Archimer
http://archimer.ifremer.fr

A Michaelis–Menten type equation for describing methylmercury dependence on inorganic mercury in aquatic sediments

Daniel Cossa[1, 2, *] , Cédric Garnier[3] , Roselyne Buscail[4] , Francoise Elbaz-Poulichet[5] , Nevenka Mikac[6] , Nathalie Patel-Sorrentino[3] , Erwan Tessier[3] , Sylvain Rigaud[7] , Véronique Lenoble[3] , Charles Gobeil[8]

[1] IFREMER, BP 330, 83507, La Seyne-sur-Mer, France
[2] ISTerre, Université J. Fourier, BP 53, 38041, Grenoble, France
[3] PROTEE, Université de Toulon, EA 3819, 83957, La Garde, France
[4] CEFREM-CNRS-UMR 5110, Université de Perpignan, 52 Avenue P. Alduy, 66860, Perpignan, France
[5] Laboratoire Hydrosciences, UMR CNRS, Universités Montpellier I & II, Place E. Bataillon, CC MSE, 34095, Montpellier Cedex 5, France
[6] Center for Marine and Environmental Research, Ruđer Bošković Institute, PO Box 1016, 10000, Zagreb, Croatia
[7] Cerege, Aix-Marseille Université, Europole Méditerranéen de l'Arbois, BP 80, 13545, Aix-en-Provence Cedex 04, France
[8] INRS-ETE, Université du Québec, 490 de la Couronne, Quebec, QC, G1K 9A9, Canada

*: Corresponding author : Daniel Cossa, email address : dcossa@ifremer.fr
cgarnier@univ-tln.fr ; buscail@univ-perp.fr ; felbaz@univ-montp2.fr ; mikac@irb.hr ; rigaud@cerege.fr ; gobeilch@ete.inrs.ca

Abstract:

Methylation of mercury (Hg) is the crucial process that controls Hg biomagnification along the aquatic food chains. Aquatic sediments are of particular interest because they constitute an essential reservoir where inorganic divalent Hg (Hg^{II}) is methylated. Methylmercury (MeHg) concentrations in sediments mainly result from the balance between methylation and demethylation reactions, two opposite natural processes primarily mediated by aquatic microorganisms. Thus, Hg availability and the activity of methylating microbial communities control the MeHg abundance in sediments. Consistently, some studies have reported a significant positive correlation between MeHg and Hg^{II} or total Hg (Hg_T), taken as a proxy for Hg^{II}, in aquatic sediments using enzyme-catalyzed methylation/demethylation mechanisms. By compiling 1,442 published and unpublished Hg_T–MeHg couples from lacustrine, riverine, estuarine and marine sediments covering various environmental conditions, from deep pristine abyssal to heavily contaminated riverine sediments, we show that a Michaelis–Menten type relationship is an appropriate model to relate the two parameters: $MeHg = aHg_T/(K_m + Hg_T)$, with $a = 0.277 \pm 0.011$ and $K_m = 188 \pm 15$ ($R^2 = 0.70$, $p < 0.001$). From K_m variations, which depend on the various encountered environmental conditions, it appears that MeHg formation and accumulation are favoured in marine sediments compared to freshwater ones, and under oxic/suboxic conditions compared to anoxic ones, with redox potential and organic matter lability being the governing factors.

Keywords: Mercury ; Methylmercury ; Aquatic sediment ; Methylation ; Demethylation

Responsible Editor: J. M. Melack

1. Introduction

Methylmercury (MeHg), mercury (Hg) most toxic form, bioconcentrates in organisms and biomagnifies along the aquatic food chains (Clarkson and Magos 2006). Although MeHg concentrations in sediments can be affected by exchanges with the water column, the main controlling factor of these concentrations appears to be the balance between Hg^{II} *in situ* methylation and MeHg demethylation reactions, two opposite natural processes primarily mediated by aquatic microorganisms (e.g., Ullrich et al. 2001; Barkay et al. 2011; Gilmour et al. 2011; Yu et al. 2012). Despite some early laboratory experiments which suggested that Hg^{II} methylation results from the activity of many aerobic and anaerobic microorganisms (Jensen and Jernelöv 1969; Vonk and Sijpesteijn 1973), more recent researches showed that methylation capacity in aquatic sediments is limited to anaerobic bacteria, including sulfate-reducing bacteria (SRB) (Compeau and Bartha 1984 and 1985; Choi et al. 1994; Baldi 1997), iron-reducing bacteria (IRB) (Fleming et al. 2006; Kerin et al. 2006; Yu et al. 2012) and methanogens (Hamelin et al. 2011). Furthermore, environmental incubations also suggested that SRB and IRB are the main mercury methylators in natural environments (Gilmour et al. 1992, 2011; Yu et al. 2010, 2012; Acha et al. 2012), with SRB being the dominant community (Choi et al. 1994; Baldi 1997; Pak and Bartha 1998; Yu et al. 2010). On the other hand, MeHg demethylation results from numerous types of microorganisms in both aerobic and anoxic environments (Oremland et al. 1991; Dahlberg and Hermansson 1995; Pearson et al. 1996; Marvin-Dipasquale and Oremland 1998; Marvin-Dipasquale et al. 2000), either by reductive or oxidative demethylation (Barkay et al. 2011; Mason 2012). In oxidative demethylation, active in SRB and methanogens, MeHg is converted into Hg^{II}, whereas in reductive demethylation, more extensively distributed throughout microbial communities, MeHg is converted into Hg^{0}.

For bacterial Hg^{II} methylation, Parks et al. (2013) recently reported a two-gene cluster *(HgcA* and *HgcB)*, suggesting a common Hg pathway in all methylating bacteria hitherto sequenced, including SRB, IRB and methanogen strains. Radiolabelled experiments suggested that a methyl group of methyltetrahydrofolate, from the acetyl-CoA pathway, is transferred to *HgcA* as CH_3^+, consistently with the enzyme-catalyzed methylation pathway earlier proposed by Choi et al. (1994). For MeHg demethylation, multiple enzymatic pathways coexist. The oxidative demethylation, primarily producing CO_2, seems ubiquitous in anaerobic sediments (Oremland et al. 1991), whereas reductive pathway, producing mainly CH_4 (*via* the organomercurial-lyase pathway, Begley et al. 1986), dominates in aerobic sediments or under anaerobic incubations of highly contaminated sediments (Marvin-Dipasquale et al. 2000; Schaefer et al. 2002; Segade et al. 2010).

All these proposed pathways suggested that an enzymatic model between MeHg net formation and its substrate concentration (Hg^{II}) should be shared in every sedimentary situation. Indeed, Gilmour et al. (2011) found a strong positive relationship between MeHg production and the log of the total Hg concentration obtained in a controlled experiment involving a SRB, *Desulfovibrio desulfuricans.* King et al. (1999) also found a nonlinear relationship between rates of MeHg formation as a function of Hg^{II} added in sediment slurries, consistent with a first-order Michaelis-Menten model. Besides the results of these experimental approaches, several field studies converged to find significant positive relationships between Hg^{II} or HgT, and MeHg in freshwater, brackish and marine sediments (e.g., Benoit et al. 2003; Hammerschmidt and Fitzgerald 2006; Drott et al. 2008; Marvin-Dipasquale et al. 2009; Gilmour et al. 2011). These empirical relationships also suggested that net MeHg production in aquatic sediments is limited by Hg^{II} availability, if HgT is assumed as a proxy for Hg^{II} substrate for methylation, which is reasonable since MeHg, in most cases, represents less than 1% of sedimentary HgT. Testing various substratum

including cinnabar, metacinnabar, Hg^{II} bound to mackinavite or organic matter (OM), Jonsson et al. (2012) reported Hg methylation rates spanning over 2 orders of magnitude, increasing with Hg^{II} dissolution or desorption from solids. Besides mobilization from solid, OM was also suggested as a key-parameter of Hg sediment-water partitioning and bioavailability, which seems to constrain MeHg/HgT in surface sediments (e.g., Sunderland et al., 2006; Hammerschmidt and Fitzgerald, 2006; Schartup et al., 2013). Lastly, large differences between estimations of Hg methylation rates exist, depending on environmental conditions, which are not directly related to Hg^{II} bioavailability, but rather to changes in the present microbial communities (Ranchou-Peyruse et al. 2009; Segade et al. 2010; Mason 2012; Husu-Kim et al. 2013).

In order to further examine the environmental conditions that control net Hg methylation and limit MeHg accumulation in aquatic sediments, we propose the use of a model resembling Michaelis–Menten kinetics synthesizing the enzymatic methylation and demethylation reactions. We thus applied this type of model to 1442 MeHg/HgT couples, collected in scientific literature or from our unpublished works. The gathered data cover sedimentary environments from surface to deep layers including abyssal, coastal, lagoonal, estuarine, lacustrine and riverine sediments, and range from pristine to heavily polluted areas and from aerobic to sulfidic environments. It appears that a Michaelis-Menten equation significantly (p< 0.001) relates MeHg to HgT concentrations accordingly to the converging hypotheses of Benoit et al. (2003), Drott et al. (2007, 2008), Hammerschmidt et al. (2008), Sparling (2009), Frohne et al. (2012) and others. Apparent half-saturation value (K_m) of the model depends on the various encountered environmental conditions, with redox potential and organic matter lability as the governing factors.

2. Material and methods

Unpublished data (N=602) originated from sediment cores collected from Mediterranean environments, including near-shore environments (Pierre-Blanche lagoon and Toulon bay), continental shelf (Rhone pro-delta), canyon (Cap de Creus), abyssal plain (Algero-Provencal and Ionian basins), and Arctic Ocean margin and deep basins. HgT and MeHg determinations were performed according to Abi-Ghanen et al. (2011).

The gathered data (N=840) used here originated from published works mentioned in Table 1. When figure data were not available, the DigiSoft program (available for free, download at: http://gss.srce.hr/pithos/rest/omanovic@irb.hr/files/Software/) software was used to convert data points of the published graphs into numerals.

The Michaelis-Menten equation is frequently used to describe enzyme-catalyzed processes, as it relates to the metabolic conversion of a compound. The Michaelis-Menten fitting has successively described the methylation rate vs Hg^{II} concentration from experiments performed either on isolated bacteria strain (e.g. the *Desulfovibrio desulfuricans* LS isolated from salt marsh sediment, Choi et al. 1994), or directly on bulk sediments (e.g. King et al. 1999). Although normally used to model kinetic results (i.e. product formation rate *vs* substrate concentration), some authors also used such relationships between product and substrate concentrations. For instance, MeHg or HgT contents in fish tissues *vs* aqueous Hg_T were properly depicted using Michaelis-Menten curves by Brent and Kain (2011) and Mathews et al. (2013), respectively. Even empirical, such fits offer the advantage of a mechanistic foundation (i.e. enzymatic processes governing Hg methylation) and provide benchmarks for maximum methylation and half-saturation constant, parameters which can

be confronted to laboratory or field experiments. Michaelis-Menten model was fit using SigmaPlot 10.0 software.

3. Results and discussion

The data couples spanned over six and five orders of magnitude for HgT and MeHg, respectively: from 3.1×10^{-3} to 1.1×10^{3} nmol g^{-1} and from 3.9×10^{-5} to 3.6×10^{-1} nmol g^{-1}, thus going from pristine to heavily contaminated sediments. Considering the entire data set, we achieved a highly significant (R^2 = 0.70, N = 1442, p < 0.001) overall relationship with the following equation:

$$MeHg = \frac{a \times Hg_T}{K_m + Hg_T} \, ,$$

where "a" is the saturation MeHg concentration (0.277 ± 0.011 nmol g^{-1}) and "K_m" estimates the HgT concentration, which corresponds to MeHg half-saturation (188 ± 15 nmol g^{-1}) (Fig. 1). As Km increases, methylation efficiency decreases.

This Michaelis-Menten function corroborates that sediment MeHg concentrations are directly dependent of HgT concentration taken as a proxy of the methylation substrate (Hg^{II}), with an asymptotic MeHg concentration (a) mainly defined by data from Sweden riverine and estuarine sediments heavily contaminated by chlor-alkali activities (Drott et al. 2008). Such a saturation point was already noticed for contaminated sediments by Benoit et al. (2003). This maximum, theoretically due to Hg^{II} saturation of the methylation enzymatic systems, illustrates a more complex situation earlier qualified as "mercury accumulation paradox" (Schaefer et al. 2004). The Michaelis-Menten type relationship calculated here is the combination of multiple counteracting enzymatic reactions. As suggested by several authors (Marvin-Dipasquale and Oremland 1998; Marvin-Dipasquale et al. 2000; Segade et al. 2010), the asymptotic MeHg concentration can be interpreted as a modification of the reductive demethylation pathways in contaminated sediments, resulting from a methanogen to SRB demethylation shift when Hg^{II} or MeHg contents exceed a threshold value. Oxidative or reductive demethylation pathways would have indeed striking different consequences in the mercury cycling in the sediment. As quoted by Segade et al. (2010), the end-product of the reductive demethylation is gaseous Hg^{0}, a species which can escape from the sediment allowing its real detoxification, whereas the oxidative demethylation generates an Hg^{II} end-product which may be recycled in the methylation pathway.

The K_m value (188 ± 15 nmol g^{-1}) constitutes a relatively stable estimate, as the coefficient of variation falls below 8%. However, keeping the asymptotic a value of the equation fixed at 0.277 nmol g^{-1}, peculiar K_m values exhibit large variations (Table 2) depending on the environmental characteristics and sites, illustrated by the K_m increase when methylation efficiency decreases. Ranking the 1442 HgT-MeHg couples according to their K_m values, it appears that (i) 5% of HgT-MeHg couples are lower than 26.5, with 93% and 75% of them are from surface sediments ($\leq$ 2 cm) and pristine sediment, respectively, and that (ii) 5% highest K_m values are higher than 825, with 97% of them correspond to deep (> 10 cm) contaminated sediments. In addition, relatively low Km values (139 ± 12 nmol g^{-1}) characterizes surface sediments ($\leq$ 2 cm) compared to that of sediments collected below 10 cm depth (223 ± 10 nmol g^{-1}, Table 2). Furthermore, the Km values for the samples collected in open ocean are much lower than those for the river samples, whereas intermediate values characterized coastal and lagoonal samples (Table 2).

Interestingly, the Michaelis-Menten model can be simplified, for HgT << K_m, as a simple ratio: $MeHg = b \times Hg_T$ with $b = \dfrac{a}{K_m} \times 100$. The calculated b value is 0.15% for our entire data set, and ranges from 0.03 to1.05% for the lowest and highest methylation efficiencies, respectively (see Supplementary information Fig. SI1). This is consistent with previous observations showing that MeHg/HgT ratios are a good proxy for net Hg methylation rates (e.g. Benoit et al. 2003; Hammerschmidt and Fitzgerald 2004, 2006; Guimarães et al. 2006; Drott et al. 2008). However, for the highest HgT values, the MeHg/HgT ratios notably decrease (Fig. SI2). This suggests that a "ratio model" does not fit the entire data set and, consequently, the diversity of the encountered environmental situations, as the Michaelis-Menten model does.

In summary, (i) sediments collected at depth > 10 cm (N=228) exhibit higher K_m than surface ones (0-2 cm), and (ii) freshwater and contaminated coastal sediments exhibit higher K_m than marine sediments. In other words, oxic/suboxic sediments and those originating from autochthonous marine settling material present higher net methylation rates than anoxic and/or continental derived sediments. It also suggests that authigenic particles from the water column may be MeHg-enriched before being deposited and incorporated in surficial sediment, as earlier suggested by Muresan et al. (2007) for a lagoonal environment. Gilmour et al. (1998) and Benoit et al. (2003) already noticed that highest MeHg/Hg ratios were found in sediments where sulfate reduction was high but sulfide accumulation was low, such as sediments' upper layers and continental slopes. Han et al. (2010) also concluded from experiments with sulfate-limited sediments that Hg^{II} active methylation "possibly occurred by syntrophic processes arising between methanogens and sulfidogens". It has been recognized that in mildly reductive conditions, HgS$^0_{(aq)}$, Hg(SH)$^0_{(aq)}$ and uncharged mercury molecules such as Hg-cysteine complexes or even HgS particles facilitate mercury uptake in methylator microorganisms (Benoit et al. 1999; Drott et al. 2007; Schaefer and Morel 2009; Graham et al. 2012). Conversely, enhancement of dissolved sulfide in anoxic sediments may inhibit MeHg production (Benoit et al. 1999; Hammerschmidt et al. 2008). It is interesting to note that high Km values for contaminated sediments are in agreement with the results of Drott et al. (2008) and Hines et al. (2000) showing that demethylation of MeHg progressively increases with depth in such sediments.

The differences between freshwater and marine sediments K_m values are likely to be interpreted in terms of OM reactivity since the organic carbon (OC) taken as a whole does not seem to be an overall explanatory factor for MeHg concentration. Indeed, from the 617 HgT-MeHg couples associated with OC contents, the significance of the MeHg vs OC relationship is not better than that of the HgT vs OC, and the MeHg/HgT ratio appeared not to be related to OC (Fig. SI3 A, B and C). It is generally observed that OM is more abundant in river and near-shore sediments than in open ocean sediments. However, it is also generally accepted that the continental OM accumulated in rivers and estuaries is more mature than the freshly deposited OM in the high productive area of the ocean margins (Arnost and Holmer 2003). This suggests that labile fresh OM present at the shelf margin is more effective in fuelling the microbial methylating activity than refractory OM, consistently with the hypothesis of Ravichandran (2004). To check the involvement of OM degradability in determining the relative MeHg abundance, we compared the MeHg/HgT ratios with the amino acid concentrations available from the sediment of Cap de Creus canyon (Fig. 2). Considering nitrogenous compounds, especially amino acid, as the most hydrolysable OM present at the N.W. Mediterranean margin (Buscail and Germain 1997), it can be deduced from Figure 2 that the OM lability is a limiting factor of the Hg methylation. This evidence agrees with an earlier study on MeHg distribution in harbour marine sediments which stated that allochthonous organic material attenuates gross and net rates of MeHg production

(Hammerschmidt et al. 2008). Drott et al. (2008) likewise showed that differences in the primary production and subsequent availability of easily degradable OM (serving as electron donor for methylating bacteria) was indicated to be the most important factor behind the observed differences in MeHg/HgT ratios and so K_m values among sites.

We conclude that, in sulfidic and non sulfidic pristine and heavily polluted sediments from both freshwater and marine environments, the abundance of MeHg relative to HgT depens on Hg availability and OM reactivity. Oxic and suboxic sediments receiving both fresh OM and high Hg deposition, such as oceanic margins, favor MeHg accumulation. Only a part of MeHg is preserved in deep anoxic sediments leading to low MeHg/HgT ratios. We can speculate furthermore that a Michaelis-Menten type model linking MeHg to inorganic Hg^{II} could also be valid for the oceanic water column.

Acknowledgements

References

Abi-Ghanem C, Nakhlé K, Khalaf G, Cossa D (2011) Mercury distribution and methylmercury mobility in the sediments of three sites on the Lebanese Coast, Eastern Mediterranean. *Arch Env Cont Tox* 60:394-405

Acha D, Hintelmann H, Pabon CA (2012) Sulfate-reducing bacteria and mercury methylation in the water column of the lake 658 of the experimental lake area. *GeoMicrob J* 29:667-674

Arnost C, Holmer M (2003) Carbon cycling in a continental margin sediment: Contrasts between organic matter characteristics and mineralization rates and pathways. *Est Cstl Shelf Sci* 58:197-208

Baldi F (1997) *Microbial transformation of mercury species and their importance in the biogeochemical cycle of mercury*. In: Sigel A, Sigel H eds. Metal Ions in Biological Systems, vol. 34. Mercury and its Effects on Environment and Biology. Marcel Dekker Inc., NY, pp. 213-257.

Barkay T, Kroer N, Poulain AJ (2011) Some like it cold: microbial transformations of mercury in polar regions. *Polar Research* 30; DOI: 10.3402/polar.v30i0.15469

Benoit JM, Gilmour CC, Mason RP, Riedel GS, Riedel GF (1998) Behavior of mercury in the Patuxent river estuary *Biogeochem* 40:249-265

Benoit JM, Gilmour CC, Mason RP, Heyes A (1999). Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. *Environ Sci Technol* 33:951-957

Benoit JM, Gilmour CC, Heyes A, Mason RP, Miller CL (2003) Geochemical and Biological Controls over Methylmercury Production and Degradation in Aquatic Ecosystems. In: Chai, Y., Braids, O.C. (Eds.), *Biogeochemistry of Environmentally Important Trace Elements*. ACS Symposium Series 835.

Begley TP, Walts AE, Walsh CT (1986) Mechanistic studies of a protonolytic organomercurial cleaving enzyme: bacterial organomercurial lyase. *Biochemistry* 25:7192–7200

Brent RN, Kain DG (2011) Development of an Empirical Nonlinear Model for Mercury Bioaccumulation in the South and South Fork Shenandoah Rivers of Virginia. *Arch Environ Contam Toxicol* 61:614–623

Buscail R, Germain C (1997) Present-day organic matter sedimentation on the NW Mediterranean margin: Importance of off-shelf export. *Limnol Oceanogr* 42:217-229

Choi SC, Chase T, Bartha R (1994) Metabolic pathways leading to mercury methylation in *Desulfovibrio desulfuricans* LS. *Appl Environl Microb* 60:4072–4077

Clarkson TW, Magos L (2006) The toxicology of mercury and its chemical compounds. *Crit Rev Toxicol* 36:609–662

Compeau GC, Bartha R (1984) Methylation and demethylation of mercury under controlled redox, pH and salinity conditions. *Appl Environ Microb* 48:1203–1207

Compeau GC, Bartha R (1985) Sulfate-reducing bacteria: principal methylators of mercury in anoxic estuarine sediment. *Appl Environ Microb* 50:498–502

Dahlberg C, Hermansson M (1995) Abundance of Tn3, Tn21, and Tn501 transposase (tnpA) sequences in bacterial community DNAfrom marine environments. *Appl Environ Microb* 61:3051–3056

Drott A, Lambertsson L, Björn E, Skyllberg U (2007) Importance of dissolved neutral mercury sulfides for methyl mercury production in contaminated sediments. *Environ Sci Technol* 41:2270–2276

Drott A, Lambertsson L, Björn E, Skyllberg U (2008) Do potential methylation rates reflect accumulated methyl mercury in contaminated sediments? *Environ Sci Technol* 42:153-158

Fleming, EJ, Mack EE, Green PG, Nelson DC (2006) Mercury methylation from unexpected sources: molybdate-inhibited freshwater sediments and an iron-reducing bacterium. *Appl Environ Microb* 72:457−464

Frohne T, Rinklebe J, Langer U, Du Laing G, Mothes S, Wennrich R (2012) Biogeochemical factors affecting mercury methylation rate in two contaminated floodplain soils. *Biogeosciences* 9:493–507

Gilmour CC, Henry EA, Mitchell R (1992) Sulfate stimulation of mercury methylation in freshwater sediments. *Environ Sci Technol* 26:2281–2287

Gilmour CC, Riedel GS, Ederington MC, Bell JT, Benoit JM, Gill GA, Stordal MC (1998) Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. *Biogeochem* 40:327-345

Gilmour CC, Elias DA, Kucken AM, Brown SD, Palumbo AV, Schadt CW, Wall JD (2011) Sulfate-reducing bacterium *Desulfovibrio desulfuricans* ND132 as a model for understanding bacterial mercury methylation. *Appl Environ Microb* 77:3938–3951

Graham AM, Aiken GR, Gilmour CC (2012) Dissolved organic matter enhances microbial mercury methylation under sulfidic conditions. *Environ Sci Technol* 46:2715–2723

Guedron S, Huguet L, Vignati DAL, Liu B, Gimbert F, Ferrari BJD, Zonta R, Dominik J (2012) Tidal cycling of mercury and methylmercury between sediments and water column in the Venice Lagoon (Italy). *Mar Chem* 130:1-11

Guimarães JR, Mauro JB, Meili M, Sundbom M, Haglund AL, Coelho-Souza SA, Hylander LD (2006) Simultaneous radioassays of bacterial production and mercury methylation in the periphyton of a tropical and a temperate wetland. *J Environ Manage* 81:95-100

Hamelin S, Amyot M, Barkay T, Wang Y, Planas D (2011) Methanogens: principal methylators of mercury in lake periphyton. *Environ Sci Technol* 45:7693–7700

Hammerschmidt CR, Fitzgerald WF (2004) Geochemical controls on the production and distribution of methylmercury in near-shore marine sediments. *Environ Sci Technol* 38 :1487-1495

Hammerschmidt CR, Fitzgerald WF (2006) Methylmercury cycling in sediments on the continental shelf of southern New England. *Geochim Cosmochim Acta* 70:918–930

Hammerschmidt CR, Fitzgerald WF, Balcom PH, Visscher PT (2008). Organic matter and sulfide inhibit methylmercury production in sediments of New York/New Jersey Harbor. *Mar Chem* 109:165–182

Han S, Obraztsova A, Pretto P, Choe KY, Gieskes J, Deheyn DD, Tebo BM (2007) Biogeochemistry factors affecting mercury methylation in sediments of the Venice lagoon, Italy. *Env Tox Chem* 26:655-663

Han S, Narasingarao P, Obraztsova A, Gieskes J, Hartmann AC, Tebo BM, Allen E, Deheyne DD (2010) Mercury Speciation in Marine Sediments under Sulfate-Limited Conditions. *Environ Sci Technol* 44:3752–3757

Hines ME, Horvat M, Faganeli J, Bonzongo JCJ, Barkay T, Majorf EB, Scott K, Bailey EA, Warwick JJ, Lyons WB (2000) Mercury Biogeochemistry in the Idrija River, Slovenia, from above the Mine into the Gulf of Trieste. *Env. Res.* 83:129–139

Hintelmann H, Wilken R-D (1995) Levels of total mercury and methylmercury compounds in sediments of the polluted Elbe River: influence of seasonally and spatially varying environmental factors. *Sci Total Environ*, 166:1-10

Hollweg TA, Gilmour CC, Mason RP (2010) Mercury and methylmercury cycling in sediments of the mid-Atlantic continental shelf and slope. *Limnol Oceanogr* 55:2703–2722

Hsu-Kim H, Kucharzyk KH, Zhang T, Deshusses MA (2013) Mechanisms Regulating Mercury Bioavailability for Methylating Microorganisms in the Aquatic Environment: A Critical Review. *Environ. Sci. Technol.*, Just Accepted Manuscript. 05 Feb 2013

Jensen S, Jernelöv A (1969) Biological methylation of mercury in aquatic organisms. *Nature* 223:753−754

Jonsson S, Skyllberg U, Nilsson MB, Westlund PO, Shchukarev A (2012) Mercury Methylation Rates for Geochemically Relevant Hg^{II} Species in Sediments. *Environ Sci Technol* 2012:11653−11659

Kerin EJ, Gilmour CC, Roden E, Suzuki MT, Coates JD, Mason RP (2006). Mercury methylation by dissimilatory iron-reducing bacteria. *Appl and Environ Microb* 72:7919 – 7921

King JK, Saunders FM, Lee RF, Jahnke RA (1999) Coupling mercury methylation rates to sulfate reduction rates in marine sediments. *Environ Toxicol Chem* 18:362–1369

Kwokal Z, Franciskovic-Bilinski S, Bilinski H, Branica M (2002) A comparison of anthropogenic mercury pollution in Kaštela Bay (Croatia) with pristine estuaries in Ore (Sweden) and Krka (Croatia). *Mar Poll Bull* 44:1152–1169

Marvin-DiPasquale MC, Oremland RS (1998) Bacterial methylmercury degradation in Florida Everglad peat sediment. *Environ Sci Technol* 32:2556-2563

Marvin-DiPasquale MC, Agee J, McGowan C, Oremland RS, Thomas M, Krabbenhoft DP, Gilmour CC (2000) Methyl-Mercury Degradation Pathways: A Comparison among Three Mercury-Impacted Ecosystems. *Environ Sci Technol* 34 :4908-4916

Marvin-DiPasquale MC, Lutz MA, Brigham ME, Krabbenhoft DP, Aiken GR, Orem WR, Hall BD (2009) Mercury cycling in stream ecosystems- 2. Benthic methylmercury production and bed sediment pore water partitioning. *Environ Sci Technol* 43:2726–2732

Mathews TJ, Southworth G, Peterson MJ, Roy WK, Ketelle RH, Valentine C, Gregory S (2013) Decreasing Aqueous Mercury Concentrations to Meet the Water Quality Criterion in Fish: Examining the Water-Fish Relationship in Two Point-Source Contaminated Streams. *Sci Total Environ* 443:836-848

Mason RP (2012) *The Methylation of Metals and Metalloids in Aquatic Systems*. Chap. 11. <u>In:</u> Methylation - From DNA, RNA and Histones to Diseases and Treatment. Dricu A editor. pp 271-301. INTECH Open Science. ISBN 978-953-51-0881-8

Mason RP, Lawrence AL (1999) Concentration, distribution, and bioavailability of mercury and methylmercury in sediments of the Baltimore harbour and Chesapeake bay, Maryland, USA. *Environ Toxicol Chem* 18:2438–2447

Mason RP, Lawson NM, Lawrence AL, Leaner JJ, Lee JG, Sheu GR (1999) Mercury in Chesapeake Bay. *Mar Chem* 65:77-86

Mikac N, Niessen S, Ouddane B, Wartel M (1999) Speciation of mercury in sediments of the Seine estuary (France). *Appl Org Chem* 13:715-725

Mikac N, Foucher D, Clarisse O, Niessen S, Lojen S, Logar M, Horvat M, Leermarkers M (2004) Relationship between mercury species and solid sulfides in aquatic sediments. *RMZ - Materials and Geoenvironment* 51 (Part 2): 1214–1217

Muhaya B, Leermakers M, Baeyens W (1997) Total mercury and methylmercury in sediments and in the polychaete Nereis diversicolor at Groot Buitenschoor (Scheldt estuary, Belgium). *Wat Air Soil Pollut* 94:109-123

Muresan B, Cossa D, Jézéquel D, Prévot F, Kerbellec S (2007) The biogeochemistry of mercury at the sediment water interface in the Thau lagoon. 1. Partition and speciation. *Est Cstl Shelf Sci* 72:472-484

Oremland RS, Culbertson CW, Winfrey MR (1991) Methylmercury decomposition in sediments and bacterial cultures: involvement of methanogens and sulfate reducers in oxidative demethylation. *Appl Environ Microb* 57:130–137

Pak KR, Bartha R (1998) Mercury Methylation by Interspecies Hydrogen and Acetate Transfer between Sulfidogens and Methanogens. *Appl Environ Microb*, 64:1987–1990

Parks JM, Johs A, Podar M, Bridou R, Hurt RA, Smith SD, Tomanicek SJ, Qian Y, Brown SD, Brandt CC, Palumbo AV, Smith JC, Wall JD, Elias DA, Liang L (2013) The Genetic Basis for Bacterial Mercury Methylation. *Science,* doi: 10.1126/science.1230667

Pearson AJ, Bruce KD, Osborn AM, Ritchie DA, Strike P (1996) Distribution of class II transposase and resolvase genes in soil bacteria and their association with mer genes. *Appl Environ Microb* 62:2961–2965

Ranchou-Peyruse M, Monperrus MR, Bridou R, Duran R, Amouroux D, Salvado JC, Guyoneaud R (2009) Overview of mercury methylation capacities among anaerobic bacteria including representatives of the sulfate-reducers: implications for environmental studies. *GeoMicrob J* 26:1–8

Ravichandran M (2004) Interactions between mercury and dissolved organic matter – a review. *Chemosphere* 55:319-331

Rigaud S, Radakovitch O, Couture R-M, Deflandre B, Cossa D, Garnier C, Garnier J-M (2013) Mobility and fluxes of trace elements and nutrients at the sediment-water interface of a lagoon under contrasting water-column oxygenation. *Appl Geochem* 31:35-51

Segade SR, Dias T, Ramalhosa E (2010) *Mercury methylation versus demethylation : Main processes involved.* Chap 7. In: Methylmercury: Formation, Sources and Health Effects. Clampet AP editor. 32p. Nova Science Publishers, Inc. ISBN: 978-1-61761-838-3

Schaefer JK, Letowski J, Barkay T (2002) mer -Mediated Resistance and Volatilization of Hg(II) Under Anaerobic Conditions. Geomicrobiol J *19:87-102*

Schaefer JK, Yagi J, Reinfelder JR, Cardona T, Ellickson KM, Tel-Or S, Barkay T (2004) Role of the bacterial organomercury lyase (MerB) in controlling methylmercury accumulation in mercury-contaminated natural waters. *Environ Sci Technol* 38:4304-4311

Schaefer JK, Morel FM (2009) High methylation rates of mercury bound to cysteine by *Geobacter sulfurreducens. Nature Geoscience* 2:123–126

Schartup AT, Mason RP, Balcom PH, Hollweg TA, Chen CY (2013). Methylmercury Production in Estuarine Sediments: Role of Organic Matter. *Environ Sci Technol* 47 :695–700

Sparling R (2009) Biogeochemistry: Mercury methylation made easy*. Nature Geoscience* 2, 92–93

Sunderland EM, Gobas FAPC, Branfireun BA, Heyes A (2006) Environmental controls on the speciation and distribution of mercury in coastal sediments. *Mar Chem* 102:11-123

Tessier E, Garnier C, Mullot J-U, Lenoble V, Arnaud M, Raynaud M, Mounier S (2011) Study of the spatial and historical distribution of sediment inorganic contamination in the Toulon bay (France). *Mar Pollut Bull* 62:2075-2086

Ullrich SM, Tanton TW Abdrashitova SA (2001) Mercury in the aquatic environment: a review of factors affecting methylation. *Crit Rev Environ Sci Technol* 31:241–293

Vonk JW, Sijpesteijn AK (1973) Studies on the methylation of mercuric chloride by pure cultures of bacteria and fungi. *Antonie van Leeuwenhoek* 39:505−513

Yu RQ, Adatto I, Montesdeoca MR, Driscoll CT, Hines ME, Barkay T (2010) Mercury methylation in sphagnum moss mats and its association with sulfate-reducing bacteria in an acidic Adirondack forest lake wetland. *FEMS Microbiology Ecology* 74:655–668

Yu RQ, Flanders JR, Mack EE, Turner R, Mirza B, Barkay T (2012) Contribution of coexisting sulfate and iron reducing bacteria to methylmercury production in freshwater river sediments. *Environ Sci Technol* 46:2684−2691

Tables

Table 1. Sources of published data used in the Michaelis-Menten model. [1]Data compilation.

Area	Reference
Lebanese coast	Abi-Ghanem et al. (2011)
Patuxent estuary	Benoit et al. (1998)
Rivers, lakes, wetlands and marine margins	Benoit et al. (2003)[1]
Karlshäll, Köpmanholmen and Skutskär estuaries	Drott et al. (2008)
Venice lagoon	Han et al. (2007), Guédron et al. (2012)
Long Island sound	Hammerschmidt and Fitzgerald (2004)
New England shelf	Hammerschmidt and Fitzgerald (2006)
Elbe river	Hintelmann and Wilken (1995)
	Hollweg et al. (2010)
Western Atlantic shelf and slope	
Kastela bay, Krka and Öre estuaries	Kwokal et al. (2002), Mikac et al. (2004)
Chesapeake bay	Mason and Lawrence (1999), Mason et al. (1999)
Seine estuary	Mikac et al. (1999)
Rupel, Deule, Seine and Soča rivers	Mikac et al. (2004)
Scheldt estuary	Muhaya et al. (1997)
Thau lagoon	Muresan et al. (2007)
Berre lagoon	Rigaud et al. (2013)
Passamaquoddy bay	Sunderland et al. (2006)

Table 2. K_m values for Michaelis-Menten type relationships between MeHg (nmol g^{-1}) and HgT (nmol g^{-1}) within various sediment types (values obtained keeping the asymptotic a parameter of the equation fixed at 0.277 nmol g^{-1}). (1) data defining the asymptotic value of the equation, i.e. from Sweden riverine and estuarine sediments, heavily contaminated by chlor-alkali activities (Drott et al. 2008).

Type of sediment	K_m	R^2 (n) p	Location	HgT level (nmol g^{-1})
Highest points (5%)	14 ± 2	0.94 (72) <0.0001	Surface sediment from various region (and heavily contaminated sediments)[1]	< 1 (and > 100)[1]
Lowest points (5%)	1870 ± 70	0.93 (72) <0.0001	Toulon and Kastela bays, Venice and Berre lagoons, Elbe, Seine and Soča rivers and estuaries	10-1000
Surface (< 2 cm)	139 ± 12	0.41 (226) <0.0001	All sites	< 1 - 1000
Deep (> 10 cm)	223 ± 10	0.75 (550) <0.0001	All sites	< 1 - 1000
Rivers	327 ± 38	0.38 (149)	Deule, Elbe, Rupel, Seine, Soča	> 1
Estuaries	159 ± 20	0.64 (171) <0.0001	Krka, Karlshäll, Köpmanholmen, Öre, Patuxent, Seine, Scheldt and Skutskär estuaries	> 0.1
Lagoons	223 ± 16	0.10 (201) <0.10	Berre, Pierre-Blanche, Thau and Venice (Mediterranean lagoons)	0.1-10
Bays and shelves	168 ± 5	0.79 (642) <0.0001	Chesapeake, Kastela, Passamaquoddy and Toulon bays, Lebanese coast, Rhone delta, Long Island Sound	< 1 - 1000
Open sea	89 ± 8	0.31 (277) <0.0001	Abyssal plains and slope (Arctic Ocean, Mediterranean Sea , Atlantic Ocean)	< 1
Abyssal plain	24.7 ± 0.2	0.99 (260) <0.0001	Mediterranean Sea and Arctic Ocean	< 0.5

Figures

Figure 1. Overall Michaelis-Menten type relationship ($p < 0.001$) between 1442 couples of MeHg and HgT concentrations in aquatic sediments. MeHg/HgT couples from various aquatic sediments including marine abyssal, coastal, lagoonal, estuarine, lacustrine and riverine sediments ranging from pristine environments to heavily polluted ones, and from aerobic to sulfidic environments. The gathered data used here originate from published (Table 1) and present authors' unpublished works. When figure data were not available the DigiSoft program (available free for download at http://gss.srce.hr/pithos/rest/omanovic@irb.hr/files/Software/) software was used to convert data points of the published graphs into numerals.
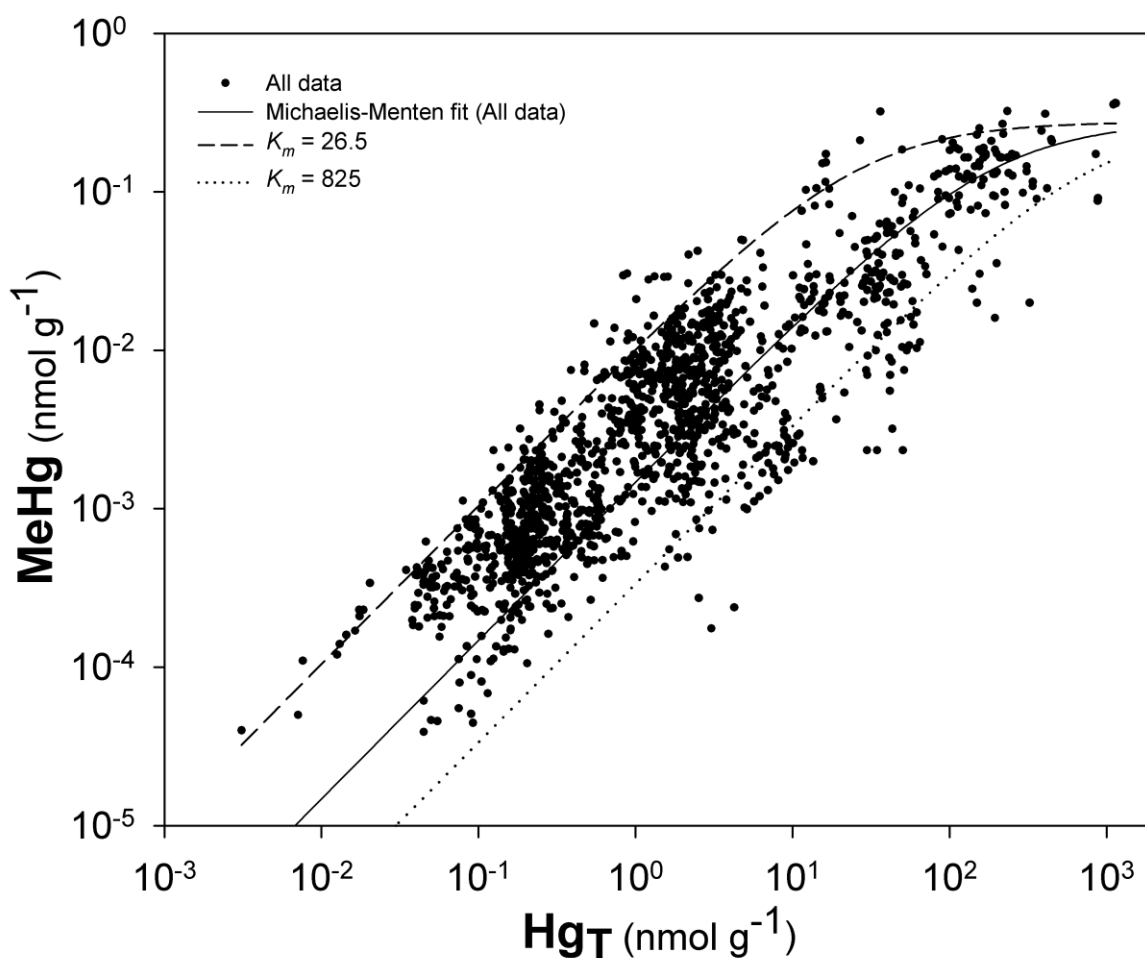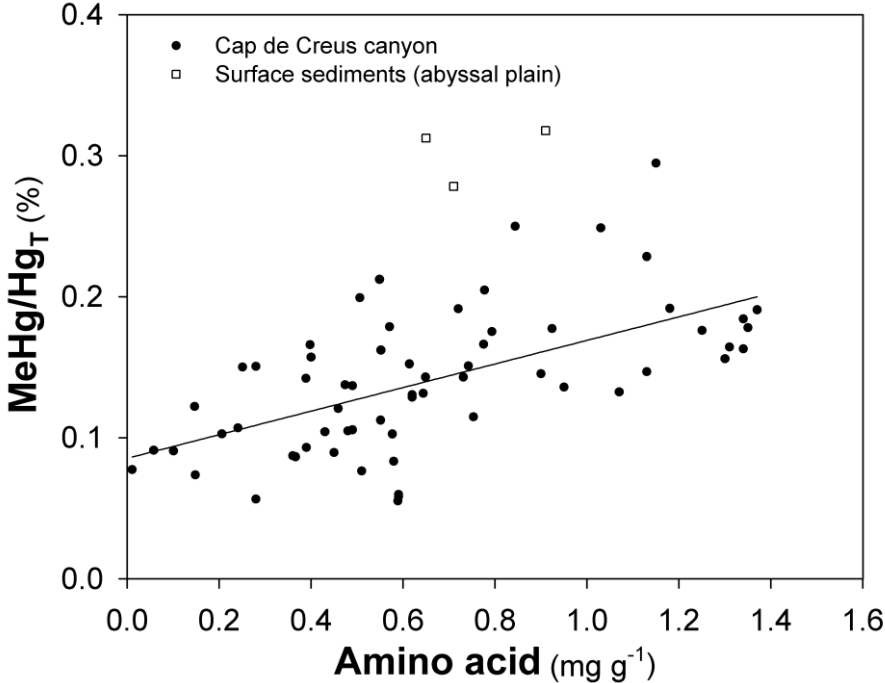
Figure 2. Relationship (p < 0.001) between MeHg/HgT ratios and amino acid (proxy of labile organic matter) in the sediment of the Cap de Creus canyon (NW Mediterranean).

Supplementary informations

Figure SI 1. The Michealis-Menten model with upper and lower ratio lines in gray (see text for more explanation)
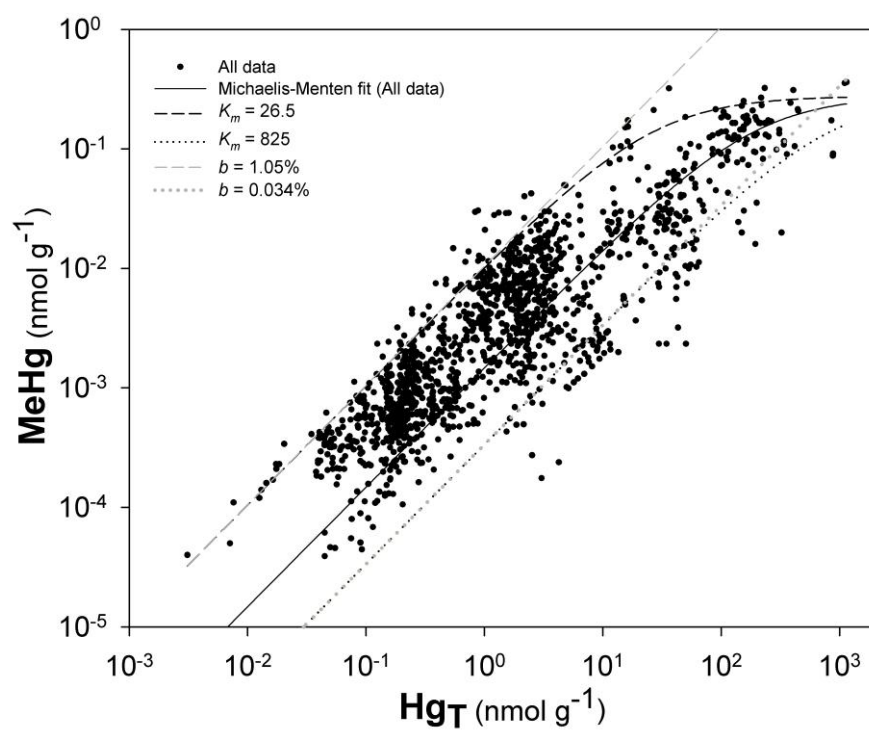
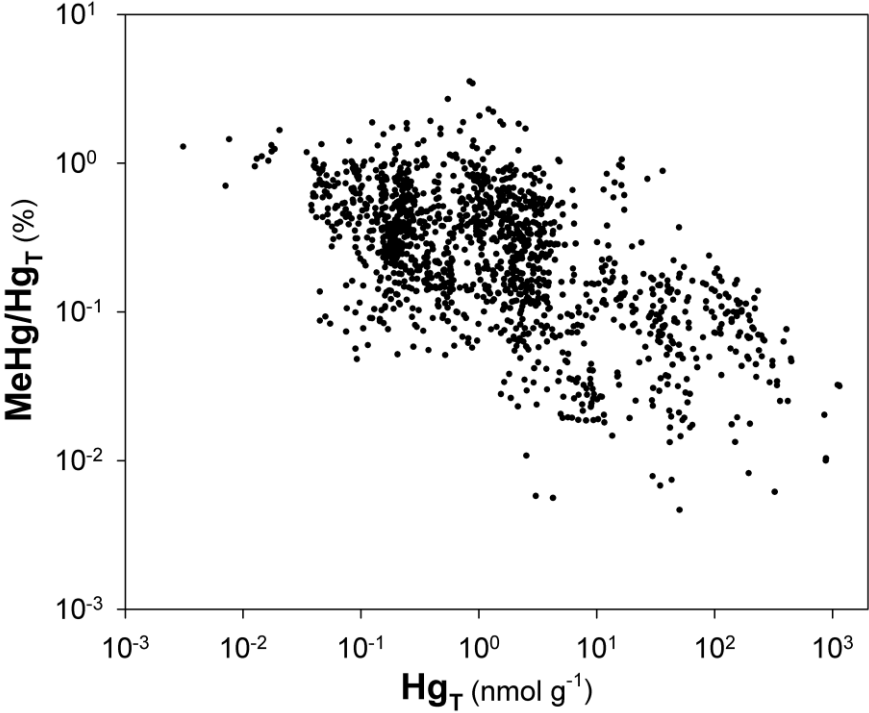**Figure SI 2.** Variation of MeHg/HgT ratio *vs* HgT concentration (N=1442)

Figure SI 3. Variation of (A) HgT, (B) MeHg and (C) MeHg/HgT ratio *vs* organic carbon content (N=617)