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Mercury methylation and demethylation in Hg-contaminated lagoon sediments (Marano and Grado Lagoon, Italy)

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ABSTRACT

Mercury (Hg) transformation activities and sulfate (SO_4^{2-}) reduction were studied in sediments of the Marano and Grado Lagoons in the Northern Adriatic Sea region as part of the "MIRACLE" project. The lagoons, which are sites of clam (*Tapes philippinarum*) farming, have been receiving excess Hg from the Isonzo River for centuries. Marano Lagoon is also contaminated from a chlor-alkali plant. Radiotracer methods were used to measure mercury methylation (^{230}Hg, ^{197}Hg), methylmercury (MeHg) demethylation (^{14}C-MeHg) and SO_4^{2-} reduction (^{35}S) in sediment cores collected in autumn, winter and summer. Mercury methylation rate constants ranged from near zero to 0.054 day^{-1}, generally decreased with depth, and were highest in summer. Demethylation rate constants were much higher than methylation reaching values of $\sim$0.6 day^{-1} in summer. Demethylation occurred via the oxidative pathway, except in winter when the reductive pathway increased in importance in surficial sediments. Sulfate reduction was also most active in summer (up to 1600 nmol mL^{-1} day^{-1}) and depth profiles reflected seasonally changing redox conditions near the surface. Methylation and demethylation rate constants correlated positively with SO_4^{2-} reduction and pore-water Hg concentrations, and inversely with Hg sediment–water partition coefficients indicating the importance of SO_4^{2-} reduction and Hg dissolution on Hg cycling. Hg transformation rates were calculated using rate constants and concentrations of Hg species. In laboratory experiments, methylation was inhibited by amendments of the SO_4^{2-}-reduction inhibitor molybdate and by nitrate. Lagoon sediments displayed a dynamic seasonal cycle in which Hg dissolution in spring/summer stimulated Hg methylation, which was followed by a net loss of MeHg in autumn from demethylation. Sulfate-reducing bacteria (SRB) tended to be responsible for methylation of Hg and the oxidative demethylation of MeHg. However, during winter in surficial sediments, iron-reducing bacteria seemed to contribute to methylation and Hg-resistant bacteria increased in importance in the reductive demethylation of MeHg. The high rates of MeHg demethylation in lagoon sediments may diminish the accumulation of MeHg.

1. Introduction

Methylmercury (MeHg) is a potent neurotoxin that adversely affects brain development, especially in fetuses and young children (Clarkson, 2002). Much of the consumption of MeHg by humans is via fish, which bioaccumulate MeHg from the food web (Fitzgerald and Clarkson, 1991; Bloom, 1992). The global use of mercury (Hg) has decreased drastically in the past several years, but it remains a threat due to releases from energy generation, incineration, and mobilization from mining activities (Fitzgerald, 1993). Mercury can also be transported hundreds of km from point sources, such as Hg mines, and this Hg can become bioavailable and methylated to MeHg, thus posing a threat to wildlife and humans (Hines et al., 2000).

Shallow coastal lagoons are ideal sites for the rapid methylation of Hg to MeHg, and its bioaccumulation in animal species consumed by humans. Lagoons are usually highly productive due to nutrient inputs from rivers and streams, they accumulate labile organic matter in sediments that fuels microbial processes responsible for Hg methylation, and they are often sinks for coastal

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pollutants such as Hg and other heavy metals (Amouroux et al., 2003). In addition, lagoon environments are often ideal for mariculture due to their high productivity and their shallow and protected surroundings, and they may produce fish and bivalves that are contaminated (Covelli et al., 2008).

Despite a recent increase in studies of Hg cycling in coastal marine systems, surprisingly little is known of the factors that control MeHg formation and degradation and how it becomes bioaccumulated. MeHg is produced anaerobically by SO_4^{2-}-reducing bacteria (SRB) (Compeau and Bartha, 1985; Gilmour et al., 1992; Choi et al., 1994) and iron-reducing bacteria (Fleming et al., 2006; Kerin et al., 2006) and it has been shown that sulfur cycling is important in controlling the bioavailability and transformation of Hg species (Benoit et al., 1999; Hammerschmidt and Fitzgerald, 2004; Han et al., 2007, 2008; Hollweg et al., 2009). In addition, Hg and MeHg are particle reactive and the partitioning between solid and dissolved phases and binding to organic matter have been shown to be strongly related to the rate of Hg methylation and the fraction of total Hg present as MeHg (Mason and Lawrence, 1999; Varekamp et al., 2000; Hammerschmidt and Fitzgerald, 2004; Han et al., 2007, 2008; Kim et al., 2011).

Bacteria are also responsible for the demethylation of MeHg, which can decrease the quantity of MeHg available for accumulation through the food web (Marvin-DiPasquale et al., 2000; Barkay and Wagner-Dobler, 2005). Bacterially mediated MeHg demethylation occurs via either a reductive process that produces Hg^0 and CH_4, or by an oxidative process that produces Hg^{2+} and CO_2 (Barkay et al., 2003). The reductive process is catalyzed by enzymes of the *mer* genetic system in Hg-resistant bacteria, and is often enhanced by increased concentrations of Hg^{2+}. The oxidative process appears to be a co-metabolism of MeHg and is similar to the use of other small organic compounds (Oremland et al., 1991).

The Marano and Grado Lagoons in the northern Adriatic Sea receive Hg from an inland mining region and also from a chlor-alkali plant effluent (Covelli et al., 2008). These lagoons are sites of local mariculture, especially clam (*Tapes philippinarum*) farming and there is concern that these operations might be impacted by Hg accumulation. The present study was part of a larger process-oriented project “Mercury Interdisciplinary Research for Appropriate Clam farming in Lagoon Environment” (MIRACLE) to better understand factors controlling Hg cycling and the potential for Hg accumulation in clams. Here we describe a study of the methylation of Hg and the demethylation of MeHg in sediment profiles from both lagoons and the relationship between these activities and sedimentary SO_4^{2-} reduction and Hg speciation. Other studies in this issue describe rates at which MeHg accumulates in growing clams (Giani et al., 2012).

2. Site description

The Grado and Marano lagoon systems (45° 40′–45° 45′ N, 13° 05′–13° 35′ E) (160 km^2) is composed of two lagoons, with an average width of 5 km extending for about 35 km, along the Northern Adriatic coast (Fig. 1). The lagoons experience semi-diurnal tides (65–105 cm range), and salinities that are low (2–7) near river mouths, but reaching between 24 and 36 at tidal inlets (Covelli et al., 2009). The lagoons are separated from the sea by a longshore bar composed of islets and sand barriers. A rather deep navigable canal separates the Marano Lagoon to the west from the Grado Lagoon to the east. The primary source of suspended sediments arrives from the sea as the contribution of river deltas and from erosion of the barrier islands (Brambati, 1970). The Grado Lagoon (76 km^2) is located in the eastern sector of the system, is very shallow (<1 m, on average), and consists of tidal flats and saltmarshes. The Marano Lagoon (84 km^2) to the west is slightly deeper than the Grado Lagoon and also contains a larger surface area that is covered with saltmarshes.

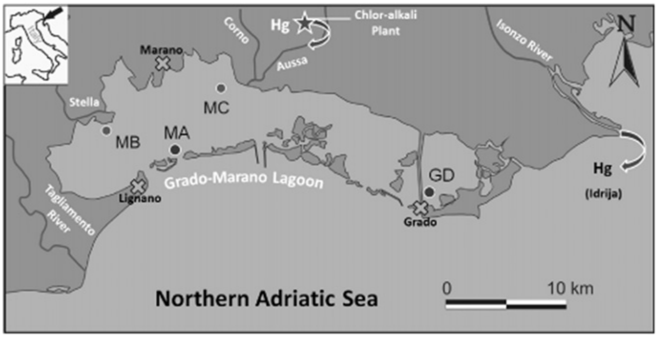

Fig. 1. Map of study sites in the Marano and Grado Lagoons, Italy.

Sediment Hg supply in the Lagoons has been historically provided by the Isonzo River located east of the lagoon system. The Isonzo River is the largest contributor of Hg into the Northern Adriatic Sea since the 16th century due to Hg mining at the Idrija mine in western Slovenia (Covelli et al., 2001). Mercury concentrations show a progressive westward decrease from about 10 $\mu g\, g^{-1}$ (Grado Lagoon) to 1 $\mu g\, g^{-1}$ (Marano Lagoon) (Brambati, 2001). In Marano Lagoon, Hg has also been delivered from the Aussa River from a chlor-alkali plant that began operation in 1949 and contributed 186,000 kg of Hg to the river (Piani et al., 2005).

Details of the geochemistry, Hg speciation and granulometric composition of the sediments in the lagoons can be found in Acquavita et al. (2012). Sites MA and GD, which are closer to sea inlets contained the highest sand content at 75% and 44%, respectively, while sites MB and MC, which were near river mouths, contained 30% and 25% sand, respectively. None of these sites was subjected to dredging.

3. Methods

3.1. Sample handling

Six-cm diameter sediment cores were collected by hand at each site in October 2008, February 2009 and July 2009. After transport to the laboratory and removal of the overlying water by siphon, cores were placed into an N_2-filled glove bag and six individual horizontal sections (0–1, 1–2, 2–3, 3–5, 5–7, 7–10 cm) from each core were removed, placed into jars, and homogenized. Sample aliquots were added to N_2-flushed serum vials for Hg transformation measurements and 5-cc syringes for SO_4^{2-}-reduction measurements.

Separate cores were used for Hg analysis, which were also extruded, sectioned, centrifuged and filtered under N_2. Pore waters were collected in pre-acid-cleaned containers and stored frozen until analysis. Solid-phase samples for Hg analyses were freeze-dried, homogenized with a mortar and pestle, and sieved through a 420-µm screen to remove coarse shell debris.

3.2. Hg transformation analyses

Sample aliquots (3 mL sediment) for Hg methylation and MeHg demethylation assays were placed in duplicate into 13 mL serum vials, which were injected with 3.0 mL of anoxic seawater to prepare slurries. All reaction vessels were sealed with butyl rubber septa and crimped with aluminum seals. Septa for methylation assays were lined with Teflon. Vials for Hg methylation were injected with 2.0 μL of a solution containing 0.5 μcuries (μCi) of $^{203}HgCl_2$, which amounted to ∼170 ng Hg(II) per vial, and which was less than the ambient Hg concentrations by ∼5–90-fold. Vials for MeHg demethylation were injected with 2.0 μL of a solution containing 0.1 μCi of $^{14}CH_3HgCl$, which amounted to ∼334 ng Hg mL^{-1} sediment, and which exceeded ambient MeHg concentrations by ∼60–370-fold. The $^{14}CH_3HgCl$ was purified before use by extraction into methylene chloride (CH_2Cl_2) followed by back extraction into distilled water. The latter extraction included the removal of CH_2Cl_2 by bubbling with N_2. Purified ^{14}C-MeHg was assayed and determined to be 100% MeHg. Vials were incubated for ∼18 h at *in situ* temperature in the dark. After incubation, vials for methylation assays received multiple injections of small volumes of 6 N HCI to stop the reaction, dissolve carbonate material, and to store samples at low pH; 4–6 mL of 6 N HCI was required to dissolve carbonates. Alkali (1.0 mL NaOH, 3 N) was added following incubation to stop the demethylation reaction and to sequester $^{14}CO_2$ in the liquid phase. Killed controls for both methylation and demethylation consisted of identical subsamples treated with acid or alkali, respectively, prior to adding radioisotopes.

For methylation assays, MeHg was extracted twice into toluene following treatment with $CuSO_4$ and KCl in H_2SO_4. Pooled toluene extracts were dehydrated using anhydrous $NaSO_4$ and radioactivity determined by scintillation counting. Methylation activity, determined from conversion of $^{203}HgCl_2$ to methyl-^{203}Hg, yielded a first order rate constant that is a measure of the fraction of inorganic ^{203}Hg converted to methyl-^{203}Hg per time. Demethylation was determined by measuring ^{14}C in CO_2 and CH_4 using a gas-stripping and trapping system similar to that described by Gray and Hines (2009). Briefly, CH_4 was flushed from the vial with air (30 mL/min for 15 min) and combusted to CO_2 in a CuO-packed quartz column at 850 °C. The resulting CO_2 was trapped in phenethylamine, methanol, and a toluene-based scintillation fluid. 1.0 mL of CH_4 was injected into each vial to facilitate removal of $^{14}CH_4$. Following $^{14}CH_4$ measurements, samples were transferred to 120 mL vials and were slowly acidified with 6.0 N HCl to convert ^{14}C to $^{14}CO_2$ that was stripped via a stream of N_2 and trapped as described above. Radioactivity was determined by scintillation counting. Demethylation activity, determined from the conversion of ^{14}C-MeHg to $^{14}CO_2$ and/or $^{14}CH_4$ also yielded a first order rate constant.

For samples collected in October 2008, Hg methylation activity was also measured using ^{197}Hg as well as ^{203}Hg. The former isotope was created in the nuclear reactor at the Jožf Stefan Institute in Ljubljana, Slovenia, and the methylation assay was conducted similarly to that described above and as previously described (Ribeiro Guevara et al., 2007).

3.3. Sulfate reduction activity

Sulfate reduction rates were determined using ^{35}S and the chromium reduction assay as described previously (Hines et al., 1997, 2001). Briefly, sample aliquots from each vertical subsection of sediment were placed anaerobically into 5.0 cc syringes (distal end removed) and sealed with rubber stoppers. $^{35}SO_4$ was injected into syringes that were then incubated overnight at *in situ* temperature in an N_2-filled jar. The reaction was stopped by freezing, and reduced S species were stripped by an N_2 gas stream while refluxing in a mixture of HCl, reduced Cr, and ethanol (Hines et al., 2001). The resulting H_2S was trapped as ZnS in a Zn-acetate solution and radioactivity of half of the material was determined by scintillation counting.

3.4. Laboratory manipulation experiments

To investigate controls on Hg methylation and MeHg demethylation in lagoon sediments, core samples from site GD were collected in June 2008 (demethylation studies) and July 2009 (methylation studies). Using anoxic procedures, the upper 4 cm of three cores were combined, and 3 mL aliquots of sediment were introduced into 13 mL serum vials that were diluted with 3 mL of deaerated water as described above. Concentrated stock solutions of Na_2MoO_4 (SO_4^{2-} reduction inhibitor), glucose, $NaNO_3$, and iron oxide were prepared on the same day and 100 μL of each was injected individually into vials to create final concentrations of 20 mM Na_2MoO_4 or 2.0 mM each of glucose, nitrate (NO_3^-), or iron (Fe) oxide. The Fe oxide was prepared by dissolving ferric chloride in weak HCl and then slowly adding NaOH until the pH reached 7.0 and the Fe had precipitated as Fe oxide. This freshly precipitated Fe oxide slurry was mixed and injected into incubation vials.

Vials were preincubated overnight to ensure that the inhibitor (MoO_4), electron donor (glucose), and electron acceptors (NO_3^- and Fe oxide) were controlling microbial processes before ^{203}Hg or ^{14}C-MeHg was added. In addition, the concentrations of amendments were sufficient to ensure that electron donors and acceptors would not be completely consumed during the experiment. After an 18-h incubation with radiotracers, reactions were terminated and samples analyzed for methylation and demethylation as described above.

3.5. Mercury speciation

Mercury speciation was determined as described in Horvat et al. (2002). Total Hg in sediment samples was determined using cold vapor atomic absorption (CVAAS) after acid digestion (Horvat, 1991). Accuracy was determined using Certified Reference Material (PACS-2 Marine Sediment, NRCC) and the relative standard deviation of at least three determinations was <5%. Samples for total dissolved (pore water) Hg were acidified and oxidized by exposure to ultraviolet light in the presence of BrCl. After a pre-reduction using $NH_2OH \cdot HCl$, Hg was determined by CVAAS after reduction by $SnCl_2$ and gold trapping (Horvat et al., 1987; Horvat, 1991). The reproducibility was 4% and BCR 579 was used as a Certified Reference material.

Total MeHg in sediment samples and dissolved MeHg in pore water were determined using solvent extraction, aqueous phase ethylation, gas chromatographic separation, pyrolysis and cold vapor atomic fluorescence (CVAFS) detection (Horvat et al., 1993). Accuracy for total MeHg analyses was determined using two Certified Reference Materials (BCR 580 Estuarine Sediment and IAEA 433 Marine Sediment). Recovery of MeHg was estimated by spiking samples with a known quantity of MeHg prior to analysis. The limit of detection was 50 pg MeHg L^{-1} for water samples and 50 pg MeHg g^{-1} for sediments.

4. Results

4.1. Microbial activities

Mercury methylation and MeHg demethylation were active at all sites and all seasons (Figs. 2–5). Methylation rate constants (K_{meth}), which are the fraction of radiotracer converted to MeHg per day, generally decreased with depth in most instances and varied from a low of near zero to a high of 0.054 day^{-1}. Highest rates were noted at site MA (Fig. 5b). Potential methylation at all three Marano lagoon sites was similar in autumn and winter, but two-times higher in summer. However, site GD exhibited high rates in autumn and summer with lower rates in winter.

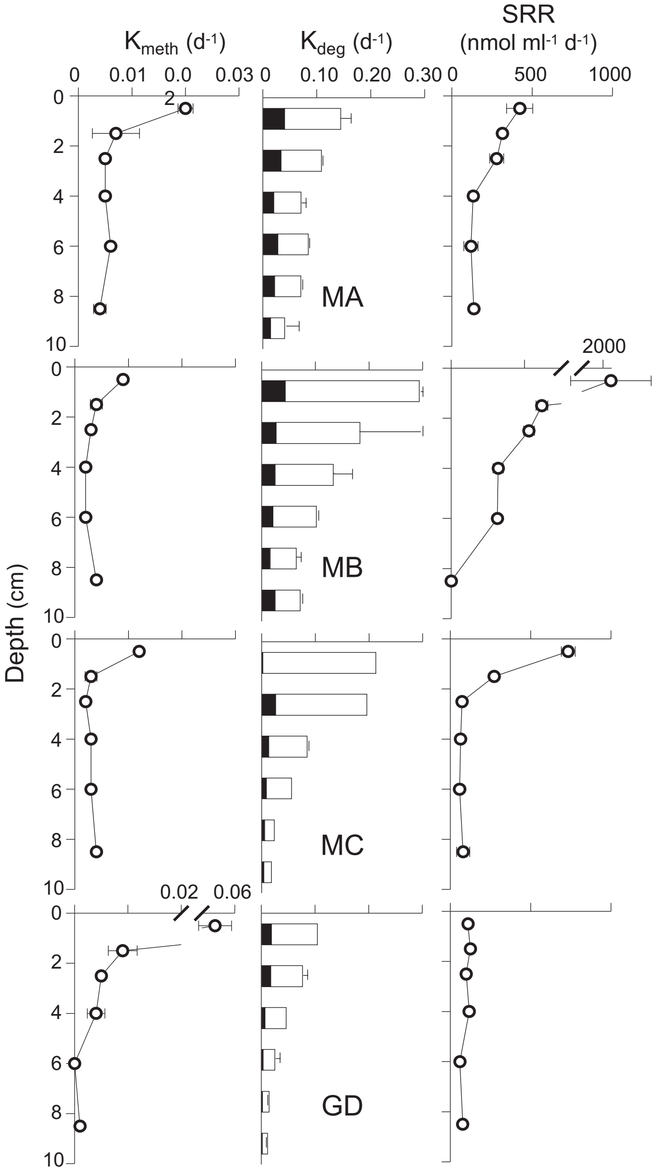

Fig. 2. Depth profiles of methylation (K_{meth}) and demethylation (K_{deg}) rate constants and sulfate reduction rates in sediments from all four sites in Marano and Grado Lagoons in autumn (October). Black bars indicate CH_4 production from MeHg whereas white bars indicate CO_2 production.

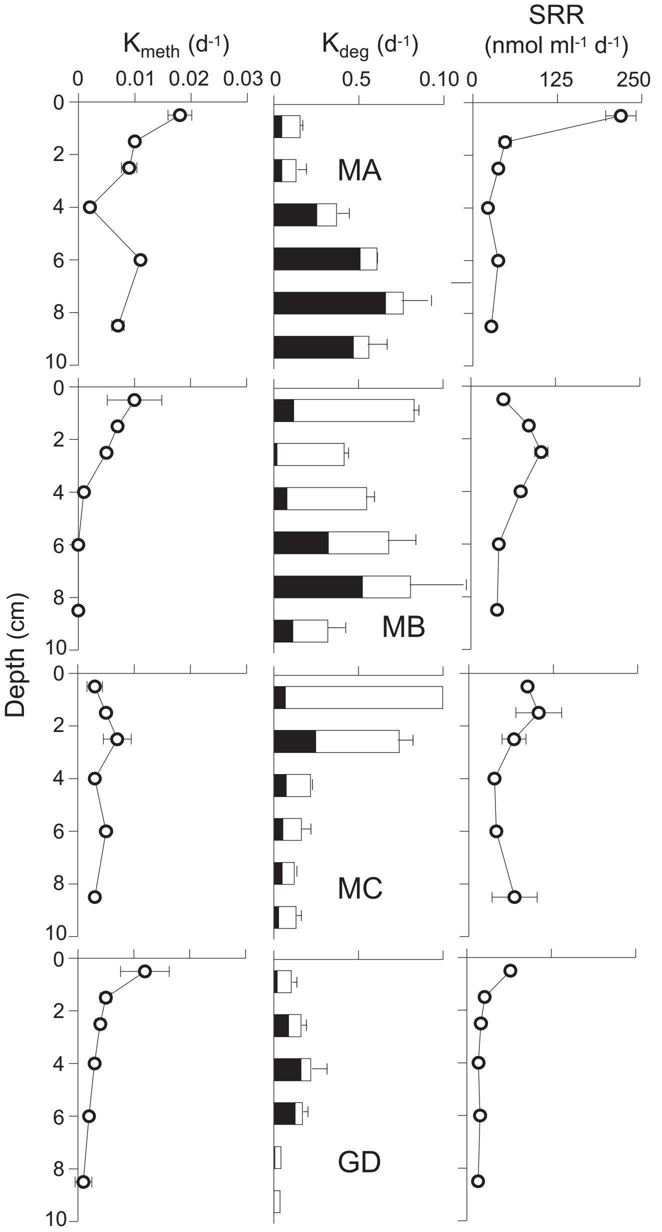

Fig. 3. Same ad Fig. 2, but for winter (February).

Demethylation rate constants (K_{deg}) ranged from ~0.01 to >0.55 day^{-1} with lowest values in winter and highest in summer (Figs. 2–5). The summer values over 0.5 day^{-1} were quite high. Rates in summer and autumn generally decreased with depth, but winter rates at all sites, except MC, exhibited subsurface maxima. In summer and autumn, the ^{14}C label liberated during demethylation was primarily recovered as $^{14}CO_2$, which is indicative of oxidative demethylation of MeHg (Figs. 2–4). However, the fraction of label recovered as $^{14}CH_4$ increased in winter suggesting that the reductive path was of increased importance at that time. When demethylation rate constants were averaged over the 10-cm depth interval, it was shown that potential demethylation activity was highest at sites MB and MC, and lowest at site GD (Fig. 5c).

Sulfate reduction rates varied greatly among sites and seasons (Figs. 2–5). In general, rates decreased with depth, but in some instances maximum rates occurred a few cm below the surface, e.g., sites MB and MC in winter and sites MB and GD in summer. Sulfate reduction was most active at sites MB and MC and lowest at GD. Rates were highest in summer at all sites except MB, which displayed highest rates in autumn. Sulfate reduction in winter was very low and about 10-fold lower than summer rates.

When data from all three seasons were compared, both methylation and demethylation rate constants significantly correlated with SO_4^{2-} reduction rates (Fig. 6) suggesting that SO_4^{2-}-reducing bacteria were responsible for at least a portion of both processes. However, demethylation varied more closely with SO_4^{2-}reduction than did methylation. Demethylation rates strongly correlated with SO_4^{2-}reduction in summer and autumn ($r^2 = 0.43$, $p = 0.0000004$), but were not correlated in winter (Fig. 6b inset), suggesting bacteria other than SRB may catalyze demethylation in winter.

Methylation and demethylation rate constants did not vary significantly with sedimentary organic carbon content, sedimentary Hg or MeHg concentrations. In addition, rate constants did not correlate with percent MeHg, i.e., the fraction of sedimentary Hg_{tot} attributable to MeHg.

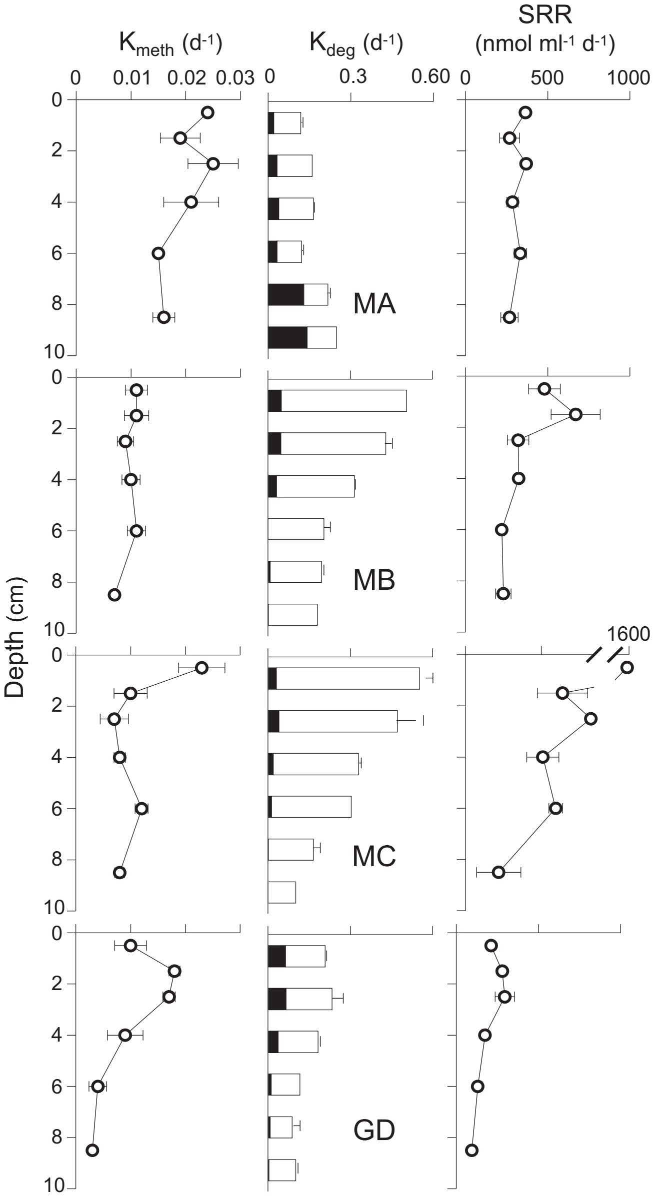

Fig. 4. Same ad Fig. 2, but for summer (July).

Sedimentary and pore-water concentrations of total Hg (Hg_{tot}) and MeHg were determined during each of the three field campaigns at sites MB and MC (Table 1). We were unable to obtain sufficient pore water to measure dissolved species at sites MA and GD due to the high sand content, so only data for MB and MC are presented here. Sedimentary concentrations of these analytes at sites MB and MC did not change appreciably throughout the year (Table 1). Concentrations of Hg_{tot} were nearly three times higher at site MC than MB, but MeHg was only slightly higher at MC. The higher Hg concentrations at site MC were due to the proximity of the site to the chlor-alkali plant in the Aussa River (Fig. 1). Pore-water Hg species varied seasonally, especially dissolved Hg that increased greatly in July compared to October and February. This increase was over two orders of magnitude at site MB and almost one order of magnitude at MC. Dissolved MeHg concentrations varied 2–3 fold through the year with highest concentrations of 0.62 and 2.4 ng L^{-1} occurring in October at sites MB and MC, respectively.

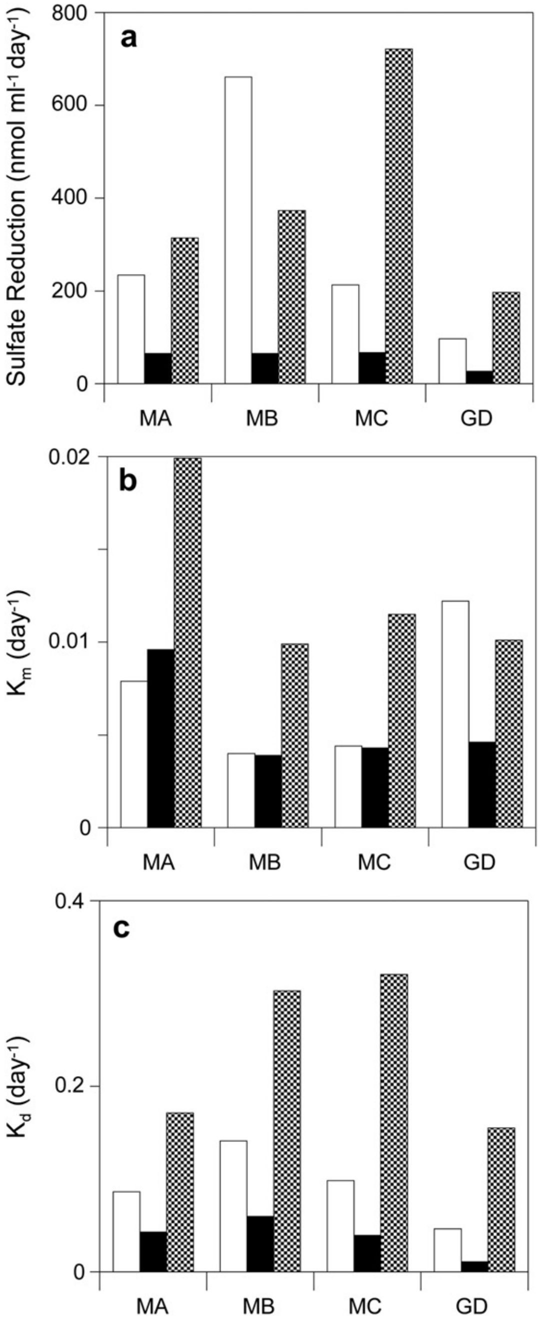

Fig. 5. Average a) sulfate reduction rates; b) methylation rate constants (K_m); and c) demethylation rate constants (K_d) for all sites in Marano and Grado Lagoons. Data are for upper 10 cm. White bars are for autumn (October), black bars are for winter (February), and gray bars are for summer (July).

Methylation rate constants (K_{meth}) increased significantly with pore-water Hg concentrations at site MB and MC ($n = 29$, $r^2 = 0.21$, $p = 0.014$), but did not correlate with pore-water MeHg. Similarly, demethylation rate constants also correlated significantly with pore-water Hg ($n = 29$, $r^2 = 0.375$, $p = 0.00041$), but not with MeHg.

Sediment–water partition coefficients (K_D; L kg^{-1}) were calculated as:

$$K_D = \frac{[Hg]_S}{[Hg]_{PW}} \qquad (1)$$

where $[Hg]_S$ is the solid-phase concentration of the Hg species in ng kg^{-1}, and $[Hg]_{PW}$ is the pore-water concentration of the Hg

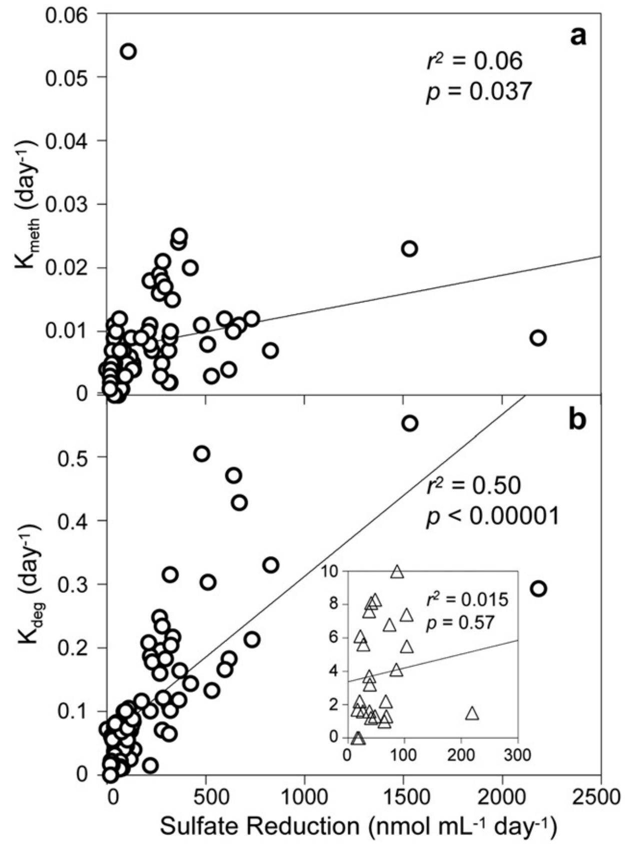

Fig. 6. Regression of (a) methylation (K_{meth}) and (b) demethylation (K_{deg}) rate constants vs. sulfate reduction rates for all sites and seasons. Data with (*) were omitted from regression analysis.

species in ng L^{-1}. Increases in K_D occur when dissolved Hg species decrease in concentration relative to solid-phase concentrations, and vice versa. Log K_D for Hg_{tot} was higher at site MC than MB reflecting the higher Hg_{tot} concentrations at MC (Table 1). Highest values occurred in autumn with lowest values in summer due to the strong increase in dissolved Hg_{tot} in summer, particularly at site MB, which exhibited very high dissolved Hg concentration. Log K_D values for MeHg were lower than for Hg_{tot} (Table 1) since MeHg is less particle reactive than Hg(II). Lowest values occurred in autumn when pore-water MeHg concentrations were at their lowest annual level. Methylation and demethylation rate constants were related inversely with the Log K_D of Hg_{tot} ($r^2 = 0.27$; $p = 0.005$, $r^2 = 0.45$; $p = 0.001$, respectively) suggesting that methylation and demethylation were controlled by the availability of dissolved Hg, and agreeing with the relation between K_{meth} and K_{deg} and pore-water Hg_{tot} described above.

4.2. Mercury speciation and transformations

To better evaluate seasonal changes in MeHg production, we multiplied MeHg production and degradation rate constants by Hg pools to obtain a crude estimate of the net production of MeHg (Table 1). We calculated rates using both total and dissolved Hg species and demethylation rates were subtracted from methylation rates to obtain a net production rate of MeHg. It is unclear what fraction of Hg or MeHg is available for bacterial transformation, but it is impossible that all of the sedimentary Hg can be readily converted to MeHg since net rates using sediment Hg pools yields rates that are unrealistically high (Table 1). We noted a similar result using sediment samples from other locations in the northern Adriatic Sea region (Hines et al., 2000, 2006), and it has been shown that an inorganic Hg tracer is more available for methylation than ambient Hg (Hintelmann et al., 2000). The results in Table 1 suggest that only a very small fraction of the total Hg could be methylated at the same rate as the ^{203}Hg tracer. Conversely, Hintelmann et al. (2000) showed evidence that the total MeHg pool has a short half-life in sediments and they suggested that added MeHg tracers may mimic this pool rather well. Our tracer was added at a very high concentration and Drott et al. (2008b) reported that increasing the concentration of MeHg can stimulate demethylation in some instances. When only pore-water Hg species were used to calculate net methylation rates, the result was obviously much smaller, but appeared to be more realistic when the ambient pools of Hg species were considered. These latter calculations resulted in a net degradation of MeHg in October, a small net production in February, and large rate of production in July. The July rates were due to the very large concentrations of dissolved Hg used to calculate them. In October, the Hg levels were much lower while the MeHg levels were the highest of the year, resulting in a net demethylation of MeHg at this time of the year.

4.3. Laboratory manipulation experiments

Methylation and demethylation activities were greatly affected by the additions of inhibitors and electron donors and acceptors (Fig. 7a). The methylation rate constant, K_{meth}, for the unamended control was 0.0063 day^{-1}. Adding the SO_4^{2-}-reduction inhibitor molybdate and the electron acceptor NO_3^- decreased methylation by 85% and 93%, respectively ($p = 0.021$, 0.0017). Conversely, the addition of glucose increased methylation by almost 3-fold, while Fe oxide amendments had no effect.

The unamended demethylation controls yielded a rate (K_{deg}) near 0.03 day^{-1} (Fig. 7b). Demethylation rates increased significantly in the presence of all amendments. Molybdate and glucose additions increased demethylation ~2.5-fold and NO_3^- and Fe oxide caused a 5.5–6.5-fold increase.

Table 1

Sediment and pore-water Hg and MeHg concentrations, Hg transformation rate constants (day^{-1}), and net production rates of MeHg (average upper 7 cm). MeHg production rates are the product of rate constants and Hg concentrations using sediment or pore-water Hg values. Demethylation is subtracted from methylation for calculation of net production rates. Rates are adjusted to account for porosity and presented on a whole sediment volume basis.

Site	Period	Sediment (ng g^{-1} d.w.)		Pore water (ng L^{-1})		K_{meth}	K_{deg}	Net MeHg production		Log K_D (L kg^{-1})	
		Hg_{tot}	MeHg	Hg_{tot}	MeHg			Sediment (pg mL^{-1} d^{-1})	Pore water (fg mL^{-1} d^{-1})	Hg_{tot}	MeHg
MB	Autumn	1640	1.03	8.4	0.62	0.40	15.5	3200	−31	5.22	3.27
	Winter	1700	1.10	14.50	0.31	0.47	6.5	3960	24	5.07	3.44
	Summer	1220	1.30	1200	0.46	1.05	32.8	6200	6300	3.34	3.47
MC	Autumn	4490	1.45	11.1	2.40	0.50	11.4	10,200	−110	5.60	2.84
	Winter	4570	2.39	22.6	0.93	0.46	4.4	10,500	31	5.31	3.77
	Summer	4410	1.26	96.9	0.19	1.22	36.5	26,700	560	4.79	4.11

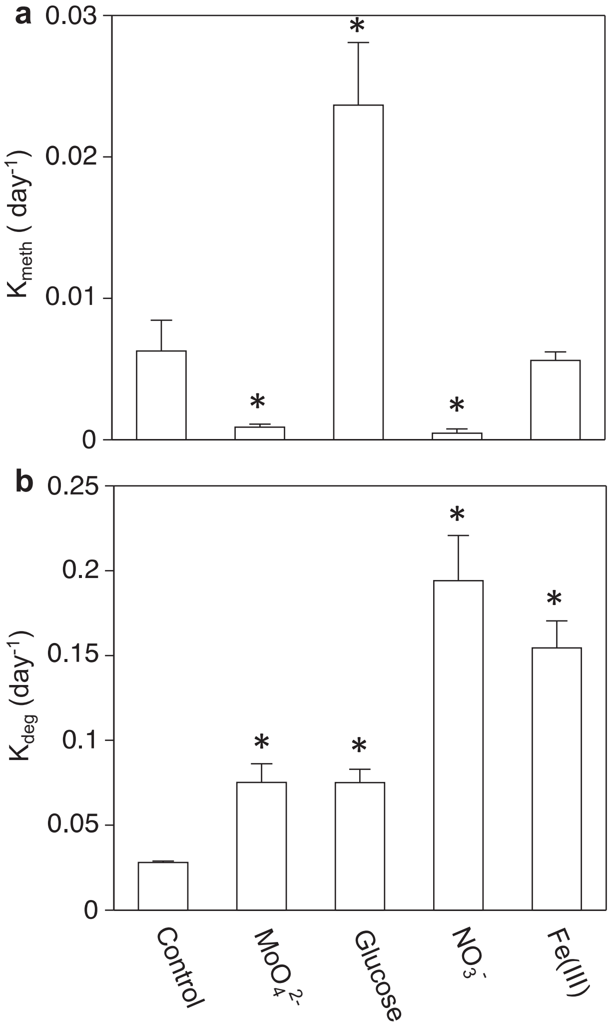

Fig. 7. Effects of various amendments on (a) Hg methylation (K_{meth}), and (b) MeHg demethylation (K_{deg}). * indicates statistically different values from controls ($p < 0.05$).

5. Discussion

5.1. Mercury transformations

Mercury transformation rate constants represented the potential for a sample to produce or degrade MeHg and were not actual fluxes of MeHg (Drott et al., 2008b). Methylation rate constants have been shown to be good indicators of the MeHg/total Hg ratio (i.e., % MeHg) in a variety of disparate sediments (Drott et al., 2008a). However, depending on the concentrations of ambient Hg and MeHg, tracers can either stimulate or inhibit the Hg transformations they intend to measure (Hintelmann et al., 2000; Drott et al., 2008b). The quantity of the ^{203}Hg tracer added was low relative to ambient Hg, but the tracer is undoubtedly more bioavailable than ambient Hg and used more readily (Hintelmann et al., 2000). The ^{14}C-MeHg tracer was much higher than ambient and it has been shown that tracer spikes like these can lead to increased rate constants, or in some instances inhibit demethylation due to toxicity (Drott et al., 2008b). Despite these limitations, comparisons of these data among sites can help elucidate areas most likely to accumulate MeHg for movement into the food chain.

Potential Hg methylation rate constants obtained using samples of lagoon sediments were similar to those reported for some other near-shore marine and freshwater habitats (0–0.05 day^{-1}) (Gilmour et al., 1998; Marvin-diPasquale and Agee, 2003; Marvin-DiPasquale et al., 2003; Gray et al., 2004; Hammerschmidt and Fitzgerald, 2004; Drott et al., 2007; Mitchell and Gilmour, 2008) including the nearby Gulf of Trieste (Hines et al., 2006) and the lagoons in Venice, Italy (Han et al., 2007). Rates were lower than those noted in a freshwater reservoir sediment (Gray and Hines, 2009) and periphyton vegetation in Brazil (Guimaraes et al., 2006).

Potential demethylation was rapid in the lagoon sediments (up to 0.55 day^{-1}) and much higher by 4–10 fold than rates reported in other areas of the region, such as marine sediments in the Gulf of Trieste and freshwater sediments in the Isonzo/Soča river system that supplies most of the Hg to the area (0–0.06 day^{-1}) (Hines et al., 2000, 2006). However, previous studies have noted high K_{deg} values of near or over 0.4 day^{-1} (e.g., Hintelmann et al., 2000) and even as high as 1.8 day^{-1} in sediments from the Carson River, Nevada (Marvin-DiPasquale et al., 2000). We also determined that potential demethylation rates in estuarine sediments in the Isonzo River near its mouth were similar in magnitude to those reported here for Grado and Marano lagoons (up to 0.4 day^{-1}) (Hines et al., unpublished). Therefore, it appears that shallow water near-shore sediments in the region have a tendency to rapidly degrade MeHg compared to the surrounding environments. Giani et al. (2012) reported that naturally occurring clams in these lagoons accumulated much more MeHg in summer. Our data show that Hg methylation was indeed more rapid in summer, underscoring the importance of sediments as sources of MeHg for biota. However, the fact that demethylation was active in these sediments may help explain the finding by Giani et al. (2012) that clams reared by Man in the lagoons never exceeded the 0.5 mg kg^{-1} Hg limit set by the European Community.

5.2. Controls on mercury methylation

Previous studies have demonstrated the importance of organic matter quantity and quality, sulfur speciation, microbial activities, and the partitioning of Hg species between particulate and dissolved phases in regulating the concentrations of MeHg in marine sediments (Benoit et al., 1999, 2003; Mason and Lawrence, 1999; Conaway et al., 2003; Hammerschmidt and Fitzgerald, 2004, 2006; Drott et al., 2007; Han et al., 2007, 2008, 2010; Hollweg et al., 2009). In general, Hg bioavailability for Hg-methylating bacteria, which is controlled to a large degree by each of the above factors, is of primary importance in controlling MeHg formation.

In the Marano and Grado Lagoon sediments, methylation potential correlated with rates of SO_4^{2-} reduction. Although statistically significant, this relationship was weak ($r^2 = 0.06$), suggesting that other factors are important in controlling Hg methylation in lagoon sediments. We did not measure Fe reduction in lagoon sediments, but pore-water concentrations of Fe and Mn were determined at sites MB and MC (Emili et al., 2012). In addition, we reported that Fe reduction was an important contributor to C mineralization in sediments in the nearby Gulf of Trieste (Hines et al., 1997) and it is likely that Fe reduction occurred in the lagoon sediments as well and may have contributed to Hg methylation. Subsurface maxima in SO_4^{2-} reduction activity were noted in winter at sites MB and MC, which suggested that microbial respiration processes in surficial sediments were dominated by electron acceptors other than SO_4^{2-}, such as O_2, NO_3^- or oxidized Fe or Mn. Dissolved Fe profiles were measured in winter at these sites (Emili et al., 2012) with concentrations similar to those noted in the Gulf of Trieste pore waters (Hines et al., 1997). Iron concentration maxima at sites MB and MC occurred at 1.5 cm in autumn and 3 cm in winter indicating that Fe reduction was occurring in the upper few cm, but O_2 penetrated deeper into sediments in winter (Emili et al., 2012). Iron-reducing bacteria have been shown to methylate Hg (Kerin et al., 2006) and evidence exists that members of this group are significant contributors to Hg methylation in nature (Fleming et al., 2006; Mitchell and Gilmour, 2008), and they may also be important in the lagoon sediments studied here, at least in winter. When the SO_4^{2-} reduction inhibitor molybdate was added to GD sediments in summer, methylation decreased by 85% indicating that SRB were the primary Hg methylators at that time and probably were throughout much of the year when SO_4^{2-} reduction was rapid, including samples we collected in autumn. However, in winter it is likely that Fe-reducing bacteria contributed to Hg methylation in the upper few cm.

We did not find a relationship between potential methylation and organic carbon content in the lagoon sediments. Total Hg in sediments is often strongly correlated with organic matter content (Lindberg and Harriss, 1974; Hammerschmidt and Fitzgerald, 2004, 2006; Miller et al., 2007; Kim et al., 2011) and some studies have shown significant correlations between methylation potential and organic C content in sediments (e.g., Hammerschmidt and Fitzgerald, 2004; Drott et al., 2008a; Marvin-Dipasquale et al., 2009). The lack of a similar relationship in lagoon sediments may simply be due to the fact that K_{meth} values and concentrations of organic C (0.4–1.15%; Emili et al., 2012) did not vary greatly throughout the lagoon system studied here, which would make it difficult to discern a mathematical connection between them. However, we did find a significant correlation between K_{meth} and the sediment–water partition coefficient K_D indicating that methylation activity is controlled by the availability of Hg due to partitioning between solid and dissolved phases. Correlations of K_{meth} with dissolved Hg agreed with this finding, indicating that dissolved Hg species represent the bioavailable pool (Benoit et al., 1999). The lack of correlation between K_{meth} and dissolved MeHg is not surprising since the product of methylation should have little control on methylation potential.

Our K_D values were similar to those reported for sediments of Lavaca Bay in Texas (Bloom et al., 1999) and the Venice Lagoon (Han et al., 2007) to the west of the Marano and Grado Lagoons studied here. However, K_D values for the latter lagoons were higher than those in Long Island Sound and the nearby New England continental shelf sediments (Hammerschmidt and Fitzgerald, 2004, 2006). These differences may be due to the fact that sediments in the Italian lagoons and Lavaca Bay have lower organic C concentrations, are in shallow waters, and are dominated by carbonate sediments, compared to sediments studied in northeast United States.

The fact that we found a relationship between K_{meth} and K_D, but not organic C, suggests that the partitioning of Hg in lagoon sediments is controlled to a significant degree by factors other than organic C. Hammerschmidt and Fitzgerald (2004) studied deeper water (18–38 m) sites with low sulfide content and low rates of SO_4^{2-} reduction (Goldhaber et al., 1977) compared to the lagoons studied here, and as will be discussed below, seasonal sulfur dynamics may be more important than organic matter in directly controlling Hg methylation since the lagoon sediments are much more microbially active than those sampled in Long Island Sound and experience large differences seasonally.

Han et al. (2007), working in the nearby Venice Lagoon system, reported that K_{meth} values did not correlate with log K_D for Hg in sediments. However, when K_{meth} data were normalized to bacterial activity by dividing by SO_4^{2-} reduction rates, they did obtain a significant relationship with log K_D. We attempted the same calculations with our data from the Marano and Grado lagoons, but did not find an improved relationship with K_D when K_{meth} data were divided by SO_4^{2-} reduction. Our K_D values were similar in magnitude to those found in the Venice Lagoon, but rates of SO_4^{2-} reduction in the Venice Lagoon (Han et al., 2007) were much more rapid than in the lagoons we studied, which may explain the need to normalize to these rates in the Venice samples.

When pore-water Hg concentration and transformation data were used to calculate net rates of MeHg production, these rates were similar to benthic fluxes of MeHg measured by Emili et al. (2012). For example, annual benthic fluxes of MeHg at sites MB and MC were 0.04–0.06 mg m^{-2} $year^{-1}$ (Fig. 9 in Emili et al., 2012), which equals 11–16.5 pg cm^{-2} d^{-1}. Our calculated production rates varied from approximately −0.7 to 44 pg cm^{-2} d^{-1} when data in Table 1 were integrated vertically over 7 cm sediment depth. This comparison is crude, but provides further evidence supporting the notion proposed by Covelli et al. (2008) that MeHg in lagoon sediments is produced within the sediment, and a large fraction is “recycled”, i.e., not buried, but lost to the water column.

5.3. *Controls on methylmercury demethylation*

Demethylation of MeHg can occur via either the reductive pathway that produces CH_4 and Hg^0 or the oxidative pathway that that produces CO_2 and presumably Hg^{2+} (Barkay and Wagner-Dobler, 2005). Demethylation of the ^{14}C-tracer in the lagoon sediments yielded primarily $^{14}CO_2$, which is indicative of oxidative demethylation. However, the fraction recovered as $^{14}CH_4$ was considerably higher in winter samples, suggesting that demethylation in winter was conducted in part by bacteria employing the reductive pathway, which is catalyzed by enzymes of the *mer* detoxification genetic system (Barkay and Wagner-Dobler, 2005). Baldi et al. (2012) reported that 53% of aerobic bacteria isolated during winter from surficial lagoon sediments were resistant to Hg, while summer samples yielded <2%. Bacteria that are resistant to Hg often harbor *mer* genes that control the reduction of Hg and sometimes the demethylation of MeHg as well (Barkay and Wagner-Dobler, 2005). During summer, the biological demethylation of MeHg in lagoon sediments is conducted oxidatively by anaerobic bacteria; primarily SRB, but in winter, the reductive process increases in importance. Values of K_{deg} correlated well with SO_4^{2-} reduction rates suggesting that SRB are important demethylators in lagoon sediments. However, no correlation occurred in winter, which agrees with the concept that demethylation was controlled by SRB during warm months, but less so in winter when Hg-resistant bacteria become more involved in the reductive degradation of MeHg. Our previous studies of sediments in the Gulf of Trieste also showed that reductive demethylation increased in importance during winter as surficial sediments became increasingly oxidized as microbial activities slowed (Hines et al., 2006). The correlation between dissolved Hg and K_{deg} may have been due in part to the fact that inorganic Hg is known to induce the *mer*-mediated reductive demethylation of MeHg (Barkay et al., 2003). However, it was not clear why dissolved MeHg did not correlate with K_{deg}. Drott et al. (2008b) reported a correlation between K_{deg} and dissolved MeHg when total Hg levels were low, but no correlation when Hg concentrations were high. They too were unable to explain these relationships. Hg concentrations at our sites were in between those of Drott et al. (2008b).

5.4. *Amendment experiments*

The amendment experiments were conducted to further investigate factors controlling methylation and demethylation of Hg in the lagoons. The data supported our other results implicating SRB as important methylators of Hg in lagoon sediments. For example, addition of the SO_4^{2-} reduction inhibitor molybdate caused an 85% decrease in Hg methylation. The lack of complete inhibition by molybdate may have been due to Hg methylation by Fe-reducing bacteria (Kerin et al., 2006), as discussed above. Additions of freshly precipitated Fe oxide did not affect Hg methylation in lagoon sediments. The stimulation of methylation by glucose indicated that methylating bacteria (presumably SRB) were limited by electron donors, which is not surprising considering that these sediments contained ∼1% organic C. The inability of Fe amendments to affect Hg methylation may have been due to the limitation of microbial activity by electron donors. If Fe reducers were limited by organic C, then additions of an electron acceptor like Fe(III) would not affect methylation. Hence, it is possible that Fe reduction contributed to methylation, but the rather strong inhibition by molybdate indicated that SRB were the primary methylators of Hg in lagoon sediments in summer when these experiments were conducted.

The inhibition of methylation by NO_3^- agrees with similar data for the Florida Everglades (Gilmour et al., 1998) and sediments from a freshwater lake in New York (Todorova et al., 2009). To date, Hg methylation has not been shown to occur during NO_3^- reduction, and in lagoon sediments, nitrate amendments inhibited Hg methylation more thoroughly than did molybdate. Therefore, at least for summer samples, NO_3^- reduction was not involved in Hg methylation.

Amendment experiments for demethylation were more difficult to interpret than methylation studies. First, molybdate caused a significant increase in demethylation, which is counter to data supporting SRB as important demethylating bacteria in lagoon sediments. Inhibition of SO_4^{2-} reduction may have allowed methanogenesis to occur, and the increased rate of MeHg demethylation in the presence of molybdate might indicate that demethylation occurs more readily during methanogenesis than during SO_4^{2-} reduction. However, methanogenic bacteria convert a significant fraction (75%; Marvin-DiPasquale and Oremland, 1998) of the methyl-C of MeHg to CH_4, and in our experiments the opposite occurred (30% $^{14}CH_4$ in controls vs. 10% $^{14}CH_4$ in molybdate treatments), suggesting that other bacteria were degrading MeHg in the presence of molybdate. Marvin-DiPasquale and Oremland (1998) found that molybdate additions to samples of Everglades freshwater sediments caused a decrease in demethylation activity and a shift to 100% $^{14}CH_4$ production from ^{14}C-MeHg. Hence, it was unclear why molybdate seemed to stimulate demethylation. It has been shown that molybdate amendments can sequester sulfide (Tonsager and Averill, 1980), which might have relieved sulfide toxicity. It has also been shown that molybdate can stimulate denitrification (Slater and Capone, 1987), and our data clearly show that NO_3^- amendments stimulated demethylation. Second, both nitrate and Fe(III) strongly stimulated demethylation indicating that NO_3^- and Fe-reducing bacteria can actively degrade MeHg in the lagoons. These experiments were conducted in summer, so it is possible that these bacterial groups were important demethylators in winter when SO_4^{2-} reduction was less important.

5.5. Seasonal effects

The seasonal changes in Hg transformations and geochemical conditions suggest dynamic temporal changes in the formation and degradation of MeHg in lagoon sediments (Fig. 8). As temperatures warm in spring and summer and reducing conditions prevail, bacterial SO_4^{2-} reduction increases, sulfide is produced, and eventually dissolved Hg is mobilized in pore water. Mercury that enters the lagoon system from the east is largely fine-grained HgS minerals (cinnabar) derived from mining activities upstream in the Isonzo/Soča River system (Hines et al., 2000). As a result, the percentage of sulfur-containing sedimentary Hg minerals in the lagoons decreases from 58% at site GD in the east to 13% at site MB to the west (Acquavita et al., 2012). Cinnabar is very stable under most conditions, but can be readily solubilized in the presence of dissolved sulfide (Paquette and Helz, 1995; Benoit et al., 1999) and can then serve as a source of bioavailable dissolved Hg; presumably as neutral sulfide complexes, HgS^0 (Benoit et al., 1999) (Fig. 8). In addition, the reduction of Fe(III) by bisulfide ion (HS^-) can rapidly solubilize Hg(II) bound to Fe(III) (Slowey and Brown, 2007) and HgS dissolution can be enhanced by dissolved organic matter (Slowey, 2010). The dissolution of Hg in spring/summer lead to a sharp increase in dissolved Hg that was partially converted to MeHg. The rapid dissolution of Hg also led to a sharp decrease in K_D values in summer (Table 1), representing a temporary disequilibrium that was relieved later in summer and was not apparent in autumn. Dissolved sulfide levels were quite low in July (1–2 μM), but sufficient to enhance the dissolution of HgS minerals. Although we have no data for later in summer, SO_4^{2-} reduction was still quite rapid in autumn, indicating that it was also rapid throughout the summer. Therefore, sulfide concentrations may have increased later in summer, removing dissolved Hg as a HgS precipitate, and leading to a decrease in the bioavailability of Hg for methylation. Hence, the spring to early autumn period exhibited a rapid dissolution and methylation of Hg, presumably followed by removal of available Hg and a decrease in methylation activity. It is possible that the bulk of MeHg formed each year occurs during a relatively short time in late spring early summer when this Hg dissolution event occurs.

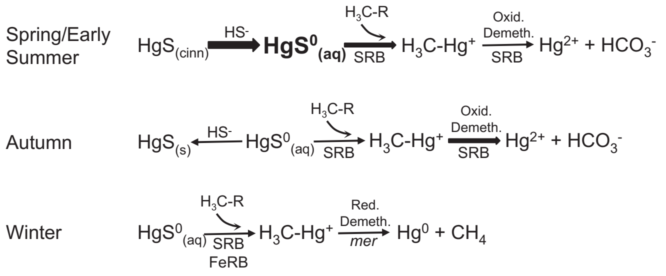

Fig. 8. Major processes occurring in lagoon sediments during three seasons. Bolder arrows and fonts refer to more rapid rates and larger pools, respectively. $HgS_{(cinn)}$ represents fine cinnabar particles. HgS^0 represents a dissolved neutral Hg-sulfur species that is presumably the form converted to MeHg by bacteria (Benoit et al., 1999). See text for details.

In autumn, SO_4^{2-} reduction and Hg methylation decreased, but the relatively high MeHg levels in pore water allowed for a net loss of MeHg due to demethylation. This loss was based on net methylation calculations using dissolved MeHg concentration data, and these calculations may be biased. First, the quantity of MeHg added as a tracer was orders of magnitude higher than ambient dissolved MeHg. Hence, the gross quantity of MeHg degraded during incubations was over 50 ng g^{-1} of sediment, most of which was the tracer. The rates in Table 1 were based on the assumption that MeHg degradation was a first order process and that the actual rate of MeHg use *in situ* was the product of the rate constant and ambient MeHg. If the fraction of ambient dissolved MeHg degraded was similar to the fraction of the tracer degraded (i.e., first order), then the rates using pore-water MeHg data would be correct. Second, unlike the methylation of Hg^{2+}, which appears to be controlled by dissolved Hg availability (Benoit et al., 1999; Hammerschmidt and Fitzgerald, 2004), studies have shown that an added MeHg tracer behaves similarly to the total MeHg pool, i.e., the combined dissolved and particle-associated MeHg pool turns over quite rapidly (Hintelmann et al., 2000). If this is the case, then calculations of net MeHg production need to be based on the product of the demethylation rate constant and total MeHg concentrations, which would be subtracted from the product of methylation rate constants and dissolved Hg_{tot}. When net production rates are calculated using this latter method, net consumption of MeHg (demethylation) occurs throughout the year at rates ranging from 36 pg mL^{-1} day^{-1} (winter) to 230 pg mL^{-1} day^{-1} (summer). These rates are unrealistic, which suggests that pore-water Hg concentrations are probably best suited for calculating actual Hg fluxes in these sediments.

The winter period behaved quite differently than summer or autumn. SO_4^{2-} reduction was less important as a source of MeHg and it was likely that Fe-reducing bacteria were of increased importance (Fig. 8). Sulfate reduction was also less important during demethylation, and it appeared that Hg-resistant bacteria that presumably harbored *mer* enzymes increased in importance as demethylators.

6. Conclusion

Sediments in Marano and Grado Lagoons are sites of active Hg cycling with year-round methylation of Hg and demethylation of MeHg. Transformations of Hg varied greatly throughout the year and were controlled by temporal changes in the sulfur cycle and the availability of dissolved Hg. Inorganic Hg apparently is mobilized in spring and early summer, as sediments become more reducing and SO_4^{2-} reduction rates and sulfide concentrations increase. The lagoon systems are sinks for cinnabar minerals from the Idrija mining region and the dissolution of fine cinnabar particles may be a source of Hg that supports rapid Hg methylation in early summer. A switch from net Hg dissolution to precipitation occurs later in summer and early autumn, which removes available Hg from solution and leads to net demethylation in autumn. Relationships between Hg transformation activities and SO_4^{2-} reduction in summer and autumn emphasize the importance of SRB during methylation and demethylation of Hg, while the link between transformation activities and sediment–water partitioning of Hg demonstrates the importance of dissolved Hg as a source for MeHg formation. The winter breakdown of the link between Hg transformations and SO_4^{2-} reduction suggests that Fe-reducing bacteria contribute to methylation in winter, and that Hg-resistant bacteria are of increased importance for demethylation.

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