The influence of mariculture on mercury distribution in sediments and fish around Hong Kong and adjacent mainland China waters

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A B S T R A C T

To study the influence of mariculture on mercury (Hg) speciation and distribution in sediments and cultured fish around Hong Kong and adjacent mainland China waters, sediment samples were collected from six mariculture sites and the corresponding reference sites, 200–300 m away from the mariculture sites. Mariculture activities increased total mercury, organic matter, carbon, nitrogen and sulfur concentrations in the surface sediments underneath mariculture sites, possibly due to the accumulation of unconsumed fish feed and fish excretion. However, methylmercury (MeHg) concentrations and the ratio of MeHg to TlHg (% MeHg) in sediments underneath mariculture sites were lower than the corresponding reference sites. The % MeHg in sediments was negatively correlated (r = −0.579, p < 0.05) with organic matter (OM) content among all sites, indicating that OM may have inhibited Hg methylation in surface sediments. Three mariculture fish species were collected from each mariculture site, including red snapper (Lutjanus campechanus), orange-spotted grouper (Epinephelus coioides) and snubnose pompano (Trachinotus blochii). The average MeHg concentration in fish muscle was 75 μg kg −1 (wet weight), and the dietary intake of MeHg through fish consumption for Hong Kong residents was 0.37 μg kg −1 week −1, which was lower than the corresponding WHO limits (500 μg kg −1 and 1.6 μg kg −1 week −1).© 2010 Elsevier Ltd. All rights reserved.

1. Introduction

Industrial and urban centers along coastal areas are major sources of mercury (Hg) pollution in oceans. Coastal sediments contain Hg concentrations that are 3–4 orders of magnitude higher than natural background levels (Mercone et al., 1999). In sediment, inorganic mercury (Hg(II)) is readily transformed by anaerobic bacteria into methylmercury (MeHg), a highly toxic and bioaccumulative form. Accumulation of MeHg in aquatic systems continues to pose a threat to fish and other biota including human beings.

The influence of aquaculture on sediment is due to deposition of organic wastes resulting from food supplies and fish excretion. Increased oxygen consumption during organic waste degradation cause progressively more anoxic conditions at the sediment/water interface, which may lead to the mobilization and potential methylation of Hg(II) (Cossa and Gobeil, 2000). However, other studies showed that organic matter (OM) can inhibit MeHg production in sediments. Degradation of OM in aquatic environments lead to production of organic ligands that can potentially form complexes with Hg(II), thereby, reducing Hg bioavailability to methylation bacteria (Ullrich et al., 2001).

Heavy metal concentrations in sediments and fish collected from Hong Kong coastal waters have previously been studied (Cheung et al., 2008). To date, no study has been conducted on Hg concentrations and speciation in sediments underneath mariculture sites of Hong Kong. This article presents Hg speciation and distribution in sediments of mariculture and reference sites, as well as Hg concentrations in different cultured fish species. The objectives of this study were to investigate: (1) the spatial Hg pollution in marine sediments around Hong Kong and adjacent mainland China waters; (2) the influence of OM enrichment due to mariculture activities on Hg speciation and distribution in sediments; and (3) the content of Hg accumulation in cultured fish and its human health risk assessment.

2. Materials and methods

2.1. Study areas

Six mariculture sites around Hong Kong and adjacent mainland China coastlines, including Tung Lung Chau (TLC), Sai Kung (SK), Sam Mum Tsai (SMT), Tsing Yi (TY), Mirs Bay (MB) and Xi Xiang...
(XX) were selected as sampling sites (Fig. 1). The detailed information for these sampling sites is shown in Table S1.

2.2. Sampling procedures

Surface sediments were collected from mariculture and the corresponding reference sites, 200–300 m away from the cages using a grabber. Triplicate sampling points, distributed under one of the floating rafts, were selected at each mariculture site (Fig. S1).

Maricultured fish were collected including Red snapper (*Lutjanus campechanus*) (RS), Orange-spotted grouper (*Epinephelus coioides*) (OG) and Snubnose pompano (*Trachinotus blochii*) (SP). Four to six replicates for each species were collected from each site except from XX. Fish samples from XX were purchased from wet markets owing to the unavailability of live fish onsite. XX has been designated as a non-mariculture zone, with mariculture activities banned since 2004 (Shenzhen Government, 2009). In order to find out the relationships between Hg concentrations and fish size, this study intentionally paid more emphasis on fish size, by grouping fish samples in the following order: the largest, the smallest, the second largest, the second smallest, etc. Fish samples were dissected in the field. Gills, liver and muscle were separately stored. Fish feed samples, originally bought from markets, were obtained from the fish farmer in TLC only. Although fish farmers from other sampling sites confirmed that their feeds were purchased from markets, they were reluctant to sell or give us the feed.

All the sediment and fish samples were stored at −20 °C immediately after collection. When shipped to the laboratory, the samples were freeze-dried, ground and homogenized.

2.3. Sample analysis

Biological and sediment samples for THg analyze were digested with concentrated nitric acid and aqua fortis in glass vials respectively. THg concentration was subsequently determined by cold-vapor atomic absorption spectrometry (Varian model VGA 77 coupled to a Varian AAS model 220FS). MeHg in sediments were extracted by HNO₃/CuSO₄/dichloromethane and back extracted to water (Liang et al., 2004). Biological samples were digested using KOH–methanol solution at 65 °C for 4 h. MeHg was determined using aqueous ethylation, purge, trap, and GC-CVAFS detection (Books Rand, MERX), following USEPA method 1630 (USEPA, 2001a). OM contents were determined using preweighed crucibles based on % of loss on ignition (% LOI), which entailed heating sediments for 6 h at 440 °C (USEPA, 2002). Sediment carbon (C), nitrogen (N) and sulfur (S) were determined by the CNS element analyzer (Elementar Vario MAX). Sediment particle size were analyzed by The Mastersizer 2000 (Malvern) and pH values were determined by ORION Model 420A.

2.4. Quality assurance of sample analysis

Two method blanks, three certified reference materials (CRMs) and 10% replicate samples accompanied each sample batch (up to 30 samples). Table S2 shows the detailed QA/QC information.

2.5. Data analyses

Difference of Hg concentrations in sediments and fish, as well as OM, C, N, S, contents and pH values in sediments among all the sites were performed by multiple-way ANOVA (data could be transformed to obtain equal variance) and K independent sample test (data could not be transformed to obtain equal variance) by SPSS 16.0 for windows. The enrichment ratio (ER) was calculated as: \[ ER = \left( \frac{X_m}{X_r} \right) \times 100/X_r, \] where \( X_m \) is the average concentration of parameters in mariculture sites, \( X_c \) is the average concentration of parameters in reference sites \( X_r \).

3. Results and discussion

3.1. Spatial distribution of THg in sediments

Table 1 shows the geochemical parameters of sediments. Two-way ANOVA (sampling sites [fixed] × mariculture effect [fixed]) was employed for testing the difference of these parameters among all sampling sites. Significant interacting effects between

![Fig. 1. Sampling sites (●) and distribution profiles of total mercury in surface sediment around Hong Kong and adjacent coastal waters. The map was constructed based on the present study, HKEPD (2007) and Shi et al. (2007).](image-url)
the two factors are exited (Table S3). Therefore, split file method was used to evaluate each factor on these parameters' distribution in sediments by one-way ANOVA.

Table S4 shows the difference of THg concentrations in sediment. Contaminant discharge location is one of the decisive factors in the geographic distribution of contaminants (Wade et al., 2008). Since XX has been designated as a pollution discharge zone (Shenzhen Government, 2009), it is expected that the rather high Hg concentration detected in XX sediments might be from sewage discharge. TY is located in the northern part of Hong Kong. The site is proximal to the third busiest port in the world with marine transportation significantly contributing to the pollution problem. TLC is a small island accessible only by ferries, with less influence imposed by human activities. The high THg concentrations observed here may be attributed to the nearby SENT Landfill. Since landfills are important Hg emission sources to the ambient environment (Feng et al., 2004), Hg emissions from the landfill may enter nearby aquatic systems by wet and dry deposition, leading to a higher Hg loading on the seabed.

Based on the present study, HKEPD (2007) and Shi et al. (2007), the ratio of MeHg to THg (% MeHg) in sediments. In corresponding reference sites, MeHg was observed in TY, MB and XX. This indicated that mariculture may increase Hg loading in sediments. The origin of environmental impact derived from intensive cage aquaculture mainly lies on the organic waste accumulation generated in fish excretion and food supply (unconsumed feed). THg concentrations in trash fish in TLC reached 79 μg kg⁻¹ (Table 3). Approximately 40% of trash fish will be unconsumed and deposited into the sediment owing to size irregularities (HKAFCD, 2009a) and their accumulation would give rise to higher THg concentration in sediments.

Table 2 shows the correlations among the geochemical parameters in surface sediments. No significant correlations were found between THg concentrations and OM, C, N and S contents as well as particle size, which were not in consistent with previous study (Hammerschmidt et al., 2008). The present results might be caused by the influence of mariculture activities. The ER of OM, C, N, and S were relatively higher than THg, indicating that mariculture activities resulted in higher loading of OM, C, N and S compared to Hg (Table 1). The disproportionate increase of these elements as well as the variance of THg concentrations in different sampling regions rendered the correlations between Hg and OM, C, N, S were not significant. Moreover, no significant correlations were noted between the ER of Hg and OM, C, N, S (Table S5), indicating that other sources of Hg are also existed in sediment underneath mariculture sites, besides mariculture activities.

3.2.2. Mariculture decreased MeHg production

MeHg concentrations in sediments ranged from 0.40 to 3.18 μg kg⁻¹ with an average of 1.27 μg kg⁻¹ (Table 1). K independent samples test was performed for analyzing the difference of MeHg concentrations among all sampling sites. For mariculture sites, MeHg concentrations in surface sediments of XX, TY and TLC were significantly higher (p < 0.05, Kruskal-Wallis H test) than the other three sites, which were consistent with THg concentrations in sediments. In corresponding reference sites, MeHg was significantly higher in TY and MB (p < 0.05, Kruskal-Wallis H test). MeHg concentrations and The ratio of MeHg to THg (% MeHg) in the surface sediments underneath mariculture site were significantly lower (p < 0.01, T-test) than the corresponding reference
sediments in SK, SMT and MB, indicating that mariculture activities may inhibit MeHg production in sediments.

MeHg formation in sediment is affected by parameters such as sulfate reducing bacteria, sulfide concentration, THg concentration, OM content, and so on (Ullrich et al., 2001). In this study, MeHg concentrations were positively correlated with THg concentrations ($r = 0.800$, $p < 0.01$) and % MeHg ($r = 0.708$, $p < 0.01$) (Table 2). This might be due to the link between THg concentrations and bioavailability of Hg to methylating bacteria (Pasquale et al., 2009). The % MeHg was negatively correlated ($r = -0.579$, $p < 0.05$, Table 2) with OM content for all sites, which indicated that OM may have inhibited Hg methylation in surface sediments. It was in agreement with previous observations on coastal sediments. The complexation of Hg with organic ligands reduced Hg bioavailability to methylation bacteria, further leading to the decline of MeHg production in sediment (Hammerschmidt et al., 2008). MeHg concentrations and the % MeHg were positively correlated with % of silt ($r = 0.609$, $p < 0.05$) and negatively correlated with % of sand ($r = -0.613$, $p < 0.05$), which indicated that the Hg adsorbed on finer-particles might be easier to be methylated. However, no relevant literatures could be cited to back up this assumption. This correlation is estimated to be related to OM content in sediments. Mariculture activities increase the % of sand in sediments by inputting organic waste with larger particle size. Thus, significant correlations observed between particle size with MeHg concentrations might be indirectly correlated, which induced by the increase of particulate OM in sediments. Accordingly, the increase of allochthonous organic compounds input with large specific surface area, e.g., black carbon, may affect the production and mobilization of MeHg by altering the bioavailability of Hg buried in the sediments (Hammerschmidt et al., 2008).

### 3.3. Hg in cultured fish

#### 3.3.1. Hg levels in tissues of cultured fish

Table 3 summarizes the mean values and ranges of THg and MeHg concentrations in the tissues of three fish species. Three-way ANOVA (sampling sites [fixed] × fish species [fixed] × fish tissues [fixed]) was used for testing the difference of THg and MeHg concentrations in fish tissues. Interacting effect was observed for every two factors (Table S6). Therefore, split file method was used for evaluating each factor on Hg distribution in fish tissues. In general, THg concentrations in OG decreased in the order of liver > gills > muscle ($F = 27.0, p < 0.001$, SNK test). THg in RS decreased in the order of liver > gills > muscle ($F = 22.0, p < 0.001$, SNK test). MeHg in OG was in the order of muscle > liver > gills ($F = 88.5, p < 0.001$, SNK test), and MeHg in muscle of RS was significantly higher than liver and gills ($F = 12.4, p < 0.001$, SNK test).

As a detoxification and storage organ, the liver is able to accumulate large quantities of pollutants, e.g., Hg, through active involvement in pollutants metabolism (Gonzalez et al., 2005). Relatively low MeHg concentrations and % MeHg were found in liver in this study ($39.8 \pm 21.3\%$ for OG and $37.6 \pm 17.0\%$ for RS), which...
might be related to detoxification process that would demethylate MeHg to Hg(II) in the liver (Eagles-Smith et al., 2009).

The present results showed that the % MeHg in muscle for OG, RS and SP were 80.8 ± 6.4%, 81.3 ± 7.2% and 66.3 ± 8.3%, respectively. In muscle, MeHg is accumulated mainly under cysteine thiol complexes (Harris et al., 2003), and is excreted slowly with a half-life of approximately 400 d (Downs et al., 1998). Thus, MeHg is the predominant species of Hg in revealed fish muscle.

Gills are considered as the main entry for Hg(II) presented in aqueous phase, as they possess wide surface areas and continuously contact with the external medium (Mieiro et al., 2009). The present data showed that fish gills contained relatively high concentrations of Hg(II) than MeHg, which may be attributed to the majority of Hg in water exists as Hg(II) (Klinck et al., 2005) as well as the fact that gills function as organs of toxicant excretion.

3.3.2. Hg level in fish feed

The diet of cultured fish in Hong Kong is commonly comprised of trash fish and feed pellet. Significantly higher THg (F = 0.986, p < 0.001, T-test) and MeHg (F = 4.39, p < 0.001, T-test) concentrations in trash fish were observed than those in feed pellet (Table 3). Trash fish are the fishery by catch supplied to operators (HKAFCD, 2009a). Although prescriptions of feed pellet are kept as commercial secrets, ingredients generally include soybean meal, fishmeal and plant by-product (Hertramf and Piedad-Pascual, 2000). Fishmeal is made by baking and grounding juvenile fish. Hg may be reduced in the production process through baking at high temperatures and mixing low Hg content ingredients into the feed pellet.

Owing to high fat content and low cost compared to feed pellets, trash fish have been used as the major feed for maricultured fish (HKAFCD, 2009a). However, using trash fish as fish feed increases Hg content in fish tissues since fish are known to readily accumulate MeHg from their diet (Hall et al., 1997), and unconsumed trash fish might increase Hg loading in the seabed. It is therefore recommended that feed pellets replace trash fish because of lower Hg contents and its ability to supply full scale nutrition by inclusion of vitamin complexes, plant proteins and minerals.

3.3.3. Correlation between Hg levels and fish size

Among the three fish species analyzed, OG and RS were generally larger than SP in terms of length and weight (Table 3). THg concentrations in muscle of OG and RS were significantly higher (F = 19.3, p < 0.011, SNK test) than SP. It has been widely accepted that larger fish tend to accumulate substantial concentrations of mercury in their muscle (Kannan et al., 2003). Thus there was no positive correlation between THg and MeHg concentrations in sediment. However, it should be noted that the variability of MeHg concentrations in the muscle of the RS collected from all the sampling sites was relatively small, without significant difference. This may be the result of the limited sample size used in this study. As mariculture environments play a fundamental role in producing quality and safe fish for human consumption, the correlations between Hg levels in sediments and fish warrant further detailed studies.

3.3.4. Do contaminated areas increase fish Hg level?

Table 4 shows the correlation between Hg concentrations in fish tissues and sediment. Data of XX were not included in the calculations since the fish samples were bought from a wet market making it difficult to trace the sources of these fish.

The relationship between Hg concentration of sediments and fish may be affected by various factors such as Hg speciation in sediment and the species-specific accumulation (Kannan et al., 1998). According to Table 4, significant correlations (p < 0.05 Two-tailed test) between MeHg concentrations in sediment and MeHg concentrations in muscle and gills of OG were observed. This relationship may be attributed to the ability of MeHg in sediments to combine effectively with extracellular thiol, e.g., cysteine, facilitating MeHg uptake by fish (Schaef and Morel, 2009). Conversely, negative correlations (p < 0.05, Two-tailed test) were observed between the MeHg concentrations in muscle of RS and the THg and MeHg concentrations in sediment. However, it should be noted that the variability of MeHg concentrations in the muscle of the RS collected from all the sampling sites was relatively small, without significant difference. This may be the result of the limited sample size used in this study. As mariculture environments play a fundamental role in producing quality and safe fish for human consumption, the correlations between Hg levels in sediments and fish warrant further detailed studies.

3.3.5. Human dietary exposure

The primary environmental source of human Hg exposure is seafood (USEPA, 2001b). Consequently, it is desirable to regulate seafood consumption to minimize the risk of Hg accumulating to toxic levels in consumer populations. Current international safety guidelines, established by the Joint FAO/WHO Expert Committee on Food Additives and Contaminants, recommend a maximum tolerable weekly intake level (TWI) for Hg of 1.6 µg kg⁻¹ (body weight) for women of childbearing age and 3.3 µg kg⁻¹ for the general population (JEFCA, 2003).

In 2008, about 300 tonnes of marine fish were consumed per day by approximately 7.0 million people in Hong Kong (HKAFCD 2009b). The present study indicated that the three species of cultured fish contained an average MeHg content of 75 µg kg⁻¹ resulting in a weekly intake of approx. 0.37 µg kg⁻¹ of MeHg (body weight as 60 kg), which amounted to 23.1% and 11.2% of the maximum tolerable level for women of childbearing age and the general population respectively. However, the average consumption rate may not accurately reflect the truth. High consumption rate group would take in much more MeHg through dietary exposure. Dickman and Leung (1998) showed that the average person in Hong Kong consumes fish or shellfish four or more times a week averaging about 60 kg of fish per year. Based on this consumption rate, the dietary intake of Hg for a person in the high fish consumption rate group in Hong Kong would reach 1.44 µg kg⁻¹ week⁻¹, almost equaled to the maximum tolerable level for women of childbearing age.

4. Conclusions

Mariculture increased Hg loading in sediment, with the major sources derived from uneaten fish feed (trash fish and fish pellet).

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**Table 4**

Correlations of mercury concentrations between sediment and fish tissues.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissues</th>
<th>n</th>
<th>Sediment</th>
<th>THg r p</th>
<th>MeHg r p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange-spotted grouper</td>
<td>Muscle</td>
<td>5</td>
<td>0.746</td>
<td>0.148</td>
<td>0.935</td>
</tr>
<tr>
<td></td>
<td>THg</td>
<td>5</td>
<td>0.831</td>
<td>0.081</td>
<td>0.958</td>
</tr>
<tr>
<td></td>
<td>MeHg</td>
<td>5</td>
<td>0.475</td>
<td>0.148</td>
<td>0.138</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>5</td>
<td>0.190</td>
<td>0.760</td>
<td>0.427</td>
</tr>
<tr>
<td></td>
<td>Gills</td>
<td>5</td>
<td>0.130</td>
<td>0.835</td>
<td>0.293</td>
</tr>
<tr>
<td>Red snapper</td>
<td>Muscle</td>
<td>4</td>
<td>-0.260</td>
<td>0.740</td>
<td>-0.473</td>
</tr>
<tr>
<td></td>
<td>THg</td>
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<td>-0.973</td>
<td>0.027</td>
<td>-0.985</td>
</tr>
<tr>
<td></td>
<td>MeHg</td>
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<td>0.887</td>
<td>0.113</td>
<td>0.859</td>
</tr>
<tr>
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<td>Liver</td>
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<td>0.527</td>
<td>0.473</td>
<td>0.718</td>
</tr>
<tr>
<td></td>
<td>Gills</td>
<td>4</td>
<td>0.843</td>
<td>0.157</td>
<td>0.581</td>
</tr>
<tr>
<td></td>
<td>MeHg</td>
<td>4</td>
<td>0.779</td>
<td>0.221</td>
<td>0.879</td>
</tr>
</tbody>
</table>
and fish excretions. However, OM beneath floating cages inhibits Hg(II) methylation. This is the first study on the MeHg concentrations in sediment underneath mariculture sites. Further studies are needed to explore the role of specific components within the organic portion of the sediments on Hg methylation and demethylation. It is recommended that trash fish should be replaced by feed pellet since Hg concentration was significantly lower (p < 0.001, T-test) in feed pellet. Hg concentrations in cultivated fish and the average dietary intake of MeHg through the consumption of cultured fish were lower than the WHO guidelines for protecting human health.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.chemosphere.2010.10.061.

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