Formation and distribution of methylmercury in sediments at a mariculture site: a mesocosm study

Peng Liang · Cheung-Lung Lam · Zhang Chen · Hong-Sheng Wang
Jian-Bo Shi · Sheng-Chun Wu · Wen-Xiong Wang · Jin Zhang ·
Hailong Wang · Ming-Hung Wong

Abstract

Purpose The aim of the present study was to investigate the differences of methylmercury (MeHg) formation and distribution between mariculture (aquaculture) sediments (MS) and reference sediments (RS) collected from a site in Hong Kong.

Materials and methods The MS and RS samples were split into four batches, three of which were spiked with $HgCl_2$ aqueous solution to a concentration of 0.8, ,2 and 8 mg k g^{-1} in sediment samples SP1, SP2, and SP3, respectively, while the rest served as a control batch (referred to as C).

Results and discussion The results showed that the highly Hg-polluted sediment produced greater amounts of MeHg. During the culture period, MeHg concentrations in sediments decreased over time. The decreasing percentage increased in the order of SP3<SP2<SP1, which might be due to the inhibition of MeHg degradation by high Hg concentrations. The mean value of MeHg concentrations and %MeHg of the total Hg (THg) in MS was significantly lower than those in RS, possibly due to the complexation of Hg with organic ligands, leading to lower Hg bioavailability for methylation bacteria. The distribution coefficient of THg (K_dT) was relatively high in MS compared to RS, indicating that the former had a greater number of binding sites for Hg adsorption.

Conclusions Methylmercury formation was inhibited in MS, probably due to increased complexation of Hg^{2+} with organic matter and adsorption of Hg to MS. Furthermore, the mean value of K_dT in MS was relatively high when compared to RS, which illustrates that MS sediments have more binding sites than RS for adsorption of Hg.

Keywords Aquaculture · Marine sediment · Mercury · Methylation rate

P. Liang · J. Zhang · H. Wang · M.-H. Wong (✉)
School of Environmental and Resource Sciences, Zhejiang Agricultural and Forestry University, Lin'an, Zhejiang, China
e-mail: mhwong@hkbu.edu.hk

P. Liang · C.-L. Lam · Z. Chen · H.-S. Wang · J.-B. Shi ·
S.-C. Wu · M.-H. Wong
Croucher Institute for Environmental Sciences, and Department of Biology, Hong Kong, People's Republic of China

J.-B. Shi
China Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China

W.-X. Wang
Section of Marine Ecology and Biotechnology, Division of Life Science, The Hong Kong University of Science and Technology, Hong Kong, China

1 Introduction

Methylmercury (MeHg) is a highly toxic and bioaccumulative compound. Its accumulation in aquatic systems continues to pose a threat to fish and other biota, including humans. The process of MeHg formation and bioaccumulation in aquatic systems has, therefore, been researched extensively over the last 40 years (Schaefer and Morel 2009). Sulfate-reducing bacteria are thought to be the principal agents responsible for MeHg production in sediments (King et al. 2000), in which they convert a small fraction of Hg^{2+} to MeHg over time. However, a number of environmental factors are known to affect MeHg formation by influencing the supply of

bioavailable Hg^{2+} and/or the activity of methylating microbes. These mainly include Hg concentrations, organic matter (OM) contents, sulfide concentrations, salinity, and redox potentials (Eh) (Ullrich et al. 2001).

Global aquaculture production has increased rapidly over the past four decades, contributing significantly to the world's supply of fish for human consumption (Subasinghe et al. 2009). Environmental issues resulting from intensive aquaculture activities are basically attributable to the deposition of OM from unconsumed fish feed and to fish metabolism (Aguado-Gimenez and Garcia-Garcia 2004). Increased oxygen consumption during OM degradation causes progressively severe anoxic conditions at the sediment–water interface, which may lead to the mobilization and potential methylation of Hg^{2+} (Cossa and Gobeil 2000); however, OM contains large amounts of functional moieties. Organic matter is also an important sediment component that is responsible for binding metal cations both in the solid phase and as soluble complexes (Hesterberg et al. 2001). Furthermore, OM acts as a major control on Hg^{2+} availability to methylating bacteria as well as influences their geographical distribution and sediment–water partitioning of Hg^{2+} by strong complexation between the reduced sulfur group with Hg^{2+} (Hammerschmidt et al. 2008). A number of studies investigated the mechanism and production rate of MeHg in different types of sediment, mainly concentrating on the influence of factors such as sulfide concentrations, OM content, and Hg^{2+} concentrations (Merritt and Amirbahman 2009). However, none of them focused on the formation and distribution of MeHg in sediments and water at mariculture (a specific form of aquaculture) sites.

Our previous results showed that MeHg concentrations in the sediments of the mariculture site of interest were lower than at the reference site, the latter being located 200–300 m away from the former (Liang et al. 2011). However, there was a lack of quantitative analysis on the production rate of MeHg in sediments and pore water as well as the distribution of the total Hg (THg) and MeHg between the sediments and water at the mariculture site. Hence, a simulative mesocosm experiment was conducted to investigate the differences in MeHg formation and distribution between mariculture sediments (MS) and reference sediments (RS).

2 Materials and methods

2.1 Experimental design

Samples of the surface layer of MS and RS (top 5 cm) were collected from a mariculture site located in Sai Kung, Hong Kong (22°20.286′ N, 114°19.143′ E) and a reference site (22°20.253′ N, 114°18.461′ E) located 200–300 m away from the mariculture site, respectively. Carnivorous fish, e.g., red snapper (*Lutjanus campechanus*), orange-spotted grouper (*Epinephelus coioides*), and snubnose pompano (*Trachinotus blochii*) are cultured in Sai Kung mariculture zones. The depth of water in the mariculture zones is around 10 m. The basic properties of MS and RS are shown in Table 1. The concentrations of THg, OM, carbon, nitrogen, and sulfur were demonstrated to be higher in MS, but MeHg was higher in RS. Samples of MS and RS were split into four equal batches, three of which were spiked with $HgCl_2$ aqueous solution at concentrations of 0.8, 2, and 8 mg k g^{-1} (dry weight basis) for samples SP1, SP2, and SP3, respectively, while the remaining samples served as the control batch (referred to as C). Each batch was further divided into two groups, the mariculture group and the reference group (Fig. 1).

$HgCl_2$ solution was prepared by dissolving $HgCl_2$ powder (M1136, Sigma-Aldrich, USA) in tap water, without any pH value adjustments. The Hg concentrations used in this study (0.8, 2, and 8 mg k g^{-1}) were adopted from previous studies that reported the ambient Hg concentrations in sediments of the Asian area (Shi et al. 2005, 2007). A 100-ml solution with adequate Hg^{2+} was mixed with 10 kg of sediment. Plastic sticks were used for mixing the sludge by stirring. Triplicate sediments for each batch were analyzed for THg concentration to check the homogeneity of sludge. The variance of all the treatments ranged from 4.31 to 17.22 %

There were two experimental periods: (1) the 4-day aging period, where the mixtures were stored in a cold room (i.e., 4 °C) (Lawrence and Mason 2001), and (2) the 56-day culture period, in which sediments (10 kg) and marine water (36 l) were placed in outdoor aquariums (90 l, 30×30×100 cm). RS were placed in each aquarium at a depth of 30 and 25 cm for the reference and mariculture group, respectively, whilst a 5-cm layer of MS was placed at the top of the mariculture group. Thus, 5 cm of MS and 25 cm of RS in the mariculture group were placed to simulate the actual mariculture environment,

Table 1 Basic properties of the sediment collected from the mariculture (MS) and reference (RS) sites, Sai Kung area, Hong Kong (mean±S.D.) (*n*=6)

	Total mercury (ng g^{-1})	Methylmercury (ng g^{-1})*	Organic matter (%)	Carbon (%)*	Nitrogen (%)*	S (%)*
MS	101±6.14	0.313±0.032	12.02±0.95	3.81±0.04	0.377±0.024	0.920±0.036
RS	95.3±5.07	0.511±0.019	10.38±0.75	2.80±0.07	0.214±0.013	0.526±0.031

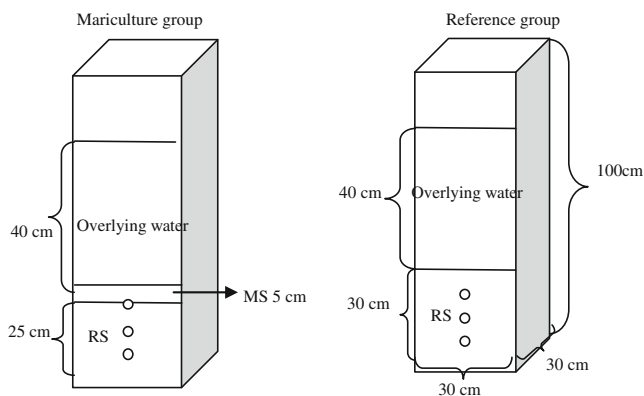*$p<0.05$ (significant difference existed between MS and RS)

Fig. 1 Design of the mesocosms. *MS* mariculture sediments, *RS* reference sediments, *unfilled circle* pore water sampling position

whereby sediments of high OM content generally cover the top 5 cm of sediments. Aluminum foil was used to cover the aquarium to keep the sediments in a photophobic condition. Marine water was prepared by dissolving commercial marine salt (Marinemix professional, Wiegand Gmbh, Germany) in tap water, with the salinity matching natural marine water (33‰). Freshwater was added on the 9th and the 41st day in order to maintain water salinity that was consistent with that of natural marine water.

2.2 Sample collection

Sediments and pore water samples were collected at the pre- and post-aging stages, with pore water samples being obtained via centrifugation of the sediments (3,500 rpm, 30 min) and the culture period lasting 56 days. The sampling frequency was higher during the initial stages since the net MeHg production in methylation experiments proved to be the highest in the first few days or weeks of equilibration (Merritt and Amirbahman 2009). In the first 2 weeks, samples were collected once every 2 days (2nd, 4th, 6th, 8th, 10th, 12th, and 14th day) and once per week for the remainder of the experiment. Sediment samples were collected using acrylic tubes and divided into three parts representing the surface (0–10 cm), middle (10–20 cm), and bottom (20–30 cm) layers. Water samples included overlying water and pore water, whereby the former was collected from the interface between sediments and water by siphon and the latter by using the rhizosphere soil moisture sampling method from different vertical layers (see Fig. 1). Sediment samples were stored at −20 °C immediately after collection and freeze-dried, homogenized, and sieved through a 2-mm mesh. Water samples were filtered through a 0.45-μm membrane filter and added to 0.2 % (v/v) of ultra-pure H_2SO_4 within 24 h.

2.3 Analytical methods

Total Hg in water samples was oxidized with 0.5 % BrCl and subsequently determined by cold vapor atomic fluorescence spectrometry (CETAC QuickTrace™ M-8000), using NH_2–OH·HCl and $SnCl_2$ for destroying free halogens and converting Hg^{2+} to volatile Hg^0, respectively (USEPA 2002). THg in sediments was detected by a direct Hg analyzer (Milstone, DMA-80) based on thermal decomposition, amalgamation, and atomic absorption spectrophotometry, following the US EPA Method 7473 (USEPA 2007).

Methylmercury (MeHg) in water was measured following the USEPA Method 1630 (USEPA 2001) in which an aliquot of 50-ml sample was placed in a 60-ml fluoropolymer distillation vessel. The distillation was carried out at 125 °C under Hg-free N_2 flow until approximately 45 ml of water was collected in the receiving vessel. MeHg in sediments was extracted by HNO_3 and $CuSO_4$ and then leached by dichloromethane and back-leached by water (Liang et al. 2004). MeHg was determined using aqueous ethylation, purge, and gas chromatography–cold vapor atomic fluorescence spectrometry detection (Books Rand, MERX). Water salinity and temperature were monitored by a portable meter (Model 1230, Thermo Orion, USA). OM was measured based on % loss on ignition (% LOI) (USEPA, 2002).

Distribution coefficients of THg and MeHg between sediment and water were calculated by using THg and MeHg concentrations in sediment compared to THg and MeHg concentrations in pore water.

2.4 Quality assurance and quality control of results

Quality assurance and quality control of THg and MeHg determinations were carried out by using duplicates, method blanks, matrix spikes, and certified reference materials, with further details shown in Table S1 of the "Electronic supplementary material".

2.5 Data analyses

The differences between the MS and RS parameters were tested by two-way ANOVA using SPSS 16.0 for windows. Two-way ANOVA (spike concentrations [fixed]×mariculture effect [fixed]) was employed for testing the difference in the parameters (THg, MeHg, %MeHg, and OM) that measured and calculated the MS and RS in sediments and pore water for all batches (Table S2, "Electronic supplementary material"). All the data were log-transferred as they did not meet the requirement of normality distribution. Significant interaction effects between the two factors (Hg concentration and sediment type) were observed; therefore, the split file method was used to evaluate each factor on the distribution of these parameters using one-way ANOVA.

3 Results and discussion

3.1 Effect of THg concentrations on Hg^{2+} methylation

Table 2 shows the concentrations of THg and MeHg as well as the percentage of MeHg to THg (%MeHg) in sediments and pore water for the pre- and post-aging periods. The average THg concentration in sediments for the spiked batches was about 70 % of the target value after the aging period, which may be due to the evaporation of Hg during the spiking procedure. After the aging period, MeHg concentrations in sediments and pore water, as well as %MeHg in pore water alone, decreased in the order of SP3>SP2>SP1>C. However, %MeHg in sediments followed the order of C=SP3<SP2<SP1. MeHg concentrations of sediments and pore water at the post-aging stage were significantly higher ($p < 0.001$, t-test) than at the pre-aging stage, indicating that MeHg was produced during the aging period.

Methylmercury formation in sediments under anoxic conditions has been demonstrated to occur when sulfate-reducing bacteria respire in the presence of Hg^{2+} (King et al. 2000; Harmon et al. 2004; Hollweg et al. 2009). The results of this study show that the higher the spiked Hg^{2+} concentration in sediment, the greater the amount of MeHg produced in sediments and pore water. This can be explained by the link between THg concentration in sediments and Hg bioavailability for bacterial methylation (Drott et al. 2008). Thus, sediment polluted with high Hg levels would be expected to produce more MeHg and pose potential risks to aquatic organisms.

3.2 MeHg degradation during the culture period

Figure 2 shows the temporal variations in the average value of the "changing ratio" for MeHg concentration and %MeHg in the surface layer sediment, where the changing ratio is calculated as the value of MeHg concentration or %MeHg on day X divided by the value of MeHg concentration or %MeHg on day 1 (data for middle and bottom sediments are shown in Figs. S1 and S2, "Electronic supplementary material"). MeHg concentrations and %MeHg in the solid phase declined slightly with time. The highest values of the spiked batches were observed on the 1st and 10th days.

Figure 3 shows the temporal variations in average value of the changing ratio for MeHg concentration and %MeHg in surface layer pore water during the culture period (data for pore water in middle and bottom sediments are shown in Figs. S3 and S4, "Electronic supplementary material"). Elevated MeHg concentrations in pore water were observed from the 10th to the 14th day. After 14 days, MeHg decreased substantially until the end of the experimental period. The %MeHg had a similar trend to MeHg concentrations during the culture period, where higher values were observed on the 1st day and from the 10th to the 28th day.

Methylmercury levels in water and sediments decreased with time during the culture period, which may be attributed to the decrease in methylation or demethylation of MeHg. The decreasing percentage of MeHg in water and sediments was calculated by dividing the difference in MeHg concentration in water and sediment between the 1st and the 56th day of the culture period by the concentration on the 1st day.

Table 2 The mean values of total mercury (THg) and methylmercury (MeHg) concentrations in mariculture (MS) and reference (RS) sediments and pore water in the aging period (mean±S.D, $n=3$)

		THg (ng g^{-1})		MeHg (ng g^{-1})		%MeHg	
		RS	MS	RS	MS	RS	MS
Sediment[a]							
Pre-aging		95.3±5.07	101±6.14	0.511±0.019	0.313±0.032	0.537±0.033 %	0.311±0.031 %
Post-aging	Control	111±26.0	95.6±26.7	0.608±0.190	0.413±0.063	0.641±0.252 %	0.464±0.162 %
	SP1	560±68.3	583±63.7	12.7±1.13	12.8±0.43	2.30±0.437 %	2.66±0.889 %
	SP2	1,348±50.5	1,453±137	21.0±5.58	14.6±3.76	1.57±0.298 %	0.991±0.569 %
	SP3	6,196±522	5,747±758	36.8±4.75	37.3±9.19	0.618±0.137 %	0.659±0.203 %
Pore water[b]							
Pre-aging		12.3±1.96	9.40±1.42	0.445±0.037	0.255±0.040	3.68±0.39 %	2.81±0.28 %
Post-aging	Control	15.6±6.62	12.6±2.40	0.650±0.621	0.351±0.140	3.79±2.87 %	2.73±0.69 %
	SP1	184±68.5	243±105	79.6±45.1	91.2±41.3	43.6±24.8 %	37.3±0.9 %
	SP2	316±98.1	314±60.8	185±90.8	130±22.4	56.8±16.3 %	43.2±15.5 %
	SP3	453±118	675±163	258±54.6	320±56.5	59.4±15.1 %	47.6±1.47 %

[a] THg and MeHg in ng g^{-1}

[b] THg and MeHg in ng l^{-1}

Fig. 2 Temporal variations in average value of changing ratio for MeHg concentration and %MeHg in surface sediment (the changing ratio was calculated as the MeHg concentration or %MeHg at the different sampling days divided by the MeHg concentration or %MeHg in day 1, respectively) for the mariculture (*MS*) and reference (*RS*) sediment
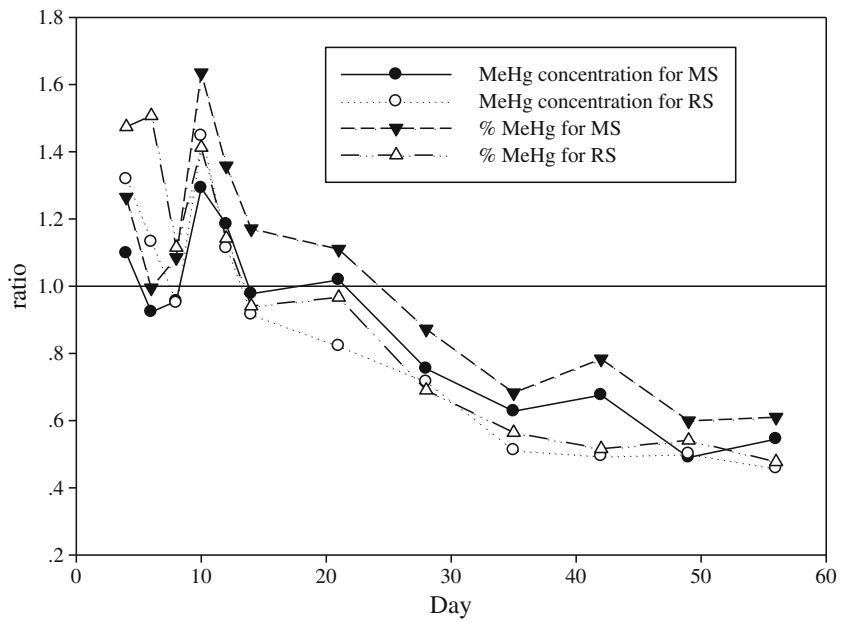
The decreasing percentages of MeHg in the pore water of RS were 96, 97, and 96 % for SP1, SP2, and SP3, respectively, and were 96, 89, and 96 % in the pore water of MS for SP1, SP2, and SP3, respectively. However, the decreasing percentages of MeHg in RS were only 64, 52, and 36 % for SP1, SP2, and SP3, respectively, and were 68, 49, and 44 % for SP1, SP2, and SP3, respectively, for MS. The decreasing percentages of MeHg in sediments were significantly lower ($p < 0.01$, *t*-test) than pore water for the spiked batches, which indicates that decreasing MeHg occurred easily in the liquid phase. The decreasing percentages of MeHg in sediments increased in the order of SP3 < SP2 < SP1. In this case, the net MeHg decrease in sediments

appears to be inhibited by higher Hg concentrations contained within the sediments, whereby the decrease occurs through microbially driven processes and abiotic pathways. The principal biotic pathways for MeHg decreases are oxidative demethylation and reductive MeHg degradation via the Hg resistance (*mer*) operon. *Mer*-specific functions are sensitive to both Hg concentration and cell density (Rasmussen et al. 1997). Higher Hg^{2+} and MeHg concentrations affect the potential for microbial Hg resistance (Merritt and Amirbahman 2009). Thus, the low decreasing percentage of MeHg in sediments highly contaminated with Hg may be due to the inhibition of *mer*-specific function by the high Hg concentrations.

Fig. 3 Temporal variations in average value of changing ratio for MeHg concentration and %MeHg in surface pore water (calculated as MeHg concentration or %MeHg at the different sampling days divided by the MeHg concentration or %MeHg in day 1, respectively) for the mariculture (*MS*) and reference (*RS*) sediment
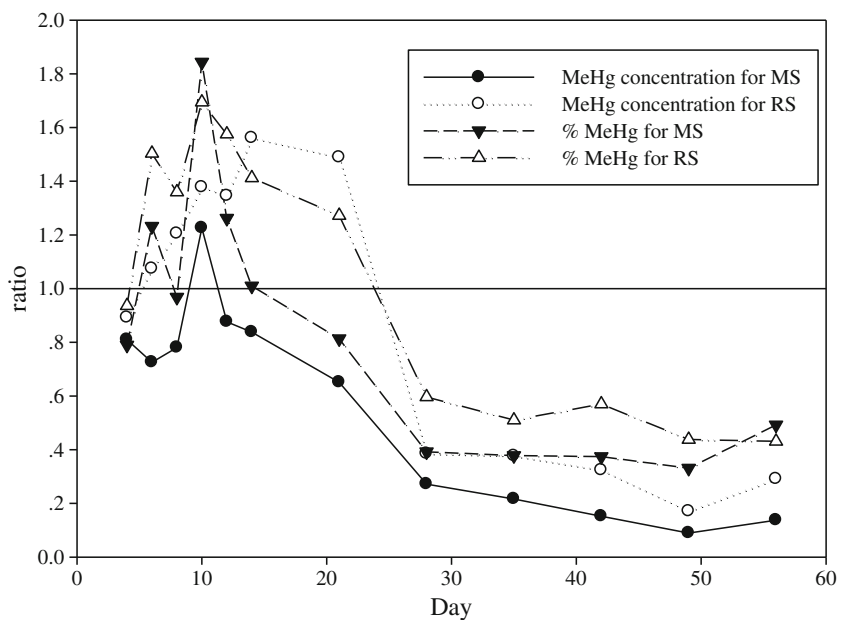
3.3 The differences between MS and RS on MeHg formation and degradation

Total Hg concentrations and OM content were significantly higher ($p < 0.05$, t-test) in MS than RS for all batches (see Table 2). MeHg concentrations and %MeHg in MS were significantly lower than RS for SP1 and SP2, but not for C and SP3. However, no significant differences were observed between MS and RS for THg concentrations, MeHg concentrations, and %MeHg in pore water. These results indicate that Hg methylation was most likely inhibited in the solid phase of MS but not in pore water, which may contribute to the OM being buried in MS. OM can inhibit MeHg formation in sediments by complexing the Hg^{2+} with organic ligands (e.g., sulfur-rich functional groups), further reducing the bioavailability of Hg^{2+} for bacterial methylation (Hammerschmidt and Fitzgerald 2004; Hammerschmidt et al. 2008). Thus, lower Hg methylation in MS may be due to the complexation role between organic ligands with Hg^{2+}.

However, higher MeHg concentration in sediment and pore water of MS than RS was observed after the aging period (see Table 2), which indicated that MeHg formation in MS was higher than RS in some situations. This was in contrast with the results during the culture period. Higher amounts of humic acid and fulvic acid might have existed in MS due to the accumulation of OM compared to RS. Humic acid and fulvic acid can also cause MeHg formation through chemical methylation (Celo et al. 2006). Thus, higher OM in MS could also contribute to Hg methylation in sediment. It should be noted that the role of OM on Hg methylation is still a debatable topic and, therefore, future work is needed to examine the role of OM on Hg methylation in more detail.

In addition, antibiotics have been used for disease prophylaxis and body growth (Holmström et al. 2003). However, it has been estimated that a minimum of 75 % of antibiotics are exported to the surrounding environment and accumulate in the sediment (Lalumera et al. 2004). The accumulation of antibiotics in sediment would have a negative effect on the structure or the activity of bacteria (Tendencia and de la Peña 2001), such as sulfate-reducing bacteria. As a consequence, Hg methylation in MS may be inhibited by the accumulation of antibiotics in sediment since sulfate-reducing bacteria are thought to be the principal agent responsible for MeHg production. It is acknowledged that the complexation role between OM and Hg^{2+}, as well as the role of antibiotics on Hg methylation, was not determined. Thus, the reasons for the inhibited MeHg production in sediment were deduced. The mechanisms of MeHg formation in MS warrant further detailed studies.

3.4 The effect of salinity and temperature on net MeHg production

Surface water evaporated during the sampling period, which led to decreased water storage and increased water salinity (Fig. 4). The substantial increase of MeHg concentrations and %MeHg in sediments and pore water on the 10th day may have occurred as a result of the influence of salinity and temperature (see Fig. 4). MeHg can be more readily produced in freshwater sediments than in marine and estuarine sediments, which is generally attributed to the salinity effect. It has been demonstrated that the sea salt anions, such as S^{2-} and CO_3^{2-}, can inhibit Hg methylation by complexing with Hg^{2+} to form inorganic complex compounds, e.g., HgS and $HgCO_3$. This can reduce the bioavailability of Hg to methylating bacteria and further reduce MeHg formation (Ullrich et al. 2001). However, higher MeHg concentrations in sediments and pore water were not recorded on the 42nd day. This may have been due to the lower temperature, which decreased MeHg production by reducing the activity of the methylating bacteria (Mauro et al. 1999).

3.5 Sediment–water partitioning of Hg species

The distribution of Hg species between the solid and liquid phase controls the toxicity, transport, and bioaccumulation of Hg in sediments (Hintelmann and Harris 2004). Figure 5 shows the distribution coefficient of THg (K_dT) and MeHg (K_dM) between sediments and pore water. The K_dT and K_dM increased after 20 days for the spiked batches, resulting from the substantial decline of THg and MeHg in pore water. The K_dT in different batches increased in the order of C<SP1<SP2<SP3, which exemplifies the strong affinity of newly added Hg^{2+} to sediments. The K_d obtained differed from one substrate to another, most likely due to the
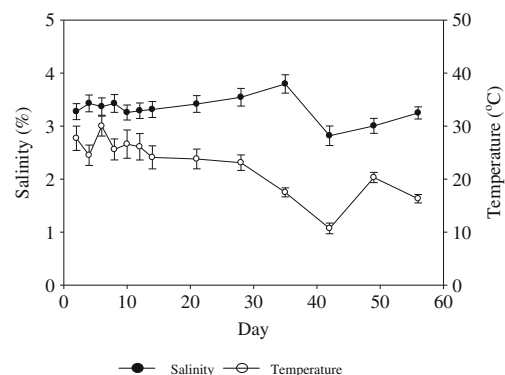

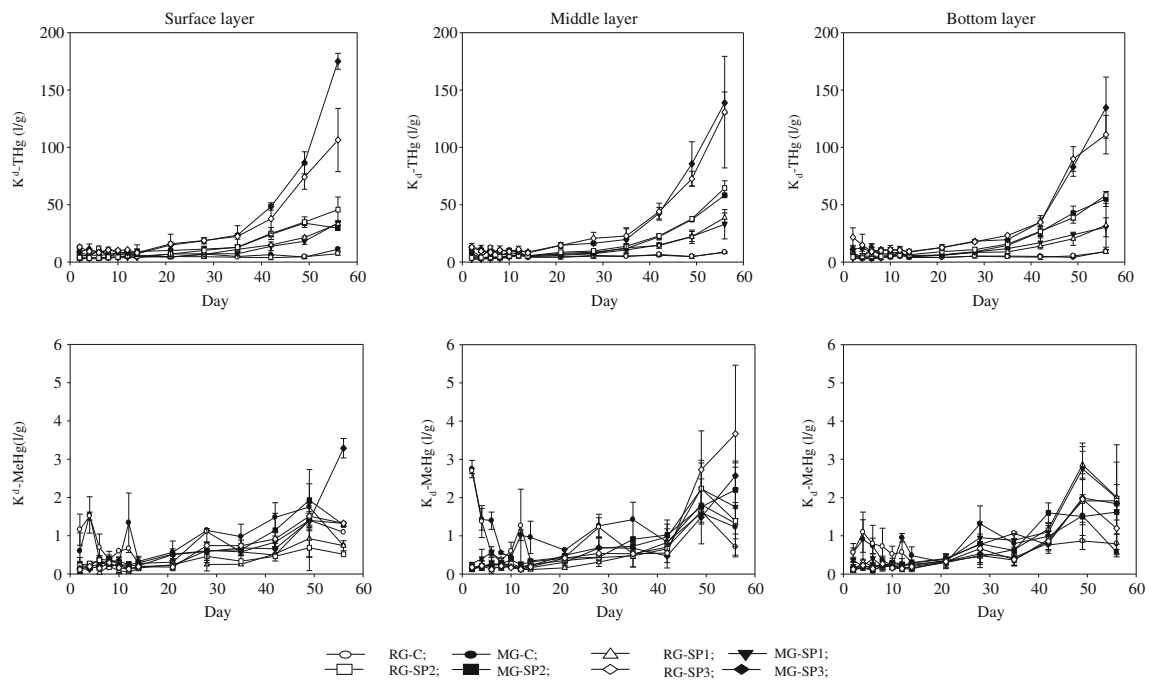Fig. 4 Temporal changes of mean values for salinity and temperature in overlying water

Fig. 5 Temporal changes of the distribution coefficients for total mercury (K_dT) and methylmercury (K_dM) for the reference group and the mariculture group

nature and abundance of the respective binding sites (Hintelmann and Harris 2004). The mean value of K_dT in MS was relatively high when compared to RS for all batches (Table 3), which illustrates that MS may have more binding sites than RS for adsorption of Hg and that the sedimentary organic materials exert a major control on the partitioning of Hg in sediments (Hammerschmidt et al. 2008).

4 Conclusions

This study evaluated MeHg formation and distribution in different sediments by using a mesocosm study. The sediment contaminated with higher Hg^{2+} levels had relatively higher MeHg potential risk since more MeHg were formed. The accumulation of OM from the unconsumed feed and fish excretion inhibited MeHg formation in sediment due to OM being complexed with Hg^{2+} reducing Hg bioavailability to methylation bacteria, leading to the further decline of MeHg production in sediment. Accordingly, the increased input of allochthonous organic compounds 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. Furthermore, the accumulation of OM in sediment promoted the adsorption of Hg^{2+} on sediment.

Table 3 Statistical results of the one-way ANOVA for the differences between mariculture sediments (MS) and reference sediments (RS) ($n=39$)

	Control	SP1		SP2		SP3	
	Results	Results	F	Results	F	Results	F
THg in sediments	MS>RS 3.68	MS>RS**	19.4	MS>RS**	10.5	MS>RS	1.71
MeHg in sediments	MS<RS 0.00	MS<RS*	5.26	MS<RS**	16.6	MS<RS	2.30
%MeHg in sediments	MS<RS 0.18	MS<RS**	10.5	MS<RS**	16.9	MS<RS	0.21
THg in pore water	MS<RS 0.05	MS>RS	1.17	MS<RS	1.48	MS>RS	1.63
MeHg in pore water	MS<RS 1.02	MS>RS	1.22	MS>RS	1.82	MS<RS	0.57
%MeHg in pore water	MS<RS 1.01	MS<RS	2.14	MS>RS	1.89	MS>RS	1.22
Organic matter	MS>RS** 98.3	MS>RS**	110	MS>RS**	133	MS>RS**	121
K_dT	MS>RS 0.17	MS>RS	0.01	MS>RS	0.30	MS<RS	0.13
K_dM	MS>RS 1.59	MS<RS	0.00	MS<RS*	4.00	MS<RS	0.82

*$p<0.05$; **$p<0.01$

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