 0.01 | 0.02 | 0.10 | 0.35 | 0.01 | 0.02 | 0.05 | 0.01 | 0.00 | 0.01 | 0.02 | nd | nd | nd | 1.35±0.11 |
| Guangzhou | GZ | 3.20 | 0.02 | 0.05 | 0.37 | 1.63 | 0.10 | 0.16 | 0.37 | 0.08 | 0.03 | 0.09 | 0.21 | nd | nd | nd | 6.33±0.62 |
| Shunde | SD | 2.42 | 0.02 | 0.10 | 0.31 | 1.60 | 0.10 | 0.09 | 0.19 | 0.08 | 0.01 | 0.04 | 0.08 | nd | nd | nd | 5.04±0.63 |
| Pok Wai | PW-1 | 3.97 | 0.04 | 0.22 | 0.79 | 6.61 | 0.16 | 0.12 | 0.31 | 0.06 | 0.03 | 0.06 | 0.12 | nd | nd | nd | 12.5±0.64 |
| | PW-2 | 4.22 | 0.03 | 0.18 | 0.79 | 2.88 | 0.08 | 0.12 | 0.29 | 0.05 | 0.02 | 0.05 | 0.09 | nd | nd | nd | 8.80±0.48 |
| Mai Po | MP-1 | 0.56 | 0.00 | 0.03 | 0.09 | 0.55 | 0.03 | 0.03 | 0.10 | 0.02 | 0.00 | 0.01 | 0.02 | nd | nd | nd | 1.43±0.16 |
| | MP-2 | 0.90 | 0.01 | 0.04 | 0.16 | 0.69 | 0.02 | 0.03 | 0.13 | 0.02 | 0.00 | 0.00 | 0.01 | nd | nd | nd | 2.02±0.44 |

Note : nd= under detection limit (0.03 ng/g); Control= commercial fish feed pellets, FW A and FW B = Food waste fish feed pellets A and B, respectively, naphthalene (Nap), acenaphthylene (Acel), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (An), fluoranthene (FlA), pyrene (Py), benz(a)anthracene (BaA), chrysene (Chry), benzo(a)- pyrene (BaP), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), indeno(1,2,3-cd)pyrene (IP), dibenz(a,h)anthracene (DahA), and benzo- (g,h,i)perylene (BghiP).The peaks of benzo(b)fluoranthene (BbF) and benzo- (k)fluoranthene (BkF) were extremely close and difficult to distinguish; therefore, these two compounds were combined as one, namely, B(bk)F.

Table 5.5 Concentrations of ΣPAHs in sediment (ng/g dw ±SD) of sampling sites in Hong Kong and Pearl River Delta (PRD)

| Area | Site | Nap | Acel | Ace | Fl | PhA | An | FlA | Py | BaA | Chry | BbkF | BaP | IP | DahA | BghiP | ΣPAHs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment sites** | | | | | | | | | | | | | | | | | |
| 1st half year | Control | 18.8 | 0.80 | 1.75 | 5.51 | 39.8 | 16.1 | 5.31 | 13.2 | 4.64 | 1.20 | 1.47 | 6.95 | nd | nd | nd | 115±50.2 |
| | FW A | 28.0 | 0.63 | 1.48 | 5.54 | 37.3 | 13.5 | 5.25 | 7.73 | 2.16 | 1.14 | 1.83 | 34.9 | nd | nd | nd | 139±21.8 |
| | FW B | 15.1 | 0.56 | 2.63 | 6.38 | 31.8 | 13.7 | 4.28 | 6.51 | 2.59 | 1.83 | 0.82 | 16.3 | nd | nd | nd | 102±24.5 |
| 2nd half year | Control | 218 | 20.1 | 62.4 | 0.51 | 4.87 | 25.0 | 0.94 | 0.30 | 2.72 | 0.65 | 8.45 | 3.66 | nd | nd | nd | 342±73.0 |
| | FW A | 165 | 19.0 | 67.2 | 0.80 | 6.62 | 26.5 | 1.24 | 0.37 | 4.37 | 0.79 | 8.81 | 6.08 | nd | nd | nd | 299±22.6 |
| | FW B | 217 | 12.4 | 58.3 | 0.93 | 7.95 | 33.3 | 1.03 | 0.41 | 3.73 | 0.83 | 8.91 | 5.74 | nd | nd | nd | 343±22.9 |
| **Control sites** | | | | | | | | | | | | | | | | | |
| Gaoyao | GY | 17.2 | 0.47 | 2.58 | 5.35 | 32.6 | 14.0 | 5.08 | 7.25 | 2.69 | 0.94 | 0.83 | nd | nd | nd | nd | 89.1±15.4 |
| Guangzhou | GZ | 64.7 | 3.67 | 9.06 | 34.5 | 184 | 78.5 | 59.5 | 71.6 | 50.8 | 27.0 | 29.8 | 14.9 | 4.86 | 3.76 | 22.5 | 627±103 |
| Shunde | SD | 28.9 | 0.54 | 2.32 | 8.20 | 42.4 | 18.2 | 6.54 | 9.40 | 3.70 | 1.40 | 0.82 | 16.2 | nd | nd | nd | 138±43.7 |
| Pok Wai | PW-1 | 26.9 | 1.49 | 4.45 | 10.6 | 39.0 | 16.8 | 16.9 | 20.1 | 12.5 | 6.94 | 7.12 | 4.41 | 1.46 | 1.93 | 7.89 | 167±12.3 |
| | PW-2 | 37.6 | 1.89 | 4.74 | 10.7 | 49.6 | 21.1 | 18.1 | 24.4 | 21.0 | 9.99 | 10.7 | 9.64 | 0.91 | 1.55 | 9.82 | 219±65.9 |
| Mei Po | MP-1 | 45.3 | 1.79 | 5.49 | 27.7 | 141 | 60.0 | 35.8 | 41.7 | 28.9 | 11.7 | 14.2 | 7.47 | 1.58 | 1.29 | 10.3 | 421±149 |
| | MP-2 | 38.7 | 2.38 | 5.17 | 13.4 | 122 | 51.9 | 21.5 | 28.4 | 21.0 | 9.15 | 11.0 | 7.41 | 2.34 | 2.68 | 15.0 | 332±55.6 |
| | MP-3 | 11.5 | 54.0 | 4.11 | 13.9 | 138 | 35.4 | 102 | 212 | 245 | 174 | 254 | 107 | 15.2 | 7.00 | 25.2 | 350±19.7 |

Note : nd= under detection limit (0.03 ng/g); Control= commercial fish feed pellets, FW A and FW B = Food waste fish feed pellets A and B, respectively, naphthalene (Nap), acenaphthylene (Acel), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (An), fluoranthene (FlA), pyrene (Py), benz(a)anthracene (BaA), chrysene (Chry), benzo(a)- pyrene (BaP), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), indeno(1,2,3-cd)pyrene (IP), dibenz(a,h)anthracene (DahA), and benzo- (g,h,i)perylene (BghiP).The peaks of benzo(b)fluoranthene (BbF) and benzo- (k)fluoranthene (BkF) were extremely close and difficult to distinguish; therefore, these two compounds were combined as one, namely, B(bk)F.

concentration in the pond sediment. However, ΣPAHs in sediments were similar to those of mangrove and mudflat located in Mai Po Marshes (260-373 ng/g dw) (Liang et al., 2007). Previous studies (Zheng et al., 2002; Liang et al., 2007) reported that local aerial deposition served as an important input of PAHs at Mai Po Mashes.

Concentrations of ΣPAHs in sediment of the present experimental ponds were similar to those of abandoned fish ponds in Hong Kong (Po Wai and Mei Po), but were higher than the farmed ponds in Gaoyao and Shunde ($p<0.05$). The Guangzhou site had the highest ΣPAHs in pond sediments (627±103 ng/g dw) ($p<0.05$), possibly due to the fact that the abandoned fish ponds in Guangzhou have never been drained, and the sediment has not been removed in the last fifteen years (Table 3.1). Furthermore, the steel works located near the ponds burnt a large amount of coal, which may generate both Hg and PAHs. Additional PAHs would be therefore accumulated in sediment through atmospheric deposition. The fish ponds in Gaoyao and Shunde were new ponds with sediment removed recently (Table 3.1), they also located far away from the urban center and manufacturing industry area. These two ponds had lower PAHs in water and sediments. Although high molecular weight PAH congeners are easily accumulated in sediment due to their hydrophobic character, some high molecular weight PAH congeners (such as Bap, IP, DahA, BghiP) were not detected in the experimental ponds in Sha Tau Kok and farmed ponds in Gaoyao and Shunde. It might indicate that InP, DahA, and BghiP, the congeners mainly derived from fuel composition were relatively lower in the study area.

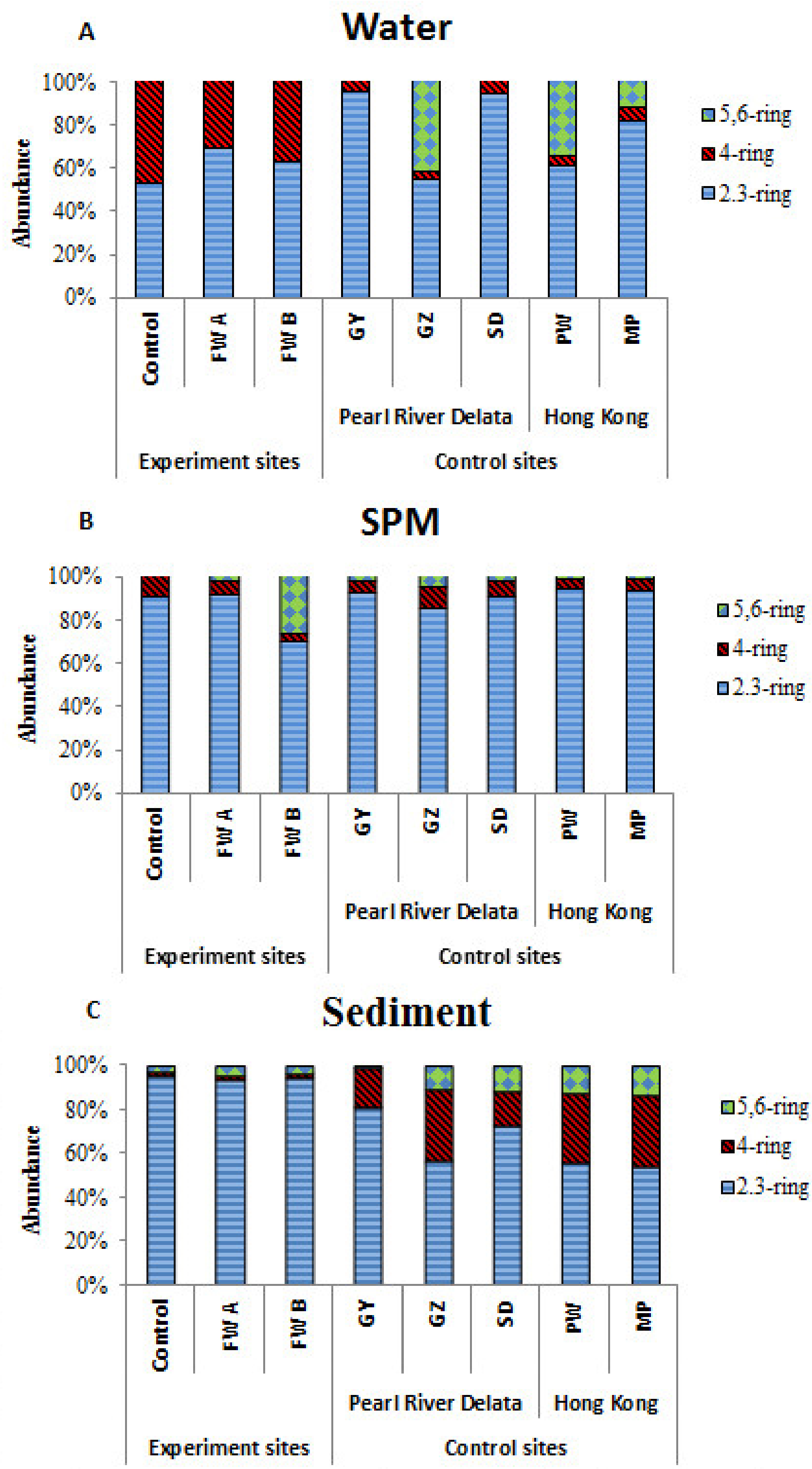



**Figure 5.1** Composition of PAHs in water, suspended particulate matter (SPM) and sediments of sampling sites in Hong Kong and Pearl River Delta (PRD).

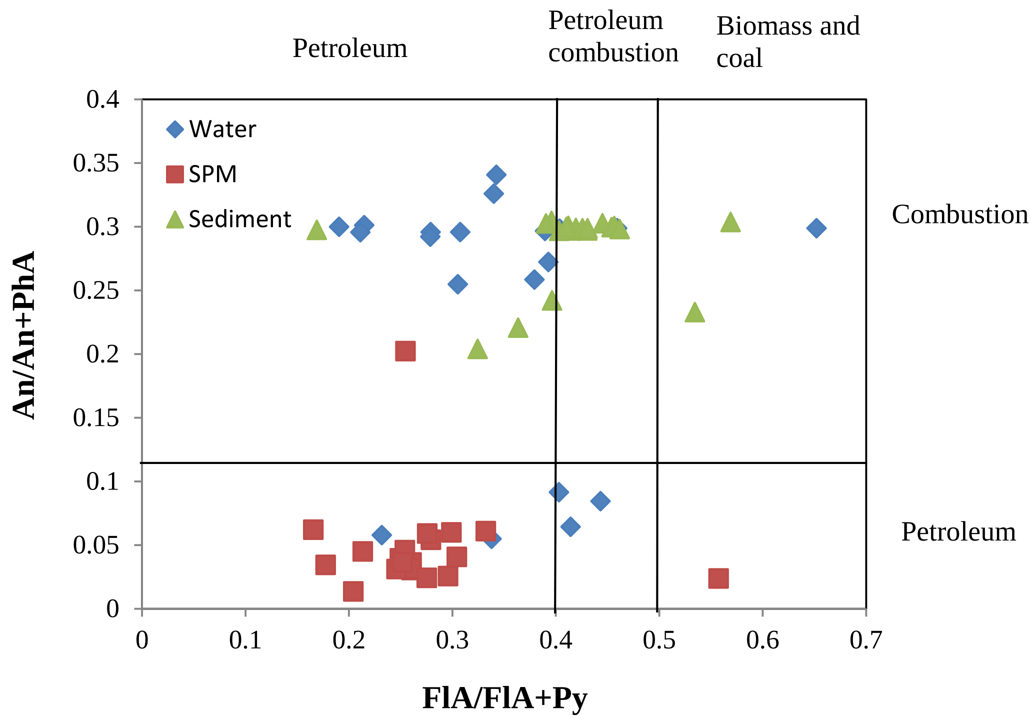



**Figure 5.2** Source analysis of PAHs in water, suspended particulate matter (SPM) and sediments of sampling sites in Hong Kong and Pearl River Delta (PRD): Isomer ratio plots of An/ An + PhA vs. FlA/FlA + Py.

Figure 5.1 shows the compositions of PAHs in water, suspended particulate matter (SPM) and sediment of sampling sites in Hong Kong and PRD. It was obvious that 2, 3-ring PAHs (Nap, Acel, Ace, Fl, PhA and An) were the most abundant congeners in the environmental samples, especially in SPM (mean 88.4%, range 69.6-94.5%) and pond sediment of the experimental sites (mean 94.8%, 92.9-95.2%). 4-ring PAHs (FlA, Py, BaA and Chry) also showed higher abundances in water of the experiment sites (mean 38.4%, range 30.8-47.2%) and sediment in control sites (mean 25.9%, range 17.9-31.7%), while 5,6-ring (BbkF, BaP, IP, DahA and BghiP) in environmental samples of control sites were higher than that of experimental sites.

The ratios of selected PAHs can be used to evaluate the source of PAHs from petrogenic and pyrogenic origin (Yunker et al., 2002). The ratios of An to An + PhA and of FlA to FlA + Py are frequently used to identify the sources of PAHs (Luo et al., 2006). An FlA / FlA + Py ratio between 0.4 and 0.5 is attributed to the combustion of liquid fossil fuels, whereas a ratio < 0.4 corresponds to petroleum contamination, and a ratio > 0.5 corresponds to combustion of grass, wood, or coal (Yunker et al., 2002). Similarly, an An/An + PhA ratio > 0.1 represents combustion processes, whereas lower values represent petroleum contamination (Wang et al., 2007). Figure 5.2 indicates that the dominant source of PAHs in SPM and water was petrogenic inputs, but that petroleum combustion was also a contributing factor, whereas fish pond sediments, was likely derived from petroleum combustion sources (FlA/(FlA+Py) between 0.4 and 0.5, An + PhA > 0.1). This is in line with the results of former studies in PRD showing combustion sources being the major source of PAHs detected in fish ponds, riverine and estuarine sediments (Kong et al., 2005; Luo et al., 2006; Luo et al., 2008).

### 5.3.3 OCPs in Environmental Samples

The main OCPs components detected in the water, SPM and sediment of sampling sites in Hong Kong and PRD included DDT and its metabolites, as well as HCHs. Because some OCPs such as CHLs, DRINs, HCB and Mirex were not detected or with very low concentrations in the environmental samples, only concentrations of DDTs and HCHs were reported herein.

According to tables 5.6 and 5.7, there were no significant differences ($p>0.05$) in concentrations of ΣHCHs in SPM and ΣDDTs in water of fish ponds collected from the experimental ponds between 1st half year and 2nd half year. On the contrary, ΣHCHs in water and ΣDDTs in SPM of these fish ponds were increased during the experimental period. There were no significant differences ($p>0.05$) in ΣOCPs in water and SPM, among the 3 ponds. In general, ΣDDTs and ΣHCHs in pond water and SPM of the experimental ponds were similar or lower than those of other fish ponds scattered around the PRD. The concentrations of ΣDDTs and ΣHCHs in the experimental pond water (ΣHCHs 11.7–147 pg/l, ΣDDTs 312-592 pg/l) and SPM (ΣHCHs 33.5–59.9 pg/l, ΣDDTs 7.24-199 pg/l ) obtained in this study were lower than that in Pearl River Estuary (Yu et al., 2008).

There were no significant ($p>0.05$) differences in ΣDDTs and ΣHCHs in sediment among three ponds during the experimental period. The ΣDDTs and ΣHCHs concentrations in sediment in the 2nd half year were higher than that in the 1st half year

Table 5.6 Concentrations of ΣHCHs and ΣDDTs in pond water (pg/l ±SD) of sampling sites in Hong Kong and Pearl River Delta (PRD)

| Area | Site | $\alpha$-HCH | $\beta$-HCH | $\gamma$-HCH | $\delta$-HCH | **ΣHCHs** | *o, p'*-DDE | *p, p'*-DDE | *o, p'*-DDD | *o, p'*-DDT & *p, p'*-DDD | *p, p'*-DDT | **ΣDDTs** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment sites** | | | | | | | | | | | | |
| 1st half year | Control | nd | 14.2 | nd | 3.90 | 18.1±6.41 | 0.98 | 1.05 | 192 | 43.2 | 74.7 | 312±24.0 |
| | FW A | nd | nd | nd | 11.7 | 11.7±6.75 | 1.16 | 1.25 | 195 | 53.1 | 78.5 | 329±20.6 |
| | FW B | nd | 7.77 | nd | 8.62 | 16.6±3.47 | 1.08 | 1.20 | 218 | 46.3 | 115 | 381±40.2 |
| 2nd half year | Control | nd | 44.7 | 36.0 | 45.6 | 126±55.8 | 4.91 | 3.44 | 248 | 293 | 42.6 | 592±48.3 |
| | FW A | nd | 36.1 | 44.0 | 13.7 | 93.7±48.8 | 2.72 | 2.83 | 245 | 42.6 | 6.82 | 300±169 |
| | FW B | nd | 54.2 | 58.4 | 34.7 | 147±16.9 | 3.23 | 3.47 | 354 | 154 | 24.5 | 540±49.0 |
| **Control sites** | | | | | | | | | | | | |
| Guangzhou | GZ | 30.3 | 91.3 | 37.2 | 87.4 | 246±85.1 | 2.70 | 2.65 | 318 | 34.2 | 147 | 505±87.7 |
| Shunde | SD | 15.3 | 58.0 | 12.1 | 68.6 | 154±46.3 | 4.53 | 4.46 | 314 | 42.9 | 149 | 515±218 |
| Gaoyao | GY | 13.1 | 64.7 | 45.4 | 71.1 | 193±78.5 | 4.36 | 5.27 | 500 | 64.8 | 195 | 769±325 |
| Mei Po | MP-1 | nd | nd | nd | 25.2 | 25.2±19.9 | 1.93 | 2.12 | 540 | 40.2 | 130 | 714±334 |
| | MP-2 | nd | 1.79 | nd | 26.7 | 28.5±14.3 | 1.36 | 2.11 | 531 | 47.8 | 124 | 706±164 |
| Pok Wai | PW-1 | nd | 14.4 | nd | nd | 14.5±10.2 | 2.28 | 2.25 | 771 | 144 | 445 | 1364±591 |
| | PW-2 | nd | 10.2 | nd | nd | 10.2±6.91 | 2.71 | 3.66 | 935 | 196 | 565 | 1702±143 |

Note: nd= under detection limit (0.05 ng/g); Control= commercial fish feed pellets, FW A and FW B = Food waste fish feed pellets A and B

Table 5.7 Concentrations of ΣHCHs and ΣDDTs in suspended particulate matter (SPM) (pg/l ±SD) of sampling sites in Hong Kong and Pearl River Delta (PRD)

| Area | Site | *α*-HCH | *β*-HCH | *γ*-HCH | *δ*-HCH | **ΣHCHs** | *o, p'*-DDE | *p, p'*-DDE | *o, p'*-DDD | *o, p'*-DDT & *p, p'*-DDD | *p, p'*-DDT | **ΣDDTs** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment sites** | | | | | | | | | | | | |
| 1st half year | Control | 9.40 | 12.1 | 20.9 | 18.1 | 60.5±22.1 | 0.39 | 2.01 | 4.54 | nd | 4.93 | 11.9±24.8 |
| | FW A | 4.35 | 5.72 | 7.11 | 16.3 | 33.5±18.6 | 0.26 | 1.94 | 2.71 | nd | 2.33 | 7.24±1.40 |
| | FW B | 5.90 | 0.24 | 14.2 | 21.3 | 41.6±13.7 | 0.64 | 2.06 | 10.5 | nd | 8.11 | 21.3±1.45 |
| 2nd half year | Control | 7.47 | 22.7 | 11.0 | 18.8 | 59.9±17.3 | 1.81 | 2.75 | 38.7 | nd | 126 | 169±23.2 |
| | FW A | 8.47 | 17.4 | 8.82 | 6.02 | 40.7±23.9 | 1.15 | 2.20 | 40.4 | nd | 146 | 189±43.8 |
| | FW B | 6.90 | 16.3 | 19.3 | 10.1 | 52.7±27.4 | 1.87 | 2.32 | 35.5 | nd | 159 | 199±30.7 |
| **Control sites** | | | | | | | | | | | | |
| Guangzhou | GZ | 12.1 | 57.5 | 20.0 | 66.6 | 156±24.2 | 2.35 | 3.14 | 80.2 | nd | 215 | 301±79.0 |
| Shunde | SD | 35.5 | 2.78 | 12.0 | 8.00 | 58.3±3.34 | 2.09 | 3.34 | 73.3 | nd | 231 | 310±22.0 |
| Gaoyao | GY | 26.8 | 5.76 | 31.2 | 6.37 | 70.1±8.82 | 0.63 | 2.27 | 16.8 | nd | 53.8 | 73.5±23.7 |
| Mei Po | MP-1 | 13.3 | 1.72 | 17.2 | 28.6 | 60.8±9.26 | 0.52 | 2.86 | 51.7 | nd | 17.3 | 72.3±12.0 |
| | MP-2 | 23.5 | 2.56 | 17.3 | 13.9 | 57.3±16.5 | 0.26 | 2.14 | 22.9 | nd | 5.20 | 30.6±12.9 |
| Pok Wai | PW-1 | 69.6 | 21.2 | 25.8 | nd | 116±50.6 | 1.40 | 3.29 | 40.0 | 76.4 | 122 | 243±18.5 |
| | PW-2 | 70.7 | 21.5 | 22.6 | nd | 115±47.4 | 0.98 | 3.55 | 43.2 | 25.0 | 133 | 205±43.2 |

Note: nd= under detection limit (0.05 ng/g); Control= commercial fish feed pellets, FW A and FW B = Food waste fish feed pellets A and B (*p>0.05*) (Table 5.8). The results indicated that pond sediment might accumulate more ΣDDTs and ΣHCHs, due to the use of more fish feeds to feed fish in the 2nd half year experimental period. The results suggested that the accumulation of OCPs in pond sediment could increase with time, which may threaten fish or wildlife health. However, the traditional practice of draining pond water after harvest, and removal of sediment regularly (used as fertilizer for cops growing on the dykes of fish ponds) (Wong et al., 2004) would reduce the concentrations of different pollutants contained in pond sediment.

The concentrations of ΣHCHs (mean 0.49 ng/g dw, range 0.40-0.55 ng/g dw) in sediment of the experimental ponds were similar to that of the abandoned fish ponds in Hong Kong and farmed ponds in PRD, except the pond in Guangzhou (ΣHCHs 1.38±0.87 ng/g dw, ΣDDTs 3.08±0.60 ng/g dw). In the present study, ΣHCHs ranged from N.D. to 2.25 ng/g dw (mean of 0.41 ng/g dw), which were significantly ($p<0.05$) lower than ΣDDTs (Table 5.8). The similar trend was reported on OCP contamination in sediments collected from the Pearl River and Pearl River Estuary (Mai et al., 2002; Yu et al., 2008). This phenomena may be due to the fact that HCHs had higher water solubility, vapor pressure, biodegradability, and lower particle affinity than DDTs (Yang et al., 2005). The ΣHCHs levels in pond sediments of the present study were lower than previous studies: fish pond sediments (mean 1.84 ng/ g dw, range ng-8.74 ng/ g dw) (Wang, 2011) and coastal sediments (1.2 to 17 ng/g dw) (Mai et al., 2002) collected from the PRD. The results indicated that HCHs are still been detected in environmental sample, but the concentrations in fish pond sediments in PRD have declined gradually.

Table 5.8 Concentrations of ΣHCHs and ΣDDTs in sediment (ng/g dw ±SD) of sampling sites in Hong Kong and Pearl River Delta (PRD)

| Area | Site | *α*-HCH | *β*-HCH | *γ*-HCH | *δ*-HCH | **ΣHCHs** | *o, p'*-DDE | *p, p'*-DDE | *o, p'*-DDD | *o, p'*-DDT & *p, p'*-DDD | *p, p'*-DDT | **ΣDDTs** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment sites** | | | | | | | | | | | | |
| 1st half year | Control | 0.03 | 0.09 | 0.01 | 0.20 | 0.30±0.10 | 1.08 | 0.09 | 0.30 | 0.00 | 0.28 | 1.75±0.26 |
| | FW A | 0.00 | 0.03 | 0.00 | 0.09 | 0.12±0.08 | 0.79 | 0.09 | 0.14 | 0.00 | 0.41 | 1.43±0.86 |
| | FW B | 0.00 | 0.02 | 0.00 | 0.14 | 0.16±0.06 | 0.81 | 0.09 | 0.23 | 0.00 | 0.39 | 1.52±0.34 |
| 2nd half year | Control | 0.05 | 0.25 | 0.12 | 0.10 | 0.52±0.23 | 0.82 | 0.10 | 0.59 | 0.00 | 0.48 | 2.01±1.11 |
| | FW A | 0.03 | 0.12 | 0.04 | 0.36 | 0.55±0.17 | 1.04 | 0.19 | 0.26 | 0.00 | 0.68 | 2.17±0.28 |
| | FW B | 0.01 | 0.18 | 0.05 | 0.16 | 0.40±0.32 | 1.04 | 0.11 | 0.35 | 0.00 | 0.31 | 1.82±0.63 |
| **Control sites** | | | | | | | | | | | | |
| Guangzhou | GZ | 0.20 | 0.46 | 0.30 | 0.42 | 1.38±0.87 | 1.63 | 0.21 | 0.22 | 0.14 | 0.88 | 3.08±0.60 |
| Shunde | SD | 0.00 | 0.25 | 0.00 | 0.08 | 0.33±0.14 | 1.44 | 0.12 | 0.16 | 0.11 | 0.40 | 2.23±0.79 |
| Gaoyao | GY | 0.00 | 0.41 | 0.00 | 0.00 | 0.41±0.11 | 1.27 | 0.11 | 0.14 | 0.09 | 0.54 | 2.16±0.15 |
| Mei Po | MP-1 | 0.00 | 0.45 | 0.00 | 0.00 | 0.45±0.29 | 1.38 | 0.11 | 0.13 | 0.00 | 0.41 | 2.04±0.49 |
| | MP-2 | 0.00 | 0.23 | 0.00 | 0.00 | 0.23±0.09 | 1.42 | 0.10 | 0.07 | 0.00 | 0.14 | 1.73±0.32 |
| Pok Wai | PW-1 | 0.00 | 0.12 | 0.00 | 0.16 | 0.28±0.06 | 1.52 | 0.15 | 0.36 | 0.00 | 1.15 | 3.18±0.28 |
| | PW-2 | 0.00 | 0.25 | 0.00 | 0.00 | 0.25±0.12 | 1.40 | 0.15 | 0.39 | 0.00 | 1.26 | 3.19±0.11 |

Note: nd= under detection limit (0.05 ng/g); Control= commercial fish feed pellets, FW A and FW B = Food waste fish feed pellets A and B.

Concentrations of ΣDDTs in sediment of the experiment ponds ranged from 0.86 to 2.45 ng/g dw, with a mean value of 2.02 ng/g dw, similar or lower that of the abandoned fish ponds in Hong Kong and farmed ponds in PRD. The DDTs concentration obtained in the present study was also similar to those in PRD fish pond sediments reported in 2010 (0.82 to 6.07 ng/g dw) (Wang, 2011), but was lower than that in 2001 (0.41 to 18.0 ng/g dw) (Zhou and Wong, 2004) and 2003 (2.87 to 27.6 ng/g dw) (Kong et al., 2005). These reflected that DDT contamination in fish pond sediments in PRD has declined gradually during the past decade (Guo et al., 2009a). This phenomenon may be due to usage of DDT and technical HCH in mainland China has been prohibited since 1983, with their residues gradually degraded in the environment (Yang et al., 2005). In addition, the traditional practice of draining pond water once every few years to maintain water quality and reduce the incidence of fish diseases, as well as the pond mud is also removed (and used as fertilizer) would reduce the concentrations of pollutants (Wong et al., 2004). The DDT levels were also much less than the sediments collected from the Pearl River (35.1-91.0 ng/g dw), Xijiang River (5.0-16.6 ng/g dw) and Lingding Bay (2.6 to 115.6 ng/g dw) (Mai et al., 2002). Generally, the DDT concentrations in sediments obtained in the present study were comparable or relatively lower amongst different sediments (river and marine) in PRD.

## 5.4 Conclusions

The analyses of PAHs and OCPs in various food waste ingredients showed that fruits, vegetables and bone meal were the major sources of PAHs and OCPs, while meat products also contributed a significant source of PAHs and DDTs for manufacturing fish pellets (FW A and FW B). No significant differences ($p>0.05$) in PAHs and OCPs were observed in water, SPM and sediment among the three ponds. The results indicated that pond sediment might accumulate more PAHs and OCPs, due to the use of more fish feeds to culture fish in the $2^{nd}$ half year, experimental period. However, the traditional practice of draining pond water and removing pond sediment regularly would avoid accumulation of PAHs and OCPs in sediments. Therefore, this traditional practice can improve the environmental quality of fish ponds, and also provide a better habitat for birds and other wildlife. The present results showed that PAHs and OCPs in pond water and sediment samples of the 3 experimental fish ponds were comparable or relatively low amongst different types of sediments in the PRD region, suggesting that the experimental site was relatively free of PAHs and OCPs, and would be suitable for farming fish.

# CHAPTER 6 HEALTH RISK ASSESSMENTS OF PAHs AND OCPs IN FRESHWATER FISH, USING FOOD WASTE AS FISH FEEDS

## 6.1 Introduction

The Pearl River Delta (PRD) was one of the most developed regions in China. Rapid industrialization and urbanization resulted in excessive releases of pollutants into the environment, and the environmental quality has been deteriorated during in the last three decades. Aquatic products are a major dietary source of protein for most of Hong Kong residents. Most of the aquatic products available in Hong Kong markets mainly come from the surrounding coastal and fresh water fish farms in PRD. Our previous studies demonstrated rather high concentrations of OCPs (Kong et al., 2005) and PAHs (Cheung et al., 2007) contained in fish collected from the fish ponds located in PRD and available in Hong Kong markets. The concentrations of OCPs and PAHs in human body (e.g. milk and plasma) were significant correlated with the frequency of fish consumption in both Hong Kong and Guangzhou populations (Wong et al., 2002a; Wang et al., 2010a; 2013a).

It is commonly regarded that the use of bioavailable pollutant concentrations to evaluate health risks is a more accurate method, because only the bioavailable portion of the contaminants will ultimately reach our bloodstream and exert adverse effects on our body (Brown et al., 1999). However, this method usually brings along ethical concerns due to the involvement of animal experiments. Therefore, using *in vitro* digestion model for conducting risk assessments to assess bioaccessible fractions of pollutants would be a suitable alternative in portraying the reality (Oomen et al., 2002). This has been used to study the bioaccessibility of pollutants (trace elements, organochlorine pesticides (OCPs), and polychlorinated biphenyl (PCBs)) (Xing et al., 2008; Tao et al., 2009; Wang et al., 2011).

The ubiquitous presence and lipophilic properties of OCPs and PAHs facilitate their accumulation in biota and subsequent biomagnification in the food chain, leading to increased concentrations with increasing trophic levels. Although pollutants in water could be bioaccumulated in fish pond food webs by way of direct uptake by plankton and forage feeding, the pollutants in sediments can be taken up through either benthic or pelagic pathways (Nfon et al., 2008; Chen et al., 2009). It is necessary to compare the concentrations of OCPs and PAHs in different fish species among different water bodies to develop models of pollutant transport, bioaccumulation, and fate. It is also necessary to quantify the bioaccumulation factor (BAF), which is defined as the field-observed ratio of the concentration of a given chemical in biota to the concentration in corresponding water expressed in equivalent units (Tomy et al., 2004). $\delta^{15}$N analysis has been used as a tool to investigate the relationship between TLs and contaminant concentration (such as PCBs and DDTs) in biota tissues (Fisk et al., 2001). It can determine trophic structures and thus facilitate interpretation of contaminant transport in aquatic communities. Most studies related to OCPs and PAHs biomagnification focused on marine and lake ecosystems (Fisk et al., 2001; Hop et al., 2002; Wan et al., 2007; Nfon et al., 2008), which have wide open spaces, complex food webs, and an abundance of different food sources available for fish species. However, information on biaccumulaition and biomagnification of OCPs and PAHs in freshwater fish ponds is scarce.

It is commonly known that conservation of fish pond habitats in northwestern New Territories is important as the fish ponds provide important ecological function. Over 380 bird species have been recorded at Mai Po, the Deep Bay mudflats and surrounding areas; about 89% of the total recorded in Hong Kong (WWF, 2006). Both migratory and resident birds utilize fish ponds as shelter and feeding sites (Young and Chan, 1997; Young, 1998). More than 40,000 of the total occurrence for 45 bird species were recorded during the 9-14 days of draining pond water in 2001 of five fish ponds located within and around the Mai Po Inner Deep Bay Ramsar Site (Lau et al., 2003). Because of the decline of pond culture activities in Hong Kong, the ecological value of fish ponds has gradually disappeared and the inactively managed fish ponds may even become a sink for various pollutants (Wong et al., 2004).

It is hypothesized that integrated pond fish farming would improve the environmental quality of fish ponds using food wastes and can also provide a better fish pond habitat for birds and other wildlife. The major objectives of the present study were to (1) analyze bioaccumulations and biomagnifications of OCPs and PAHs in food chains; (2) use *in vitro* digestion method for analyzing the bioaccessibilities of these pollutants contained in fish muscle; and (3) assess potential health risks based on the pollutant concentrations detected in fish muscle.

## 6.2 Materials and Methods

### 6.2.1 Experiment Design

The experimental design followed Section 3.2.1 of Chapter 3

### 6.2.2 Sampling

Sampling of water, sediment and fish followed Section 4.2.2 of Chapter 4.

### 6.2.3 Chemical Analyses

Fish samples (2-3 g) were extracted with a mixture of acetone, dichloromethane (DCM), n-hexane (v/v/v 1:1:1, 120 ml) in a Soxhlet apparatus for 18 h (USEPA, 1996b). The extracts were concentrated to 2 ml. A series of chromatographic columns were applied for sample cleanup, using an activated copper/sodium sulfate anhydrous/florisil column and eluted with 100 ml n-hexane (USEPA, 1996c). The eluant was concentrated to 1 ml. The instrumental analyses and QA/QC for PAHs and OCPs quantification were the same as described in Sections 5.2.3 and 5.2.4 of Chapter 5.

### 6.2.4 Digestible Fraction of PAHs and OCPs

The *in vitro* digestion test was performed according to the methods described by Moreda-Pineiro et al. (2011) and our previous study (Wang et al., 2010a), with slight modifications. The entire digestion process was performed in capped Teflon centrifuge tubes (50 ml) in the dark to simulate the anaerobic condition of the stomach. Briefly, 3 g of freeze-dried samples were first added into 30 ml of synthetic gastric juice (2.0 g/l pepsin in 0.15 M NaCl, acidified with HCl to pH 1.8) and shaken at 100 rpm for 2 h at 37 ℃. Afterward, the mixture was centrifuged (15 min, 37 ℃, 1500 rpm) and the supernatant was filtered through a 0.45 mm glass fiber filter. Artificial intestinal juice (30 ml, 2.0 g/l pancreatin, 2.0 g/ l amylase and 5 g/l bile salts, in 0.15 M NaCl, pH 6.8) was added. Then, the mixture was resuspended and shaken at 30 rpm for 6 h at 37 ℃. Finally, the tubes were centrifuged at 1500 rpm at 37 ℃ for 15 min to separate supernatant, and solids and the supernatant was filtered through a 0.45 mm glass fiber filter. The filtrate (gastric and intestinal) was both extracted with 40 ml n-hexane/acetone (3:1, v/v) for 10 min in a 250 ml separatory funnel. Extraction with 40 mL of n-hexane was carried out two more times. All the extracts were combined into one mixture extract, which was dried with 5 g of anhydrous sodium sulfate. The extract was reduced to 2 ml using a rotary evaporator and purified by Florisil cleanup method (USEPA, 1996c). The solution was then concentrated to 200 μl for phthalate esters analyses. Deuterated PAHs (acenaphthene-$d^{10}$, phenanthrene-$d^{10}$, chrysene-$d^{12}$, and perylene-$d^{12}$) were then added as internal standards and OCP internal standard (2, 4, 5, 6-Tetrachloro-mxylene, TCmX) for quantification.

### 6.2.5 Calculation

The bioaccessbility (%BA) of pollutant was calculated as the ratio of the amount of pollutant in the liquid phase (stomach phase+intestinal phase) to total pollutant (Oomen et al., 2002).

$$\% \text{ BA} = (\text{BA extracted pollutant} / \text{total pollutant}) \times 100\ \% \qquad (6.1)$$

US EPA Standard Equations for evaluating non-cancer exerted on humans via fish consumption were adopted in this study (USEPA, 1989b, 2000a). For non-carcinogenic effects, the estimated daily intake was compared with the recommended reference doses (RfD) ($6.0\times10^{-2}$ mg/kg-day for Nap, $6.0\times10^{-2}$ mg/kg-day for Ace, $4.0\times10^{-2}$ mg/kg-day for Fl, $3.0\times10^{-1}$ mg/kg-day for An, $4.0\times10^{-2}$ mg/kg-day for FlA, $3.0\times10^{-2}$ mg/kg-day for Py,

$5.0\times10^{-4}$ mg/kg-day for DDT, $3.0\times10^{-4}$ mg/kg-day for $\gamma$-HCHs) (USEPA, 2012a) as stated in Eq. (6.2) and (6.3):

$$EDI = C_{fish} \times DR / BW \qquad (6.2)$$

where $C_{fish}$ is measured concentration in a given species of fish, DR daily consumption rate (g/ d) and BW body weight (kg). In order to suit local conditions, BW of 60 kg was used for adults (Cheung et al., 2007) and 21.2 kg for preschool children (Leung et al., 2000), and DR was 142.2 g/d for adults and 50 g/d for children (Leung et al., 2000; USEPA, 2000a), respectively.

$$\text{Hazard Ratio (HR)} = EDI / RfD \qquad (6.3)$$

The HR $\leq$ 1, it indicates no adverse health effects, whereas HR > 1 indicates that there is potential risk to human health (USEPA, 1989b).

The potency equivalent concentration (PEC) of carcinogenic PAHs was calculated for each sample for comparison with the screening value of BaP according to the guideline (USEPA, 1993). The toxic equivalency factor (TEF) of BaP, DahA, IP, BbK, BfK, BaA and Chry was used in accordance with USEPA (Nisbet and LaGoy, 1992). The PEC of each fish sample was calculated using Equation 6.4 (USEPA, 2000a): where TEF is toxic equivalency factor compared with BaP for carcinogenic PAH congeners and C is the concentration (ng/g, ww).

$$PEC = \Sigma\, (TEF \times C) \qquad (6.4)$$

To derive advisory consumption recommendations for multiple carcinogens in fish, the equation developed by USEPA (2000b) was calculated according to the equation, based on the cancer slop factor for each compound and calculated to prevent 1 excess cancer in 1,000,000 over a life exposure:

$$CR_{max}= RL \times BW / \Sigma C_d \times OSF_d \quad (6.5)$$

where $CR_{max}$ is the maximum allowable fish consumption rate (g/d ), $C_d$ is the measured concentration of contaminant d in a given species of fish (ng/g) and the oral slop factor ($OSF_d$) for contaminant (mg/kg-d). RL is maximum acceptable risk level (diemsionless), which is defined as a person weighing 60kg who consumed 93g of fish per day (USEPA, 2000a) containing the same concentration of contaminant, for 70 years, the increased risk would be at most one additional cancer death per 1,000,000 persons (USEPA, 1989a).

### 6.2.6. Bioaccumulation and Biomagnification

The calculation of bioaccumulation and biomagnification followed Sections 4.2.6, 4.2.7 and 4.2.8 of Chapter 4

### 6.2.7 Statistical Analyses

All of the statistical tests were performed using SPSS 19.0 for Windows. Normality was confirmed by the Kolmogorov-Smirnov test. Data of PAHs and OCPs concentrations and bioaccessibility in gastric and intestinal condition were analyzed using two independent t-test, Wilconxon rank sum test, one-way NOVA and Kruskal-Wallis test.

## 6.3 Results and Discussion

### 6.3.1 OCPs and PAHs Concentrations

According to Table 6.1, the values of length, weight, feed conversion ratio, specific growth rate, and protein efficiency ratio in grass carp in Sha Tau Kok fish ponds, fed with

FW A were significantly better than those fed with FW B and control diet ($P<0.05$). Cereal food waste was used as the major protein sources of FW A (53%), and meat waste was used to replace parts of cereals (25%) in FW B, and also used as major ingredients (28%) in FW B which were similar to the control feed which contained mainly of fish meal, wheat middling, flour, bean pulp, and rapeseed meal. Cereals could be easily digested by grass carp (herbivores), which can explain that fish fed with FW A was the best among the three treatments. These results indicated that the fish fed with food waste feeds achieved better or similar growth performance and feed conversion ratio, and therefore food wastes can serve as an alternative source of protein for culturing grass carp.

Figure 6.1 shows that, during the 1$^{st}$ half year to 2$^{nd}$ half year of the experiment period, ΣPAHs concentrations in grass carp, bighead carp and mud carp reared in Sha Tau Kok fish ponds increased ($p<0.05$), accompanied with significant increases of 4-ring and 5,6-ring PAHs ratio in ΣPAHs ($p<0.05$). However, 2, 3-ring PAHs were still most abundant in the fish collected in the 2$^{nd}$ half year of the experiment (except bighead carp fed with control diet). In Sha Tau Kok, ΣPAHs detected in grass carp (23.4±2.26 ng/g ww) and bighead carp (23.5±0.54 ng/g ww) fed with control diet were higher than that fed with food waste fish feed ($p<0.05$), but no significant difference ($p>0.05$) was obtained for ΣPAHs concentrations in mud carp among the three experiment fish ponds (Table 6.2). There was also no significant difference ($p>0.05$) on ΣPAHs in grass carp and bighead carp, between treatments with food waste A and food waste B. When compared with other fish ponds (control sites), it was observed that ΣPAHs in the fish collected from abandoned fish ponds in Hong Kong and farmed ponds in PRD were similar or higher

**Table 6.1** Growth performance and nutrient utilisation in freshwater fish (fed with food waste feed pellets and control feed pellets) from Sha Tau Kok

| | Grass carp (*Ctenopharyngodon idellus*) | | | Bighead carp (*Aristichtys nobilis*) | | | Mud carp (*Cirrhina molitorella*) | | |
|---|---|---|---|---|---|---|---|---|---|
| Diet | Control | FW A | FW B | Control | FW A | FW B | Control | FW A | FW B |
| Length (cm) | 20.7±1.10$^{a}$ | 23.8±2.81$^{b}$ | 21.9±2.23$^{a}$ | 16.4±1.26$^{c}$ | 16.0±1.19$^{c}$ | 19.6±2.50$^{ad}$ | 13.3±1.26$^{e}$ | 16.0±0.33$^{c}$ | 13.0±4.24$^{e}$ |
| Weight (g) | 85.3±10.9$^{a}$ | 151±56.2$^{b}$ | 91.3±33.3$^{a}$ | 39.4±9.46$^{c}$ | 36.4±9.28$^{c}$ | 64.7±24.2$^{a}$ | 18.3±1.83$^{d}$ | 22.1±11.2$^{cd}$ | 17.4±5.91$^{d}$ |
| Weight gain (%)[1] | 46.6±18.8$^{a}$ | 159±26.5$^{b}$ | 64.0±38.1$^{a}$ | 60.6±39.4$^{a}$ | 49.3±38.8$^{a}$ | 164±99.0$^{b}$ | 251±35.3$^{bc}$ | 323±43.2$^{c}$ | 232±104$^{bc}$ |
| Feed conversion ratio[2] | 4.68±1.46$^{a}$ | 1.92±0.63$^{b}$ | 4.21±1.20$^{a}$ | | | | | | |
| Specific growth rate (%)[3] | 15.1±6.07$^{a}$ | 51.4±13.2$^{b}$ | 20.7±14.8$^{a}$ | | | | | | |
| Protein efficiency ratio (%)[4] | 8.04±3.24$^{a}$ | 27.5±6.71$^{b}$ | 11.0±6.01$^{a}$ | | | | | | |

Note: Control: commercial fish feed- Jinfeng®, 613; FW A: food waste A; FW B: food waste B.

[1]Weight gain (%) = [final weight (g) – initial weight (g)]/initial weight (g) ×100 (Bake et al., 2009)

[2]Feed conversion ratio = feed intake (g) / [Final biomass – Initial biomass (g)] (Bake et al., 2009)

[3]Specific growth rate (%) = [ln final weight (g) – ln initial weight (g)]/feeding period (day)] ×100 (Bake et al., 2009)

[4]Protein efficiency ratio (%) = wet body weight gain (g)/protein intake (g) ×100 (Kaushik et al., 2004)

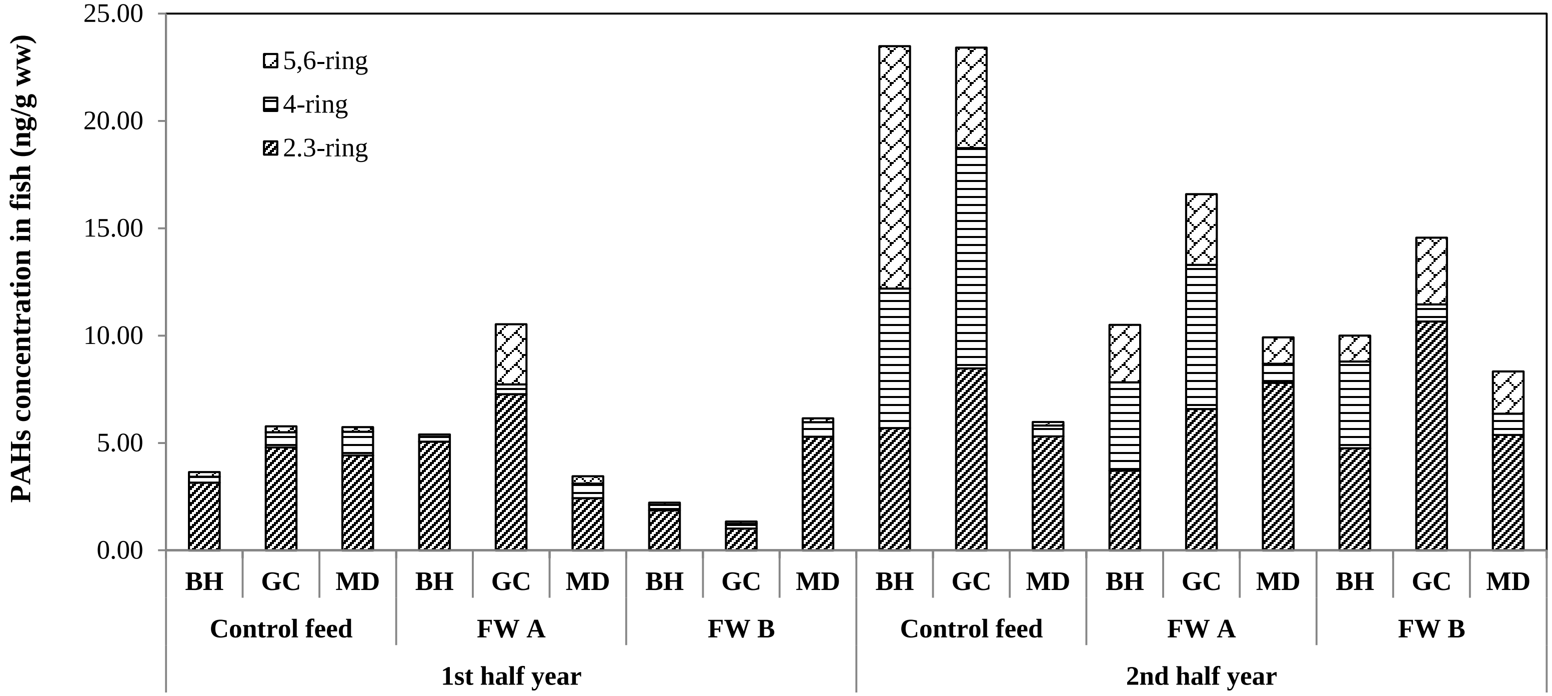



**Figure 6.1** PAHs concentrations (ng/g ww) in fish sampled during the experimental period (October 2011-December 2012).

Note: GC: grass carp; BH: bighead carp; MD: mud carp; FW A: food waste A, FW B: food waste B, Control feed: commercial fish feed- Jinfeng®, 613 formulated feed.

**Table 6.2** PAHs concentrations (ng/g ww) in fish (fed with food waste feed pellets and control fish feed pellets) from Sha Tau Kok and other fish ponds (control)

| | | n | Nap | Acel | Ace | Fl | PhA | An | FlA | Py | BaA | Chry | BbkF | BaP | IP | DahA | BghiP | **ΣPAHs** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sha Tau Kok (experiment pond)** | | | | | | | | | | | | | | | | | | |
| Control feed | BH | 11 | 1.15 | 0.18 | 0.36 | 0.76 | 2.87 | 0.37 | 0.13 | 1.53 | 4.09 | 0.75 | 5.13 | 6.16 | nd | nd | nd | $23.5\pm0.54^{a}$ |
| | GC | 19 | 1.18 | 0.08 | 0.34 | 0.74 | 5.39 | 0.74 | 0.88 | 0.92 | 6.27 | 2.19 | 3.18 | 1.50 | nd | nd | nd | $23.4\pm2.26^{a}$ |
| | MD | 6 | 1.32 | 0.03 | 0.52 | 0.45 | 2.09 | 0.89 | 0.14 | 0.08 | 0.14 | 0.15 | 0.16 | 0.01 | nd | nd | nd | $5.99\pm3.45^{b}$ |
| FW A | BH | 9 | 1.09 | 0.02 | 0.19 | 0.70 | 1.65 | 0.07 | 0.57 | 0.72 | 2.59 | 0.24 | 2.67 | nd | nd | nd | nd | $10.5\pm5.02^{bc}$ |
| | GC | 25 | 2.28 | 0.24 | 0.32 | 0.14 | 3.32 | 0.28 | 1.29 | 0.91 | 3.33 | 1.18 | 2.28 | 1.02 | nd | nd | nd | $16.6\pm2.21^{c}$ |
| | MD | 6 | 1.69 | 1.04 | 0.18 | 0.36 | 4.25 | 0.27 | 0.12 | 0.23 | 0.44 | 0.11 | 0.01 | 0.01 | 1.20 | nd | nd | $9.92\pm1.80^{bc}$ |
| FW B | BH | 10 | 1.37 | 0.11 | 1.12 | 0.43 | 0.55 | 1.18 | 0.43 | 0.40 | 0.70 | 2.51 | 1.21 | nd | nd | nd | nd | $10.0\pm4.10^{bc}$ |
| | GC | 21 | 3.32 | 0.13 | 0.14 | 1.38 | 4.64 | 1.04 | 0.57 | 0.11 | 0.07 | 0.06 | 2.97 | 0.13 | nd | nd | nd | $14.6\pm2.02^{c}$ |
| | MD | 6 | 1.16 | 0.11 | 0.43 | 0.30 | 2.36 | 1.00 | 0.17 | 0.19 | 0.54 | 0.10 | 0.25 | 0.28 | 1.44 | nd | nd | $8.33\pm1.82^{b}$ |
| **Other fish ponds (control)** | | | | | | | | | | | | | | | | | | |
| Shunde | GC | 5 | 1.43 | 1.82 | 0.51 | 2.24 | 6.11 | 2.58 | 0.90 | 0.84 | 0.80 | 0.29 | 0.20 | 0.38 | 0.12 | nd | 0.14 | $18.3\pm1.61^{c}$ |
| | BH | 3 | 2.03 | 0.33 | 0.35 | 1.14 | 6.83 | 2.88 | 0.85 | 1.00 | 2.11 | 0.38 | 0.33 | 0.40 | nd | 0.22 | 0.11 | $18.9\pm3.81^{ac}$ |
| Guangzhou | GC | 3 | 2.52 | 0.21 | 0.54 | 1.31 | 5.44 | 2.35 | 1.15 | 1.04 | 1.00 | 0.28 | 0.26 | 0.59 | nd | nd | nd | $16.7\pm7.07^{c}$ |
| Gaoyao | LB | 6 | 3.88 | 0.21 | 0.88 | 1.29 | 6.26 | 2.63 | 1.25 | 0.96 | 1.89 | 0.59 | 0.35 | 0.69 | nd | nd | nd | $20.9\pm2.27^{a}$ |
| Hong Kong | T | 8 | 0.91 | 0.08 | 0.45 | 0.27 | 1.19 | 0.62 | 0.19 | 0.32 | 0.73 | 0.20 | 0.08 | 0.18 | nd | nd | nd | $5.23\pm3.09^{b}$ |

Note: GC: grass carp; BH: bighead carp; MD: mud carp; LB: largemouth bass; T: tilapia; FW A: food waste A, FW B: food waste B, Control feed: commercial fish feed- Jinfeng®, 613 formulated feed; different superscripts (a, b, c) between feeding groups are significantly different ($p< 0.05$), nd= under detection limit (0.05 ng/g).

than that from the experimental ponds which fed with food waste feeds. The PAH concentrations obtained in the experimental in ponds fed with food waste feeds were lower than those collected from fish ponds in the PRD in 2003 (25.8-77.1 ng/g ww) (Kong et al., 2005) and Mai Po Mashes in 2004 (184-854 ng/g dw) (Liang et al., 2007), but similar to that collected in Hong Kong markets in 2010 (8.94-54.4 ng/g ww) (Wang et al., 2010a) and in 2004 (1.57-24.8 ng/g ww) (Cheung et al., 2007).

In the present study, HCHs in the fish collected from experimental ponds were under the delectation limit (0.10 ng/g). Figure 6.2 shows that, during the 1st half year to 2$^{nd}$ half year of the experiment period, ΣDDTs in grass carp, bighead carp and mud carp in Sha Tau Kok fish ponds significantly increased ($p<0.05$), while *p, p'*-DDD ratio in ΣDDTs were significant increased ($p<0.05$), as well as *p, p'*-DDD and *p, p'*-DDT which were major compounds contained in ΣDDTs in the fish. In Sha Tau Kok, ΣDDTs in bighead carp (1.55±0.64 ng/g ww) and mud carp (1.48±0.46 ng/g ww) fed with food waste A feed, and grass carp (1.42±0.03 ng/g ww) fed with food waste B feed were lower than the same species of fish in experimental fish ponds, respectively ($p<0.05$) (Table 6.3). When compared with other fish ponds, it was observed that ΣDDT in the fish collected from abandoned fish ponds in Hong Kong were similar to that from experimental ponds which fed with food waste feeds. However, ΣDDTs in the fish collected from farmed ponds were significantly higher ($p<0.05$) than that from experimental ponds which fed with food waste feeds. The DDT concentrations obtained in the experimental ponds fed with food waste feeds were lower than those collected from fish ponds in the PRD in 2003 (7.93 - 32.4 ng/g ww) (Kong et al., 2005), but similar to that collected from Hong Kong markets in 2010 (0.81-34.8 ng/g ww, mean 1.92 ng/g ww) (Wang et al., 2011).

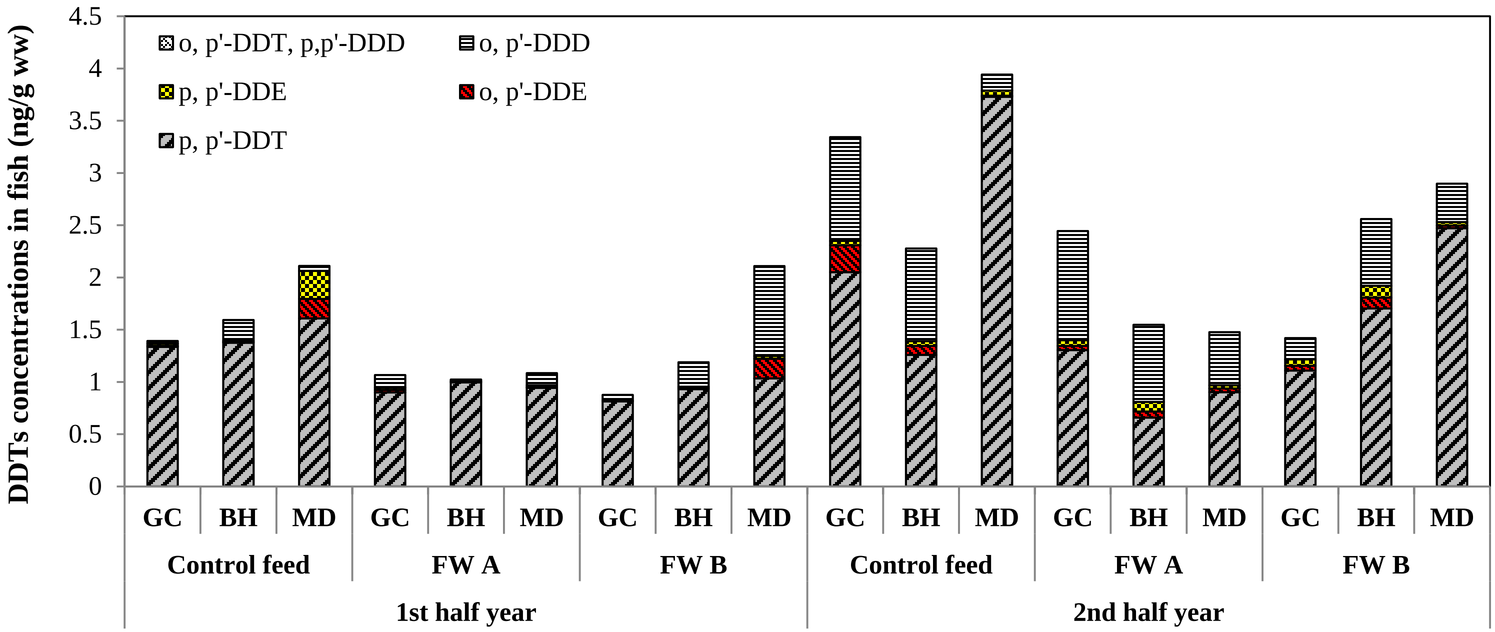



**Figure 6.2** DDTs concentrations (ng/g ww) in fish sampled during the experimental period (October 2011-December 2012).

Note: GC: grass carp; BH: bighead carp; MD: mud carp; FW A: food waste A, FW B: food waste B, Control feed: commercial fish feed- Jinfeng®, 613 formulated feed.

**Table 6.3** DDTs concentrations (ng/g ww) in fish (fed with food waste feed pellets and control fish feed pellets) from Sha Tau Kok and other fish ponds (control)

| | | *α*-HCH | *β*-HCH | *γ*-HCH | *δ*-HCH | **Σ HCHs** | *o,p'*-DDE | *p, p'*-DDE | *o, p'*-DDD | *o, p'*-DDT & *p,p'*-DDD | *p, p'*-DDT | **Σ DDTs** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Sha Tau Kok (experiment pond)** | | | | | | | | | | | | |
| Control feed | GC | nd | nd | nd | nd | 0.00 | 0.26 | 0.04 | 0.99 | nd | 2.05 | 3.34±1.98[ab] |
| | BH | nd | nd | nd | nd | 0.00 | 0.09 | 0.05 | 0.89 | nd | 1.26 | 2.28±0.11[a] |
| | MD | nd | nd | nd | nd | 0.00 | 0.01 | 0.05 | 0.15 | nd | 3.73 | 3.94±0.78[be] |
| FW A | GC | nd | nd | nd | nd | 0.00 | 0.04 | 0.05 | 1.05 | nd | 1.31 | 2.45±1.09[abd] |
| | BH | nd | nd | nd | nd | 0.00 | 0.06 | 0.09 | 0.74 | nd | 0.66 | 1.55±0.64[cd] |
| | MD | nd | nd | nd | nd | 0.00 | 0.03 | 0.03 | 0.51 | nd | 0.90 | 1.48±0.46[cd] |
| FW B | GC | nd | nd | nd | nd | 0.00 | 0.05 | 0.06 | 0.20 | nd | 1.11 | 1.42±0.03[cd] |
| | BH | nd | nd | nd | nd | 0.00 | 0.10 | 0.11 | 0.64 | nd | 1.70 | 2.56±0.21[a] |
| | MD | nd | nd | nd | nd | 0.00 | 0.02 | 0.03 | 0.37 | nd | 2.47 | 2.90±0.24[ab] |
| **Other fish ponds (control)** | | | | | | | | | | | | |
| Shunde | GC | 0.05 | 0.01 | 0.03 | 0.01 | 0.10±0.04[a] | 0.25 | 0.23 | 2.39 | nd | 1.60 | 4.47±0.79[e] |
| | BH | 0.21 | 0.01 | 0.01 | 0.06 | 0.30±0.20[ab] | 0.04 | 0.06 | 3.11 | nd | 2.48 | 5.70±0.81[ef] |
| Guangzhou | GC | 0.08 | 0.11 | 0.02 | 0.22 | 0.43±0.26[ab] | 0.06 | 0.10 | 2.47 | nd | 2.26 | 4.88±1.95[ef] |
| Gaoyao | LB | 0.07 | 0.01 | 0.02 | 0.09 | 0.18±0.02[b] | 1.04 | 0.32 | 2.14 | nd | 2.45 | 5.95±0.08[f] |
| Hong Kong | T | 0.04 | 0.13 | 0.05 | 0.05 | 0.27±0.20[ab] | 0.20 | 0.12 | 1.01 | nd | 0.74 | 2.07±0.42[ad] |

Note: GC: grass carp; BH: bighead carp; MD: mud carp; LB: largemouth bass; T: tilapia; FW A: food waste A, FW B: food waste B, Control feed: commercial fish feed- Jinfeng®, 613 formulated feed, nd: not detected; different superscripts (a, b, c) between feeding groups are significantly different ($p < 0.05$), nd= under detection limit (0.05 ng/g)

Concentrations of HCHs in the fish collected from abandoned ponds in Hong Kong and farmed ponds in PRD were similar to that collected from Hong Kong markets in 2010 (0.33-3.09 ng/g ww, mean 1.22 ng/g ww) (Wang et al., 2011).

In fish, bioconcentration from water via the gill skin, and ingestion of contaminated food are possible routes for PAHs and DDTs to accumulate in tissues and the route depends mainly on their feeding preference, general behaviour and trophic level (Baumard et al., 1998; Fisk et al., 2001). The present study indicated that fish fed with food waste feeds had a lower tendency to bioaccumulate PAHs and OCPs than fish fed with control feeds, and those collected from control sites (presumably fed by various commercial feeds). Previous studies in China and European counties demonstrated that fish meal derived from trash fish for producing fed pellets generally contained rather high concentrations of POPs such as PBDEs, PCBs and OCPs (Guo et al., 2009b; Suominen et al., 2011).

In this study, food wastes replaced part of the fish meal used in fish feeds. Although food waste feeds have higher or similar concentrations of PAHs and OCPs than the control diet, but due to the lower bioavailability of these pollutants, fish fed with food waste feeds had relatively lower PAHs and OCPs than those fed with the control diet. The control diet is feed Jinfeng®, 613 formulated feed, a common commercial fish feed used in aquaculture in PRD and Hong Kong. Rapid industrialization and urbanization in PRD resulted in excessive releases of pollutants into the river. The fish farmers are still practicing the traditional way of filling up fish ponds using river water (Ruddle and Zhung, 1988). On the contrary, the experimental ponds were located near the Mai Po Nature Reserve far away from the industrial and urban areas. It is expected that adoption of the practice traditional of draining pond water after harvest, and removal of sediment regularly would reduce the pollutant concentrations in pond sediment (Wong et al., 2004).

### 6.3.2 Bioaccessibilities and Health Risk Assessment

In the present study, digestible concentrations of HCHs in all fish samples were under detection limits. Figure 6.3 shows that the bioaccessibilities of total PAHs and DDTs based on the gastrointestinal model for the fish (fed with food waste feed pellets and commercial feed pellets) from Sha Tau Kok and other fish ponds (control sites). The digestible concentrations of PAHs ranged from 1.40 - 4.99 ng/g ww (mean 3.2 ng/g ww), accounted for 10.2 – 64.0% (mean 33.0%) for the raw concentration of PAHs. The digestible concentrations of DDTs ranged from 0.34 – 1.66 ng/g ww (mean 0.77 ng/g ww), account for 22.9 – 87.7 % (mean 53.0 %) accounted for the raw concentration of DDTs. Grass carp (13.1%), and bighead carp (10.2 %) from experimental ponds fed with commercial feed had the lowest bioaccessibility for PAHs, as well as that the lowest bioaccessibility for DDT (22.9 %). This may indicate that the total PAHs and DDTs intake via fish consumption detected by conventional procedures with organic solvent is considerably overestimated the actual human exposure to PAHs and DDTs via food intake.

Persistent organic pollutants (POPs) such as PAHs, OCPs and PCBs are normally associated with high lipid foods such as fish, and therefore these high lipid foods were a major source for human exposure to POPs (Carlson and Hites, 2005). In reality, lipid is difficult to digest, and therefore POPs would be difficult to be released from food matrix (Tao et al., 2009). It has been shown that lipids may decrease the intake of benzo(a)pyrene *in vitro* digest model (Weber and Lanno, 2001), and high lipid food may

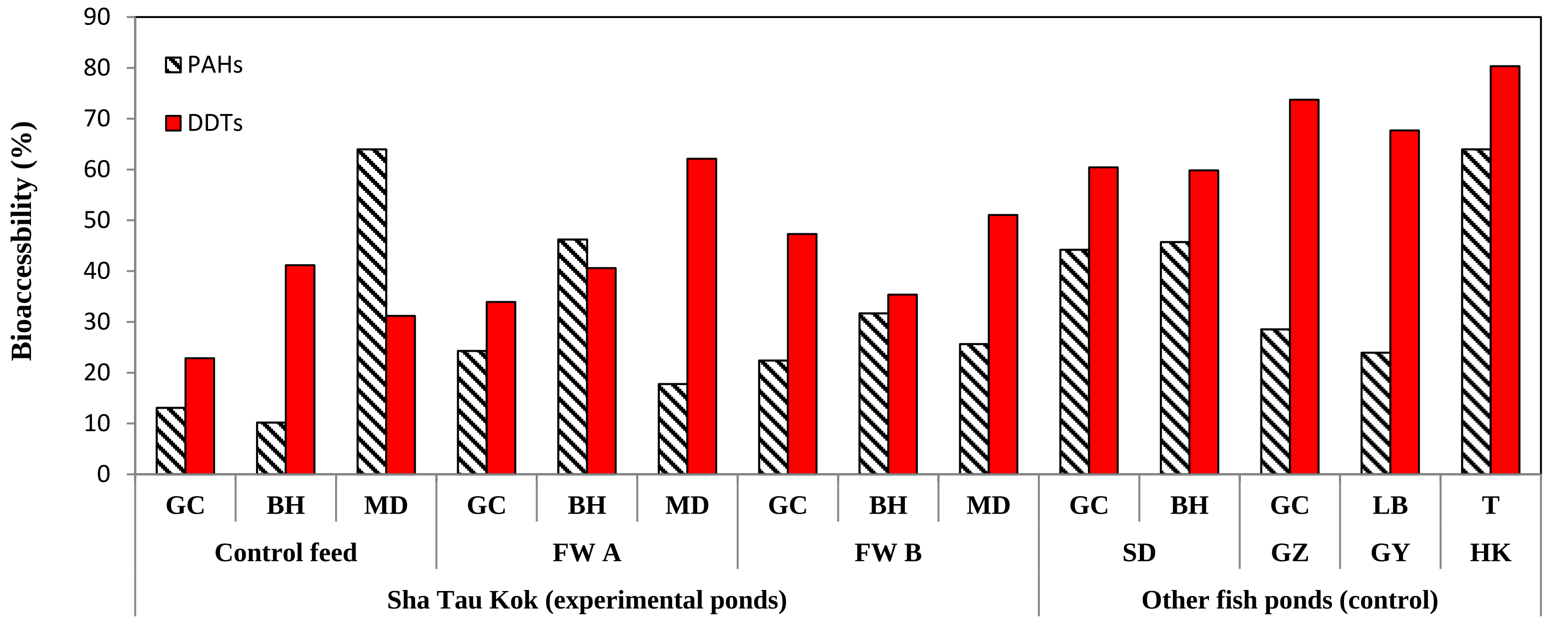



**Figure 6.3** Average bioaccessibilities of PAHs and DDTs in fish muscles collected from Sha Tau Kok experimental fish ponds and other fish ponds in gastrointestinal conditions.

Note: GC: grass carp; BH: bighead carp; MD: mud carp; LB: largemouth bass; T: tilapia; FW A: food waste A, FW B: food waste B, Control feed: commercial fish feed- Jinfeng®, 613 formulated feed.

have a much lower bioaccessibility of PCB than low lipid food (Xing et al., 2008). This suggested that the lipid content may be a major factor to affect the bioaccessbility of organic pollutants. In the present study, no linear correlation was observed between the bioaccessibilities of PAHs and DDTs and lipid content in fish muscle. The role of lipid content in food matrix on the bioaccessibility of organic pollutants needs further investigation.

Consumption of fish and seafood is a major source of dietary intake of organic contaminants including PAHs (Wang et al., 2010a) and OCPs (Dickman and Leung, 1998). Our previous study showed a significant correlation of OCPs in human milk with the frequency of fish consumption in both populations of Guangdong and Hong Kong (Wong et al., 2002a). Considering that the average fish consumption rate (164.4 g per day per person) (Dickman and Leung, 1998) of Hong Kong residents is higher than 142.2 g/d recommended by USEPA for subsistence consumers (USEPA, 2000b), this might under estimate the actual exposure based on the more conservative consumption rate of 142.2 g/d.

Figure 6.4 shows the hazard quotients of PAHs through fresh water fish consumption for Hong Kong residents. A HR higher than 1 implies that the EDI exceeds the RfD for the contaminant of interest which may exert potential harmful health effects. There was unlikely non-cancer risk for PAHs exerted on adults and children via consumption of the fish collected from experimental ponds and other fish ponds. A risk above $10^{-6}$, value is considered by USEPA as an acceptable risk for cancer when estimating the lifetime excess cancer risks of PAHs (USEPA, 1989b). The cancer risk values for fish were all below $10^{-6}$, suggesting there was a very low cancer risk for PAHs exerted on humans via

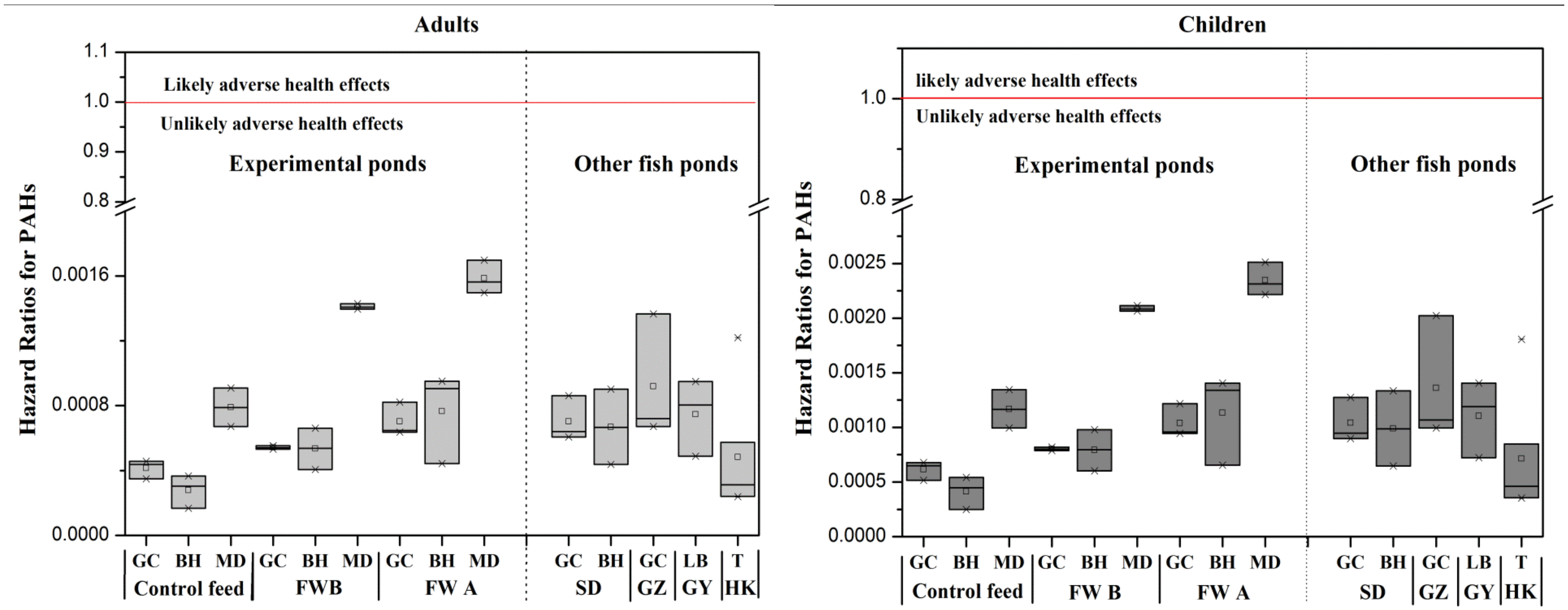



**Figure 6.4** Hazard ratios of PAHs through freshwater fish consumption by adults and children in Hong Kong. The consumption rates are 93 g $d^{-1}$ for adults and 50 g $d^{-1}$ for children (Leung et al., 2000), respectively. Each box represents interquartile range (25th and 75th percentile) of hazard ratios.

Note: MD: mud carp, T: tilapia, LB: largemouth bass, BC: bighead carp, GC: grass carp, GZ: Guangzhou, SD: Shunde, GY: Gaoyao, HK: Hong Kong, FW A: food waste A, FW B: food waste B, Control feed: commercial fish feed- Jinfeng®, 613 formulated feed.

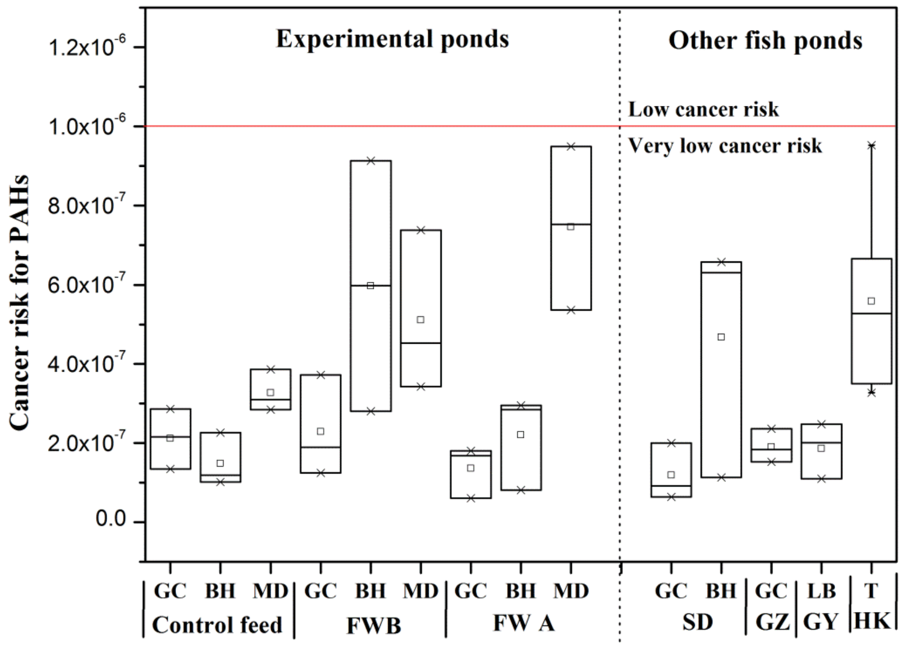



**Figure 6.5** Cancer risks of PAHs through freshwater fish consumption by adults and children in Hong Kong. The consumption rates are 93 g $d^{-1}$ for adults and 50 g $d^{-1}$ (Leung et al., 2000) for children, respectively. Each box represents interquartile range (25th and 75th percentile) of cancer risk of each fish.

Note: MD: mud carp, T: tilapia, LB: largemouth bass, BC: bighead carp, GC: grass carp, GZ: Guangzhou, SD: Shunde, GY: Gaoyao, HK: Hong Kong, FW A: food waste A, FW B: food waste B, Control: Commercial pellet; Control feed: commercial fish feed- Jinfeng®, 613 formulated feed.

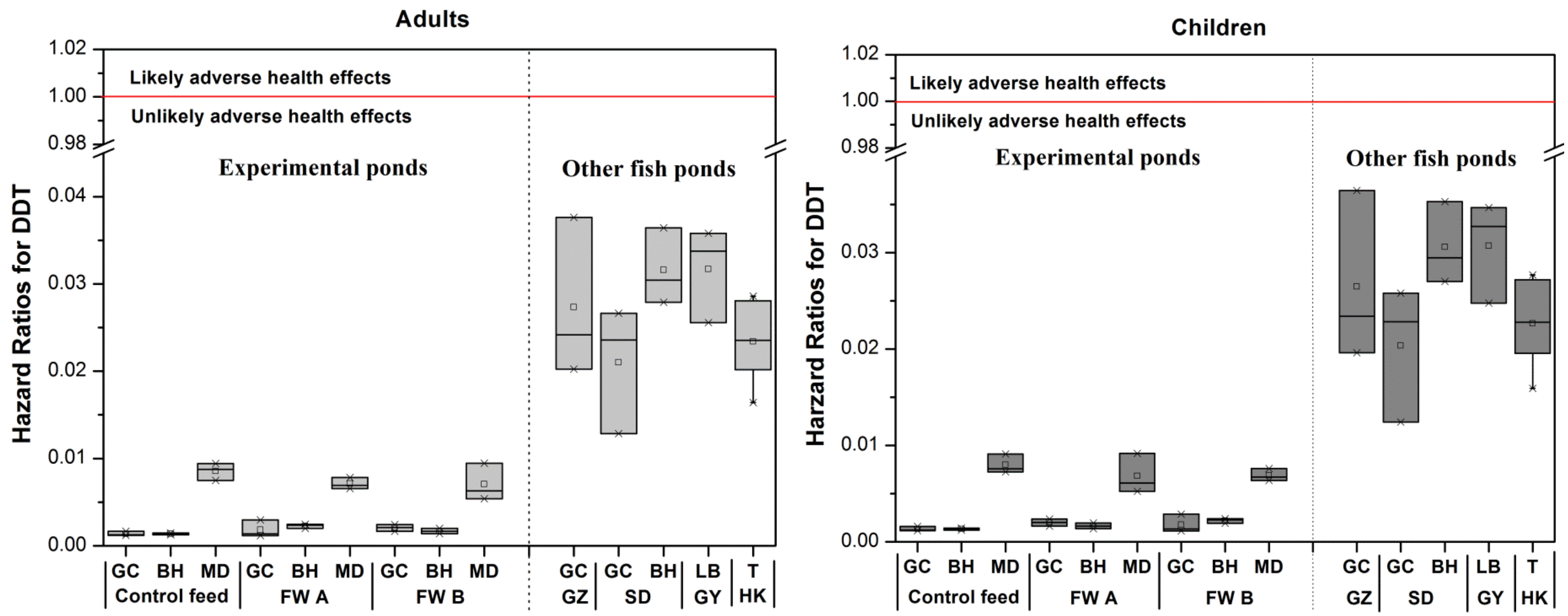



**Figure 6.6** Hazard ratios of DDT through freshwater fish consumption by adults and children in Hong Kong. The consumption rates are 93 g $d^{-1}$ for adults and 50 g $d^{-1}$ (Leung et al., 2000) for children, respectively. Each box represents interquartile range (25th and 75th percentile) of hazard ratios.

Note: MD: mud carp, T: tilapia, LB: largemouth bass, BC: bighead carp, GC: grass carp, GZ: Guangzhou, SD: Shunde, GY: Gaoyao, HK: Hong Kong, FW A: food waste A, FW B: food waste B, Control: Commercial pellet; Control feed: commercial fish feed- Jinfeng®, 613 formulated feed.

consumption of bighead carp, grass carp and mud carp (fed with food waste feed pellets and commercial feed pellets) reared in Sha Tau Kok and other fish ponds (Figure 6.5).

Figure 6.6 shows the hazard quotients of DDT through fresh water fish consumption for Hong Kong residents. There were also unlikely non- cancer risk for DDTs exerted on adults and children via consumption of all the fish tested in the present study, as well as the fish collected from experiment ponds in Sha Tau Kok which possessed lower HR levels than that from abandoned fish ponds in Hong Kong and farmed ponds in PRD. The maximum allowable fish consumption rate ($CR_{max}$, g/d) of each species was calculated, based on the raw and digestible DDTs concentration to prevent 1 excess cancer in 1,000,000 over a lifetime exposure (Figure 6.7). Based on the total raw concentrations, the $CR_{max}$ ranged from 297 (Largemouth bass) to 683 g/d (Grass carp fed with food waste B fish feed) in experimental ponds and other fish ponds. When bioaccessibility was taken into consideration, the $CR_{max}$ increased sharply and all fish species exceeded the undigested average consumption rate. All the $CR_{max}$ values were higher than the USEPA recommended limit of 142.2 g/d (USEPA, 2000a). These results indicated that human consumption of the fish (grass carp, bighead carp and mud carp) fed with food waste feeds in Sha Tau Kok experimental fish ponds were safe, in terms of PAHs and DDTs.

### 6.3.3 Bioaccumulation and Biomagnification

In the present study, BAF or BSAF is defined as the observed ratio of biota tissue concentration to dissolved water or sediment concentration. It is assumed that aquatic organisms only accumulated PAHs and OCPs from water and sediment for both BAF and BSAF (Lawrence and Mason, 2001; Streets et al., 2006). As shown in Table 6.4, log BAF was higher than log BSAF in all the samples, suggesting that these containments

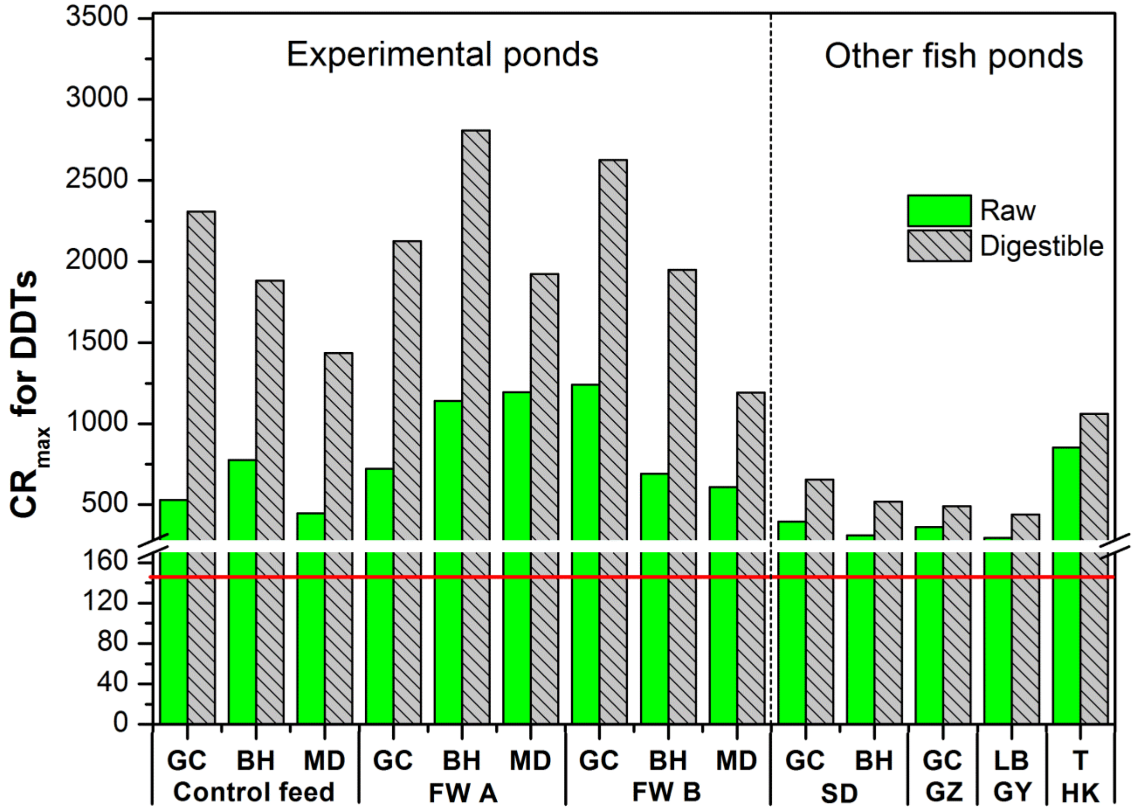



**Figure 6.7** The expected maximum allowable fish consumption rate ($CR_{max}$, g/d) of each species based on the raw and digestible DDTs concentrations for Hong Kong residents. Red line shows the recommended average consumption rate for Hong Kong residents (93 g/d).

Note: MD: mud carp, T: tilapia, LB: largemouth bass, BC: bighead carp, GC: grass carp, GZ: Guangzhou, SD: Shunde, GY: Gaoyao, HK: Hong Kong, FW A: food waste A, FW B: food waste B, Control: Commercial pellet; Control feed: commercial fish feed - Jinfeng®, 613 formulated feed.

accumulated in fish were mainly derived from the surrounding water, even though sediment contained higher concentrations of PAHs and OCPs than water. The BAF values of PAHs were lower than that of OCPs, indicating OCPs in water could be accumulated more easily by fish. The highest BAF values of PAHs (2.75) and OCPs (4.91), and BSAF values of DDTs (0.44) were obtained in largemouth bass collected from the farmed ponds in Gaoyao. The BSAF values of PAHs and DDTs in the grass carp and bighead carp fed with food waste feeds in Sha Tau Kok were lower than that in the fish collected from farmed ponds in PRD. The BSAF values of PAHs obtained in the fish fed with food waste fish feeds were lower than those collected from other fish ponds in PRD in 2003 (0.19-0.77) (Kong et al., 2005) and Mai Po Mashes (0.27- 0.95) (Liang et al., 2007). The BSAF values of OCPs obtained in the fish fed with food waste feeds were also lower than those collected from fish ponds in PRD in 2003 (0.59-1.05) (Kong et al., 2005). The results indicated ΣPAHs and ΣOCPs in the experimental ponds (water and sediment) using food waste feeds would have a relatively lower impact on the reared fish.

Recently, stable isotopes of nitrogen have been used to assess food web transfer of organic and inorganic contaminants in aquatic food webs. The ratio of the heavier to lighter stable isotopes of nitrogen ($^{15}N/^{14}N$), expressed relative to a standard as $\delta^{15}N$, generally increases with trophic position in aquatic food webs (Deniro and Epstein, 1981; Minagawa and Wada, 1984). This technique has been used as a tool to determine the trophic relationships and to estimate the biomagnifications of POPs in aquatic ecosystems (Fisk et al., 2001; Hop et al., 2002; Nfon et al., 2008). In this study, two typical food chains of freshwater fish pond in Sha Tau Kok and PRD were selected for investigation:

**Table 6.4** Bioaccumulation (BAF) and biota-sediment accumulation (BSAF) of freshwater fish (fed with food waste fish feed pellets and commercial fish feed pellets) from experimental ponds and other fish ponds (control).

| | | Log BAF | | | Log BSAF | | |
|---|---|---|---|---|---|---|---|
| | | ΣPAHs | Σ HCHs | ΣDDTs | ΣPAHs | ΣHCHs | ΣDDTs |
| **Experiment ponds** | | | | | | | |
| Control | GC | 2.54 | - | 4.30 | -1.17 | - | 0.22 |
| | BH | 2.54 | - | 4.13 | -1.17 | - | 0.06 |
| | MD | 1.95 | - | 4.37 | -1.76 | - | 0.29 |
| FW A | GC | 2.23 | - | 4.11 | -1.47 | - | 0.05 |
| | BH | 2.43 | - | 3.91 | -1.27 | - | -0.15 |
| | MD | 2.21 | - | 3.89 | -1.49 | - | -0.17 |
| FW B | GC | 2.12 | - | 3.85 | -1.55 | - | -0.11 |
| | BH | 2.28 | - | 4.11 | -1.38 | - | 0.15 |
| | MD | 2.04 | - | 4.16 | -1.62 | - | 0.20 |
| **Other fish ponds** | | | | | | | |
| Shunde | GC | 2.70 | 3.24 | 4.16 | -0.88 | -0.51 | 0.30 |
| | BH | 2.71 | 3.71 | 4.26 | -0.86 | -0.04 | 0.41 |
| Guangzhou | GC | 2.42 | 3.44 | 4.21 | -1.60 | -0.51 | 0.20 |
| Gaoyao | LB | 2.75 | 3.42 | 4.91 | -0.63 | -0.35 | 0.44 |
| Hong Kong | T | 1.98 | 3.37 | 3.93 | -1.53 | -0.01 | -0.19 |

Note: GC: grass carp; BH: bighead carp; MD: mud carp; LB: largemouth bass; T: tilapia; FW: A food waste A, FW B: food waste B, Control feed: commercial fish feed - Jinfeng®, 613 formulated feed.

**Table 6.5** Trophic magnification factor (TMF) in freshwater fish (fed with food waste feed pellets and commercial feed pellets) from experimental ponds and other fish ponds (control) (*p<0.05*).

| | | **ΣPAHs** | | | **ΣDDTs** | | | **ΣHCHs** | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | TMF | Equation (log TL vs ΣPAHs) | $R^2$ | TMF | Equation (log TL vs ΣDDTs) | $R^2$ | TMF | Equation (log TL vs ΣHCHs) | $R^2$ |
| **Sha Tau Kok** | | | | | | | | | | |
| Control | O | 0.80 | y = -0.10x + 2.98 | 0.33 | 1.31 | y = 0.12x - 0.33 | 0.41 | | | |
| FW A | O | 0.73 | y = -0.13x + 3.25 | 0.64 | 1.25 | y = 0.10x - 0.34 | 0.47 | | | |
| FW B | O | 0.75 | y = -0.13x + 3.22 | 0.48 | 1.33 | y = 0.12x - 0.46 | 0.63 | | | |
| **Control area** | | | | | | | | | | |
| PRD | O | 0.91 | y = -0.04x + 2.42 | 0.47 | 1.27 | y = 0.10x - 0.19 | 0.44 | 1.05 | y = 0.02x - 0.74 | 0.03 |
| PRD | P | 0.94 | y = -0.03x + 2.14 | 0.06 | 1.24 | y = 0.09x - 0.29 | 0.74 | 1.07 | y = 0.03x - 0.54 | 0.12 |

Note: TL: trophic levels, O: Omnivorous food chains (plankton, grass carp, bighead carp and mud carp); P: Predatory food chains (plankton , trash fish, largemouth bass), FW A: food waste A, FW B: food waste B, Control feed: commercial fish feed - Jinfeng®, 613 formulated feed.

the omnivorous (plankton, grass carp, bighead carp, mud carp and tilapia) and predatory food chains (plankton, trash fish, largemouth bass). Five significant linear relationships were obtained between log TL and log ΣPAHs concentration (*$p<0.05$*), and TMF as biomagnification power (Table 6.5). All the TMF were lower than one suggesting PAHs were generally not biomagnified or biodiluted trough the food chains in the Sha Tau Kok experimental ponds and other fish ponds. Similar phenomenon was also observed in Baltic Sea (Nfon et al., 2008) and Northwater Polynya (Canada) (Fisk et al., 2001). Although PAHs has similar hydrophobic as PCBs, they are easily metabolized and eliminated from higher trophic level aquatic organisms (Näf et al., 1992; Selck et al., 2003), thus limiting food chain biomagnification.

On the contrary, five significant linear relationships were obtained between log TL and log ΣOCPs concentration (TMF range: 1.6-10.5) was smaller than those reported for marine and freshwater ecosystems (Fisk et al., 2001; Hop et al., 2002; Guo et al., 2008). These fish pond food chains are substantially shorter and simpler than those of other marine and fresh water ecosystems, and with short culture period (from 4 to 6 months). These would also lead to decreased accumulation of OCPs through the food chain (Hop et al., 2002). According to the present results, birds feeding on the fish reared in the fish ponds would not impose any health risks caused by the studied PAHs and OCPs.

In this study, the experimental ponds would provide shelters, water and food for birds and other wildlife. In general, the ponds are relatively free of PAHs and OCPs and the fish produced contained lower pollutants. In addition, lower bioaccumulation and biomagnificantion of these pollutants in the food chains of experimental ponds were noted. The pond water was drained after fish harvest which attracted bird foraging on the exposed soft mud floor of the drained fish ponds. According to the present results, the traditional fish farming practice using food waste can provide a better fish pond habitat for birds and other wildlife.

## 6.4 Conclusions

Concentrations of PAHs and DDTs in the three different fish species cultured in Sha Tau Kok experimental fish ponds were similar or lower than that collected from abandoned fish ponds in Hong Kong and farmed ponds in PRD. The results of health risk assessment showed that consumption of grass carp, bighead and mud carp fed with food waste pellets was safe for Hong Kong residents. In addition, as there were no or lower biomagnifications of PAHs and OCPs, and low concentrations of the PAHs and OCPs contained in the fish ponds (both water and sediment) indicated the traditional fish farming using food wastes can provide a better fish pond habitat for birds and other wildlife.

# CHAPTER 7 GENERAL DISSCCUSION AND CONCLUSIONS

## 7.1 Introduction

Freshwater fish products for local consumption mainly depend on import, in which food safety and quality have become issues of recent public concern (CFS, 2006d, c). On the other hand, due to the decline of pond fish culture in Hong Kong, inactively managed fish ponds may become a sink for various pollutants (Wong et al., 2004) and also impair pond use for wildlife (Young, 1998, 2004).

Generally, fish could accumulate heavy metals and POPs from their living environment and foods, and some of these contaminants can be biomagnified through the food chains, then posing risks to human health (Fisk et al., 2001; Hop et al., 2002; Cheung et al., 2007; Cheung et al., 2008). With this in mind, the purpose of this study was to evaluate the environmental and health effects of using food wastes as fish feeds, focusing on the concentrations and variations of the metalloid/metals (As Hg, Cd, Cr, Zn, Pb, Cu and Ni), OCPs and PAHs in the experimental freshwater fish ponds in Sha Tau Kok; assessing bioaccumulation and biomagnification of these pollutants in the experimental ponds in Sha Tau Kok, famed ponds in Pearl River Delta, and abundant fish ponds in Hong Kong; and finally investigating the bioaccessibility of these pollutants through fish consumption, using an *in vitro* gastrointestinal digestion model.

**Table 7.1** Composite analysis of experimental feeds

| **Formulation** | **Control feed** | **FW A** | **FW B** |
|---|---|---|---|
| Dry matter (%) | 93.7±0.20$^{a}$ | 95.7±0.02$^{a}$ | 93.2±0.05$^{a}$ |
| Ash (%) | 8.24±0.09$^{a}$ | 9.18±0.46$^{a}$ | 18.9±0.03$^{b}$ |
| Protein (%) | 30.2±1.55$^{a}$ | 31.4±0.44$^{a}$ | 31.1±3.36$^{a}$ |
| Lipid (%) | 5.17±0.94$^{a}$ | 6.12±1.66$^{a}$ | 13.3±1.81$^{b}$ |
| Fibre (%) | 9.57±0.21$^{a}$ | 10.1±0.63$^{a}$ | 5.72±0.87$^{b}$ |
| Carbohydrates (%)[1] | 40.5 | 39.9 | 24.2 |
| Energy (kJ/g diet)[2] | 16.2 | 16.6 | 16.8 |
| CHO/L ratio [3] | 7.84 | 6.36 | 1.83 |
| P/E (mg/kJ)[4] | 1865 | 1898 | 1857 |
| Protein solubility (%)[5] | 60.6±2.64$^{a}$ | 51.8±1.43$^{b}$ | 38.7±1.35$^{c}$ |

Note: Different superscripts (a, b, c) between feeding groups are significantly different ($p< 0.05$). Control feed: commercial fish feed- Jinfeng®, 613; FWA: food waste A; FWB: food waste B.

[1]Carbohydrates (%) = 100 − (crude protein % + crude lipid % + moisture% + ash %+ fibre %) (Castell and Tiews, 1980)

[2]Energy (KJ/g diet) = (% crude protein × 23.6) + (% crude lipids × 39.5) + (%carbohydrates × 17.3) (Chatzifotis et al., 2010)

[3] Carbohydrates to Lipid (CHO: L) ratio = % wt. in CHO/ % wt. in lipid

[4] Protein to energy (P/E) **(**mg/kJ) = crude protein (%) / Energy

[5] Protein solubility (%) = Protein in KOH / Protein in Sample x 100% (Araba and Dale, 1990)

It was hypothesized that (1) food waste can replace part of the fish meal used in fish feeds to produce quality fish, so that the local inland fisheries could be reactivated sustainably; (2) the use of food wastes for fish culture could ease part of the waste disposal pressure at local landfills; (3) the use of food wastes and adoption of the traditional fish farming practice could improve the environmental quality of fish ponds; and (4) actively managed fish ponds would provide better water, food and shelter for for birds and other wildlife.

## 7.2 Growth Performance, Feed Nutrient Utilization, and Levels of Environmental Pollutants in Fish Feeds

In the present study, experimental fish feed pellets containing about 75% food wastes were used for maintaining feed quality (Table 7.1). FWB contained the highest ash and lipid contents ($p<0.05$) and the lowest fibre and soluble protein contents ($p<0.05$). No significant differences ($p>0.05$) in protein and energy contents were found between FW A and FW B, and the commercial fish feed (Table 7.1). According to Table 6.1, the values of length, weight and nutrient utilisation in grass carp reared in Sha Tau Kok fish ponds, fed with FW A were significantly higher than those fed with FW B and control diet ($P<0.05$). These results indicated that the fish fed with food waste feeds achieved better or similar growth performance and feed conversion ratio, indicating food wastes can serve as an alternative source of protein for fish culture.

The lowest concentrations of As, Pb, Cr, Cu, Ni, Zn and ΣPAHs were detected in the control diet ($p<0.05$) among all feeds. There was no significant difference ($p>0.05$) in concentrations of Hg and ΣDDT in FW A and FW B, whereas significantly ($p<0.05$)

lower ΣHCHs were found in FW A and FW B, when compared with the control diet. The analyses of metalloid/metals in various food waste ingredients for making fish pellets (FW A and FW B) showed that vegetables, cereals and bone meal were major sources of metalloid/metals (except Hg), ΣPAHs and ΣOCPs contamination for FW A and FW B. The analyses of THg and MeHg in various food waste ingredients (FW A and FW B) showed that fish meal was the major source of Hg (THg and MeHg: 149 ± 80.9 and 15.4 ± 2.5 ng/g, respectively in making FW A and FW B. The results revealed that metalloid/metals, OCPs and PAHs in food waste fish feeds and control diet were significantly lower *($p<0.05$)* than that in commercial feeds collected from PRD in the present study and previous studies (Guo et al., 2009b). Therefore, these two kinds of food waste feeds can provide a high quality food source for fish.

## 7.3 The Levels of Environmental Pollutants in Water, SPM, and Sediments Collected from Experimental Ponds and Other Fish Ponds (Control Sites)

In experimental ponds, concentrations of As, Cd and ΣHCHs in pond water, Cd, Cr, Cu, Zn, MeHg and ΣDDTs in pond SPM were increased ($p<0.05$) during the experimental period. On the contrary, there were no significant differences ($p>0.05$) in ΣPAHs in water and SPM among the three experimental ponds. Metalloid/metal concentrations (except Cu) in sediment of the experimental ponds did not change significantly ($p>0.05$) during the experimental period. However, ΣPAHs and ΣOCPs in sediment in the second half year were higher than that in the first half year ($p>0.05$). The results indicated that pond sediment might accumulate more ΣDDTs and ΣHCHs due to the use of more fish in the second half year. There was no significant difference (*$p>0.05$*) in metalloid/metals, ΣPAHs and ΣOCPs of water, SPM and sediment among the three experimental ponds in Sha Tau Kok during the experiment period.

Concentrations of metalloid/metals, ΣPAHs and ΣOCPs in water and sediment of the experimental ponds were similar or lower than that of the abandoned ponds in Hong Kong and farmed ponds in PRD, and lower than that reported previously on fish ponds sediment collected from the PRD (Zhou and Wong, 2000, 2004; Kong et al., 2005; Cheung et al., 2008; Cheng et al., 2011; Wang, 2011). Generally, our results suggested that the experimental site was relatively free of metalloid/metals, ΣPAHs and ΣOCPs, and would be suitable for farming fish. It is envisaged that when fish ponds are drained and sediment removed during each harvest, will avoid accumulation of the contaminations in sediment.

## 7.4 The Levels of Environmental Pollutants in Fish Collected from Experimental Ponds and Other Fish Ponds (Control Sites)

During the first half year to second half year of the experiment, As, Cu, Ni, and Zn concentrations in grass carp, bighead carp and mud carp reared in Sha Tau Kok fish ponds decreased ($p<0.05$), while Pb in grass carp fed with food waste pellets and Hg in the three fish species of all treatments increased ($p<0.05$). Figures 6.1 and 6.2 show that, during the 1st half year to 2nd half year of the experimental period, ΣPAHs and ΣDDTs in grass carp, bighead carp and mud carp collected from Sha Tau Kok experimental fish ponds increased ($p<0.05$). 4-ring and 5, 6-ring PAHs ratio in ΣPAHs and *p, p'*-DDD ratio in ΣDDTs (with *p, p'*-DDD and *p, p'*-DDT, and 2, 3-ring PAHs the major compounds) and ΣPAHs in the fish in 2nd half year of experiment were significantly increased ($p<0.05$), respectively. In the present study, HCHs in the fish collected from experimental ponds were under detection limits.

In Sha Tau Kok, concentrations of As and Cu in grass carp fed with FW A, and Pb in grass carp and As and Cu in bighead carp fed with all food waste feed pellets, were higher than in the relevant fish species which fed with the control diet, respectively ($p<0.05$). However, bighead and mud carp fed with control pellets contained the highest MeHg and THg concentrations in muscle ($p<0.05$). ΣPAHs concentration in grass carp and bighead carp fed with control diet were higher than that fed with food waste feeds ($p<0.05$). ΣDDTs in bighead carp and mud carp fed with food waste A fish feed, and grass carp fed with food waste B fish feed were lower than that same fish species obtained in experimental fish ponds, respectively ($p<0.05$) (Table 6.3). It was observed that metalloid/metals, ΣPAH and ΣDDT in the fish collected from experimental ponds which fed with food waste feeds were similar or lower than that from abandoned fish ponds in Hong Kong and farmed ponds in PRD, and lower than those reported by previous studies on the fish pond sediment collected from PRD (Zhou and Wong, 2004; Cheung et al., 2007; Cheung et al., 2008; Wang et al., 2010a; Wang et al., 2011).

In fish, bioaccumulation from water via gill and skin, and ingestion of contaminated food are possible routes for environmental pollutants to accumulate in tissue. Therefore, the feeding preference, behaviour, and trophic level of fish were the main factors to determine the accumulation of pollutants in fish tissue (Baumard et al., 1998; Fisk et al., 2001). The present study indicates that fish fed with food waste feeds have relatively lower concentrations of metalloid/metals, PAHs and OCPs than fish fed with control feeds and those collected from other fish ponds. The experimental ponds were located near the Mai Po Marshes far from the industrial and urban areas. It is envisaged adoption of the traditional practice of draining pond water after harvest, and removal of sediment regularly could avoid accumulation of pollutants in pond sediment (Wong et al., 2004). On the contrary, rapid industrialization and urbanization in PRD resulted in the excessive release of pollutants into the river. The fish farmers are still practicing the traditional way of filling up fish ponds using river water (Ruddle and Zhung, 1988), and this will exacerbate the impact of environmental pollutants on the cultured fish. In addition, it has been demonstrated that fish meal derived from the trash fish for production feed pellets contained rather high levels of pollutants such as Hg, PBDEs, PCBs and OCPs (Guo et al., 2009b; Liang et al., 2011; Suominen et al., 2011).

In this study, part of the fish meal used in fish feeds could be replaced by food waste (up to 75%). Although food waste feeds contained similar or higher concentrations of metalloid/metals, PAHs and OCPs than control diet, fish fed with food waste feeds had relatively lower concentrations of these pollutants than fed with control diet. This indicated these pollutants in food waste feeds had a lower bioavailability than control diet, using fish meal as the main protein source.

## 7.5 Bioaccessibilities and Health Risk Assessments of Consumption The Fish Fed with Food Waste Feeds

In the present study, digestible concentrations of HCHs in all fish samples were under detection limits. The bioaccessibilities of metalloid/metals, $\Sigma$PAH and $\Sigma$DDT in all fish samples ranged from 10.2% to 87.7% (mean 33.4 %). Previous studies revealed that bioaccessbility ratios of these pollutants exhibited positive correlations with carbohydrate and dietary fibre contents, and negative correlations with protein content (Cabanero et al., 2007; Moreda-Pineiro et al., 2012). Therefore, the fish species may be an important factor for the difference obtained in bioaccessibility. The low bioaccessibility may indicate that contaminant intake via fish consumption detected by conventional procedures with organic solvent is considerably overestimated the actual human exposure to metalloid/metals, ΣPAH and ΣDDT via food intake.

There were unlikely non-cancer risk for metalloid/metals, PAHs and DDT exerted on adults and children via consumption of the three fish species fed with food waste pellets and commercial pellets in Sha Tau Kok experimental ponds. A risk above $10^{-6}$, value is considered by USEPA (USEPA, 1989b), as an acceptable risk for cancer when estimating the lifetime excess cancer risks of As, PAHs and DDT. For PAHs and DDTs, the cancer risk values for fish were all below $10^{-6}$, suggesting there was a very low cancer risk for PAHs and DDT exerted on humans via consumption of bighead carp, grass carp and mud carp (fed with food waste and commercial feed pellets) in experimental fish ponds and other fish ponds (Figure 6.5). As to As, the CR values of fish were all above $10^{-6}$, and only tilapia ($2.34\times10^{-4}$) was higher than the upper limit of the acceptable risk levels ($10^{-4}$) (USEPA, 2012a), based on the 10% of organic As out of total As condition (USFDA, 1993). In reality, due to different dietary habits of residents, the species and frequency of fish consumption varied tremendously, and many other factors such as cooking method, doneness level, and food processing, could all affect the final lifetime cancer risk for Hong Kong residents by ingesting these freshwater fish. However, the present results provided some useful information showing that consumption of grass carp, bighead and mud carp which fed food waste feed pellets was safe and more importantly, commercial fish pellets could be partially replaced by food waste for culturing fish in order to reducing the cost of fish feeds and ease the disposal pressure of food wastes at local landfills.

## 7.6 Comparison of using two different fish consumption rates for the health risk assessment of consumption of fish fed with FWA, FWB and control feed

Leung et al. (2000) suggested a fish/seafood consumption rate of 93 g/day for adults (aged 25-64) in Hong Kong. Recently, the Centre for Food Safety has published a new fish consumption rate of 57.5 g/day for adults (20-84 age), based on the Hong Kong population-based food consumption survey 2005-2007 (CFS, 2010). In this study, the consumption rates of 93 g/day was adopted for evaluating the health risks of consumption of freshwater fish (fed with FWA, FWB and control feed), for providing better protection for the general public (Leung et al., 2000). However, the new fish consumption rate of 57.5 g/day for adults was also included for comparison, in order to obtain a more comprehensive and updated health risk assessment.

Figures 7.1 and 7.2 compare the two different fish consumption rates: 93 g/day (Leung et al., 2000) and 54.5 g/day (CFS, 2010), for estimating EDI, HI and cancer risk values for the metalloid/metals (As, Cd, Pb, Cr, Cu, Ni, Zn and Hg), PAHs and OCPs concentrations in GC BH and MD (fed with FWA, FWB and control feed) cultured in Sha Tau Kok. In general, there were no health risks exerted on adults via consumption of freshwater fish (fed with FWA, FWB and control feed) by using the aforementioned two different fish consumption rates. However, it is worth nothing that using fish

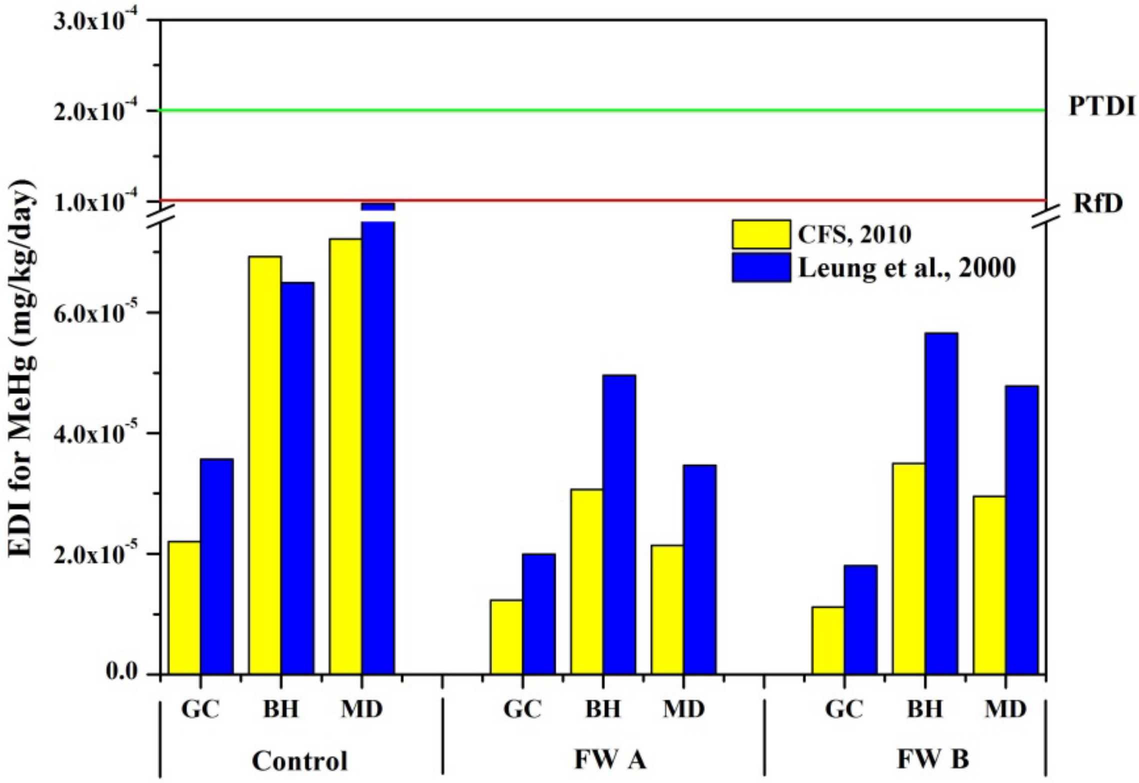



**Figure 7.1** Compare the two different fish consumption rates: 93 g/day (Leung et al., 2000) and 54.5 g/day (CFS, 2010), for estimating for estimated daily intakes (EDI) of bioaccessible MeHg through consumption of freshwater fish by adults in Hong Kong.

Note: RfD= reference dose of (1.00E-04 mg/kg/day) (USEPA, 2012a); PTDI= provisional tolerable daily intake ( 2.29E-04 mg/kg/day) (JECFA, 2003); Control commercial pellets, FW A and FW B food waste feed pellets A and B, GC grass carp, BH bighead carp, MD mud carp.

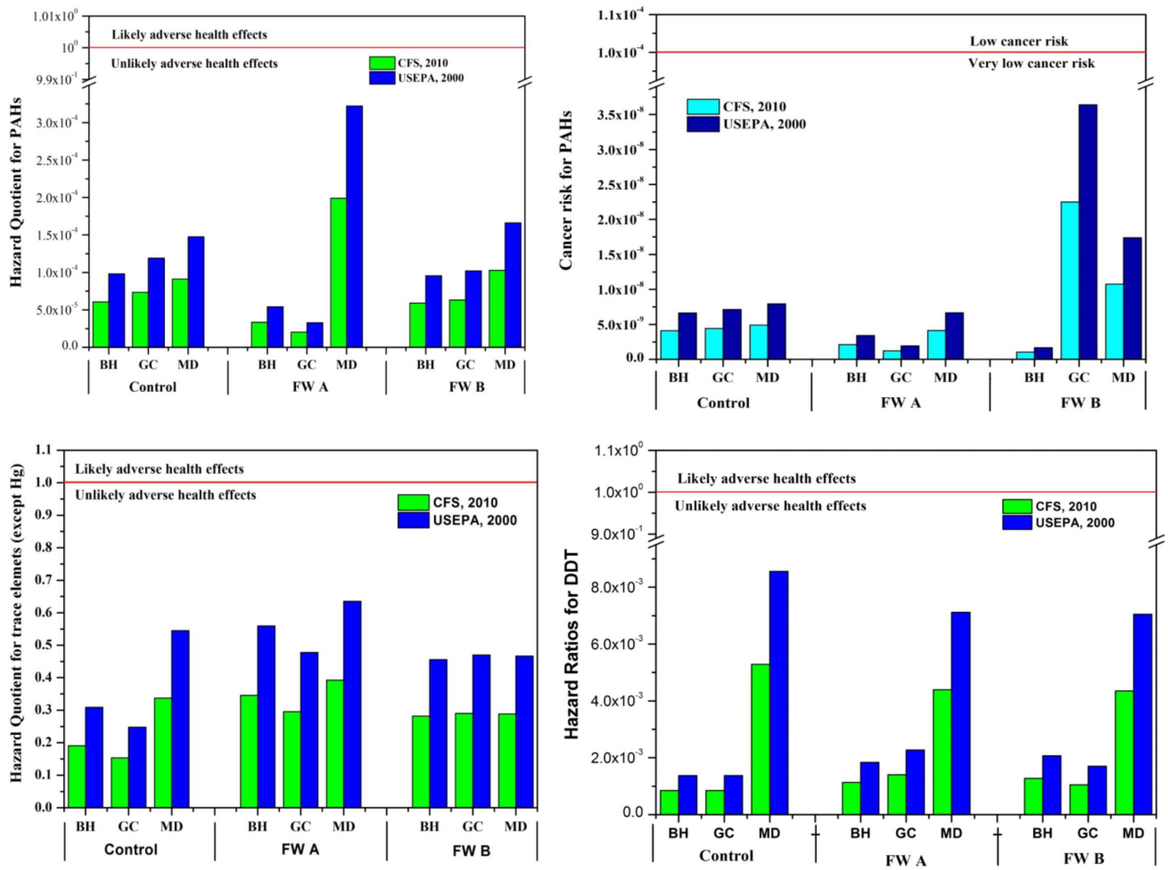



**Figure 7.2** Compare the two different fish consumption rates: 93 g/day (Leung et al., 2000) and 54.5 g/day (CFS, 2010), for estimating Hazard Quotient (HQ) and Cancer risk for PAHs and trace elements exerted on adults via consumption of bighead carp (BH), grass carp (GC) and mud carp (MD) (fed with food waste fish feed pellets and commercial fish feed pellets) in Sha Tau Kok

Note: Control commercial pellets, FW A and FW B Food waste feed pellets A and B consumption rate of 93 g/day (Leung et al., 2000) for evaluating the health risk via fish consumption resulted in a risk value of 1.62 times higher than the recent fish consummation rate of 54.5 g/day (CFS, 2010).

## 7.7 Bioaccumulation and Biomagnification of Environmental Pollutants in Fresh Water Fish, Using Food Waste as Fish Feed

According to Tables 4.2 and 6.4, log BAF was higher than log BSAF in all the fish samples, suggesting that these pollutants accumulated in fish were mainly derived from the surrounding water, even though sediment contained higher metalloid/metals, PAHs and OCPs than water. The BAF values of As, Pb, Cr, Cd, Zn, Ni, Cu and PAHs were lower than that of Hg and OCPs, indicating Hg and OCPs in water could be more easily accumulated by fish. The present study observed positive correlations ($p<0.05$) between TOC levels in sediment and BSAF of As, Cu, Ni, Zn, THg, PAHs and DDTs (Table 7.2). This phenomenon is also observed in previous studies in the aquatic ecosystem of PRD (Zhou and Wong, 2000; Cheung et al., 2008; Wang et al., 2010b), suggesting that organic matter plays a significant role in controlling the transport of these pollutants in sediment. In this study, the mean BAF for MeHg, PAHs and DDTs in omnivorous food chains (plankton, grass carp, bighead carp and mud carp) is higher than in predatory food chains (plankton, trash fish and largemouth bass). The same trend is also noted for BSAF. These indicated that omnivorous food chains may possess a higher bioaccumulation ability of these lipophilic contaminants than predatory food chains.

**Table 7.2** Correlation matrix among TOC in sediment and the BSAF of metalloid/metals, ΣPAH and ΣDDT (n=15) (*$p<0.05$*)

| | As | Cd | Pb | Cr | Cu | Ni | Zn | THg | MeHg | PAHs | DDTs |
|---|---|---|---|---|---|---|---|---|---|---|---|
| As | 1.00 | | | | | | | | | | |
| Cd | 0.59 | 1.00 | | | | | | | | | |
| Pb | 0.69 | 0.78 | 1.00 | | | | | | | | |
| Cr | 0.75 | 0.81 | 0.91 | 1.00 | | | | | | | |
| Cu | 0.87 | 0.57 | 0.59 | 0.68 | 1.00 | | | | | | |
| Ni | 0.98 | 0.56 | 0.61 | 0.73 | 0.92 | 1.00 | | | | | |
| Zn | 0.89 | 0.78 | 0.82 | 0.93 | 0.88 | 0.90 | 1.00 | | | | |
| THg | 0.80 | 0.85 | 0.79 | 0.83 | 0.73 | 0.79 | 0.86 | 1.00 | | | |
| MeHg | 0.74 | 0.89 | 0.77 | 0.79 | 0.74 | 0.69 | 0.82 | 0.89 | 1.00 | | |
| PAHs | 0.88 | 0.58 | 0.68 | 0.65 | 0.71 | 0.84 | 0.76 | 0.86 | 0.68 | 1.00 | |
| DDTs | 0.90 | 0.54 | 0.68 | 0.71 | 0.76 | 0.89 | 0.82 | 0.86 | 0.66 | 0.97 | 1.00 |
| TOC | 0.68 | 0.10 | 0.30 | 0.32 | 0.62 | 0.68 | 0.50 | 0.50 | 0.34 | 0.77 | 0.81 |

In this study, two typical food chains of freshwater fish pond in Sha Tau Kok and PRD were selected for investigation: the omnivorous (plankton, grass carp, bighead carp and mud carp) and predatory food chains (plankton, trash fish, largemouth bass).. Five significant linear relationships were obtained between $\delta^{15}N$ and log metalloid/metals, between $\delta^{15}N$ and log $\Sigma$PAHs concentrations, and the slop of the regression as biomagnification power (Table 4.3), all the slops (expect THg and MeHg) were lower than zero suggesting the metalloid/metals were generally not biomagnified or biodiluted through the food chains in the Sha Tau Kok experimental ponds and other fish ponds (control). On the contrary, five significant linear relationships were obtained between log TL and log $\Sigma$OCPs concentration, between log THg and $\delta^{15}N$ and between log MeHg and $\delta^{15}N$, and TMF was smaller than those reported for marine and freshwater ecosystems (Fisk et al., 2001; Hop et al., 2002; Campbell et al., 2005; Guo et al., 2008; Ikemoto et al., 2008a). These fish pond food chains are substantially shorter and simpler than those of other marine and fresh water ecosystems, and with short culture periods (from 4 to 6 months). These would also lead to decreased accumulation of contaminants through the food chains (Hop et al., 2002).

The Sha Tau Kok experimental fish ponds are located near the Mai Po Nature Reserve located at northwestern New Territories, and these ponds should provide shelter and feed for waterbirds. As a traditional fish farming model, the pond water drained after each harvest to maintain water quality, which would also reduce the incidence of fish diseases (Wong et al., 2004). During this time, it will attract birds foraging on the exposed soft mud floor of the drained fish ponds. The present study indicated the experimental fish ponds (water and sediment) receiving food waste feeds were relative free of metalloid/metals, OCPs and PAHs. In addition, the fish reared by food waste feeds contained lower levels of these pollutants in fish tissues. Furthermore, weaker bioaccumulation and biomagnification of these pollutants in the food chains of the experimental ponds than those of farmed ponds in PRD and other river and marine ecosystems were noted. Therefore, the traditional fish farming practice using food waste feeds can provide a better fish pond habitat for birds and other wildlife.

## 7.8 General Conclusions

A schematic diagram showing the trophic linkages (omnivorous food chains, involving: plankton, grass carp, bighead carp and mud carp) of this study is presented in Figure 7.1. Conclusions can be drawn from the results derived from all the experiment:

Experimental feed pellets comprised of about 75% food wastes seemed appropriable for maintaining feed quality. Similarly protein and energy contents were found among FW A, FW B, and the commercial feeds. The results indicated that the fish fed with food waste feeds achieved better or similar growth performance and feed conversion ratio when compared with control (fish fed with commercial feeds). It was also revealed that concentrations of metalloid/metals, OCPs and PAHs contained in food waste feeds were significantly lower than that in commercial fish feed collected from PRD (data obtained in the present study and previous studies). Therefore, food wastes can serve as an alternative source of protein for fish culture and food waste feeds can provide a high quality feed for freshwater fish.

There were no significant differences ($p>0.05$) in metalloid/metals, PAHs and OCPs of water, SPM and sediment among the three experimental ponds in Sha Tau Kok during

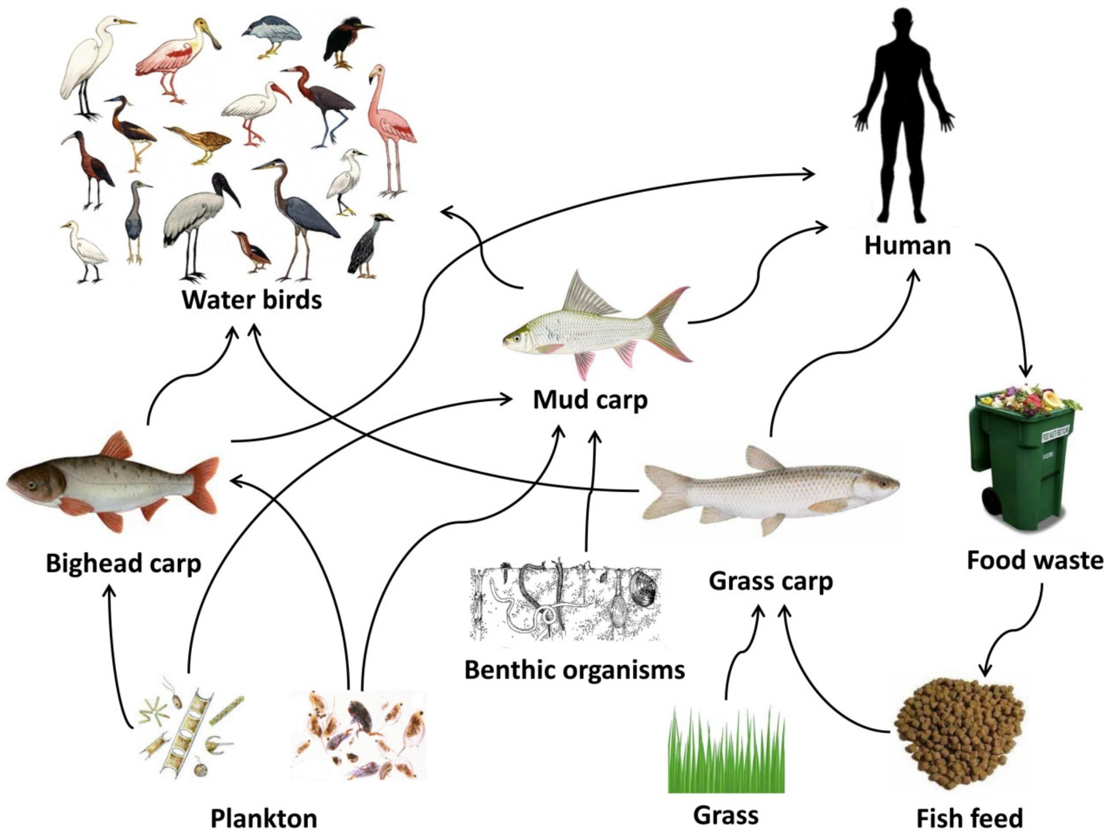



**Figure 7.3** Schematic diagrams of the omnivorous food chains used in the present study and some farmed fish pond in PRD.

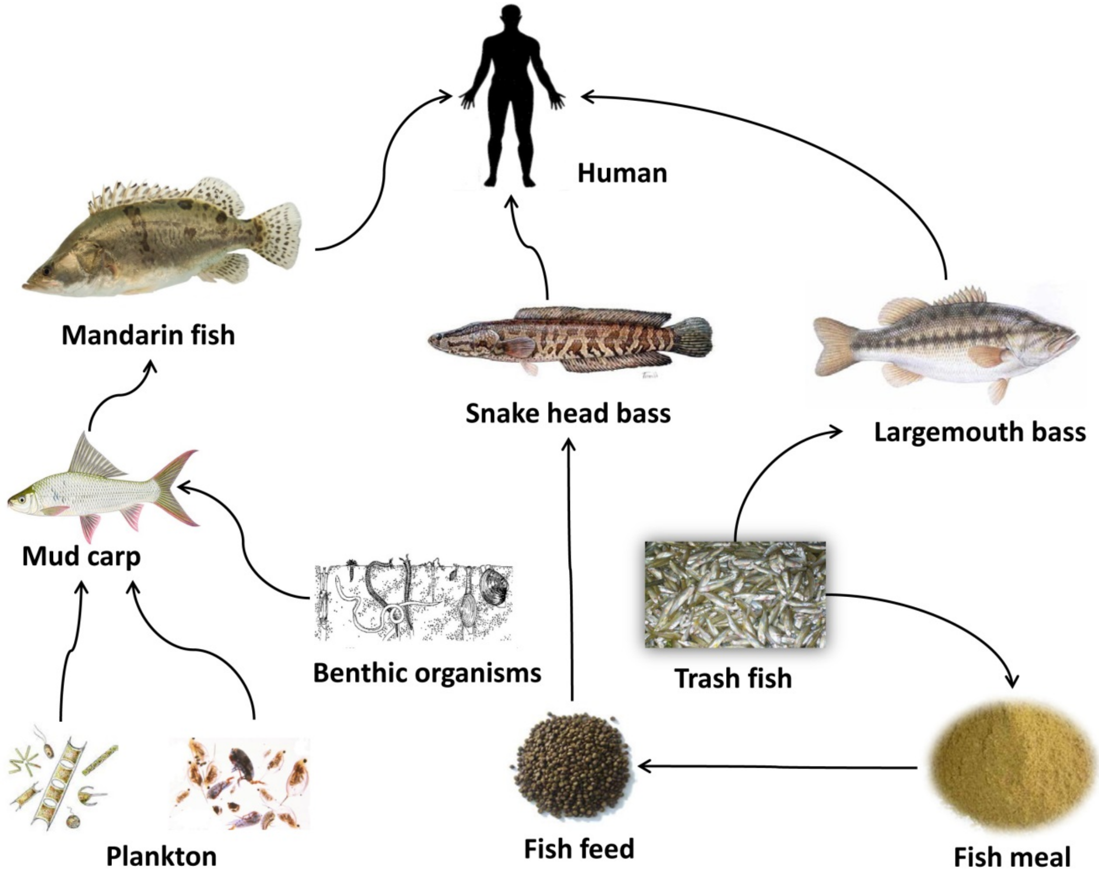



**Figure 7.4** Schematic diagrams of the predatory food chains commonly observed in some farmed ponds in PRD.

the experimental period. The the experimental ponds were relatively free of these pollutants. In this study, the traditional fish farming model was used to culture low trophic level fish e.g. filter feeders (bighead), herbivores (grass carp), bottom feeders (mud carp) of this omnivorous food chain, which are more environmental friendly as they can utilize more solar energy (Figure 7.1). The results indicated that grass carp and bighead carp fed with food waste feeds were relatively free of metalloid/metals, PAHs and OCPs. The results of health risk assessment showed that the fish fed with food waste feeds were safe for consumption.

The lower bioaccumulation and biomagnification of these pollutants in the experimental ponds indicated (Cheng et al., 2011; Cheng et al., 2013b) that the use of food wastes, when compare to those predatory food chain (involving trash fish, mud carp and largemouth bass) in farmed ponds of PRD (Figure 7.2) and marine and fresh water ecosystems. In addition, the practice of traditional pond management by draining pond water and removing pond sediment regularly can provide a better fish pond habitat for birds and other wildlife.

The use of food waste instead of fish meal further reduced accumulation of metalloid/metals, PAHs and OCPs in the cultured fish. It can also lower the cost of fish farming, and at the same time, partially ease the disposal pressure of food waste, and conserve the ecological value of fish ponds

## 7.9 Limitations of the Present Study

The present study only focused on metalloid/metals, PAHs, HCHs and DDTs. However, other pollutants such as polybrominated diphenyl ethers (PBDEs), phthalate esters (PAEs) and perfluorinated compound were not monitored. These pollutants have their own toxicity and will generate synergistic effects when they are taken up simultaneously. Furthermore, pollutant concentrations were only determined in fish muscle, but not fish skin, head and internal organs (such as intestine) are also consumed by some of the local residents.

## 7.10 Future Work

Chemical analyses alone may not be sufficient to evaluate the adverse effects of the complex mixtures of chemicals present at contaminated sites. The use of biomarkers in various native species (such as mosquitofish and tilapia) is considered a useful and effective tool in obtaining information about the environment quality (especially pollutant levels) and the effects of pollutants on living organisms (May et al., 2004). Therefore, it is suggested to use oxidative metabolism enzyme to evaluate potential combined toxic effects of different pollutants accumulated in fish. The results would supplement chemical analyses for assessing the toxicity of multi-compounds in the aquatic environment.

Previous studies reported the rate of breast cancer has a positive correlation with fish in take (Stripp et al., 2003). Hong Kong registered the highest breast cancer incidence in Asia between 1973 and 1999 (Leung et al., 2002), and fish are major dietary source of protein for most of the residents. Hong Kong person consumes fish or shellfish four or more times per week, about 164.4 g per day (Dickman and Leung, 1998). Therefore, it is suggested to use E-screen and vitellogenin assays to assess the estrogen-like activities of contaminants extracted from the fish by gastric and intestinal digestion juice. The results would provide more direct evidence about food contaminants on human health.

In the present study, 10 % (w/w) of the ingredients used for making food waste pellets were fish meal. This portion of fish meal acts as nutrient supplement to provide amino acids source for fish. However, using fish meal as part of the fish meal pellets increases cost and introduces pollutants into fish feed. It has been reported that, during the black solider fly (*Hermetia illucens*) larvae growth process can quickly consume large amounts of food wastes (Craig Sheppard et al., 1994; Zheng et al., 2012). The larvae also contained higher levels of amino acids (33.8 g/100g) than commercial fish meal (Sealey et al., 2011). Therefore, it is proposed to use black solider fly larvae for converting food waste into their biomass to replace fish meal.

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1. **Cheng, Z.,** Nie, X.P., Wang, H.S., Wong, M.H., 2013 Risk assessments of human exposure to Bioaccessible Phthalate Esters through market fish consumption. Environment International 57-58C:75-78

2. **Cheng, Z.,** Wang, H.S., Wong, M.H., 2013 Dietary Exposure and Risk Assessment of Mercury via Total Diet Study in Cambodia. Chemosphere. 92, 143–149

3. **Cheng, Z**., Man, Y.B.,Nie, X.P., Wong, M.H., 2012 Trophic relationships and health risk assessments of trace metals in the aquaculture pond ecosystem of Pearl River Delta, China. Chemosphere. 90:2142-2148

4. **Cheng, Z**., Nie, X.P., Chen, K.C., Li, K.B., Wong, M.H., 2012 Arsenic contamination in the freshwater fish ponds of Pearl River Delta: Bioaccumulation and health risk assessment. Environmental Science and Pollution Research. 20, 4484-4495

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13. He, X.T., Wang, Z.H., Nie, X.P., Yang, Y.F., Pan, D.B., Leung, A.O.W., **Cheng, Z**., Yang, Y.T., Li, K.B., Chen, K.C., 2012b. Residues of fluoroquinolones in marine aquaculture environment of the Pearl River Delta, South China. Environmental Geochemistry and Health. 34, 323-335.

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15. **Cheng Z**, Nie X., Wang F, Li K.B., 2009. Cloning and sequence analysis of P-gp in *Mugilogobius abei*. Journal of Safety and Environment. 9(1): 4-8.

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17. Zhao X.Y., Liu L.J., Nie X.P., He X.T., **Cheng Z**, 2010. Online biomonitoring for waterborne antibiotics and organophosphorous using the Multispecies Freshwater Biomonitor. Acta Scientiae Circumstantuae. 30:180-185.

## Manuscripts under review：

1. **Cheng, Z.,** Mo, W. Y., Choi, W. M., Man, Y.B., Nie, X.P., Wong, M.H., 2013 Replacing fish meal by food waste to produce quality fish with lower mercury levels: the case of ployculture of lower trophic level fish. Environmental Science & Technology.

2. **Cheng, Z.,** Mo, W. Y., Choi, W. M., Man, Y.B., Nie, X.P., Wong, M.H., 2013 Replacing fish meal by food waste can produce quality fish with trace element levels: the case of ployculture of lower trophic level fish. Chemosphere.

3. **Cheng, Z.,** Mo, W. Y., Choi, W. M., Wong, M.H., 2013 Replacing fish meal by food waste can produce quality fish with lower polycyclic aromatic hydrocarbon levels: the case of ployculture of lower trophic level fish. Marine Pollution Bulletin.

## Scientific Meetings and Workshops Attended:

1. 7th International Conference on Marine Pollution and Ecotoxicology. 17- 21 June, 2013, Hong Kong,

Paper presented during the meeting:

**Cheng, Z.,** Mo, W. Y., Choi, W. M., Wong, M.H., 2013 Replacing fish meal by food waste can produce quality fish with lower polycyclic aromatic hydrocarbon levels: the case of ployculture of lower trophic level fish.

2. Croucher Advanced Study Institute Innovative Technologies for Soil Remediation, December 1-5, 2008, Hong Kong

3. The Progress and Challenge of Effectiveness Evaluation of Persistent Organic Pollutants (POPs) in Asia-Pacific Region under Stockholm Convention, 27 February 2009, Hong Kong

4. Workshop on Persistent Organic Pollutants (POPs) New Development and Challenges in the Implementation of the Stockholm Convention, 10-11 April 2012, Hong Kong

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