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A laboratory-incubated redox oscillation experiment to investigate Hg fluxes from highly contaminated coastal marine sediments (Gulf of Trieste, Northern Adriatic Sea)

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Abstract Mercury (Hg) mobility at the sediment–water interface was investigated during a laboratory incubation experiment conducted with highly contaminated sediments (13 μg g^{-1}) of the Gulf of Trieste. Undisturbed sediment was collected in front of the Isonzo River mouth, which inflows Hg-rich suspended material originating from the Idrija (NW Slovenia) mining district. Since hypoxic and anoxic conditions at the bottom are frequently observed and can influence the Hg biogeochemical behavior, a redox oscillation was simulated in the laboratory, at in situ temperature, using a dark flux chamber. Temporal variations of several parameters were monitored simultaneously: dissolved Hg (DHg) and methylmercury (MeHg), O_2, NH_4^+, NO_3^- + NO_2^-, PO_4^{3-}, H_2S, dissolved Mn^{2+}, dissolved inorganic and organic carbon (DIC and DOC). Under anoxic conditions, both Hg (665 ng m^2 day^{-1}) and MeHg (550 ng m^2 day^{-1}) fluxed from sediments into the water column, whereas re-oxygenation caused concentrations of MeHg and Hg to rapidly drop, probably due to re-adsorption onto Fe/Mn-oxyhydroxides and enhanced demethylation processes. Hence, during anoxic events, sediments of the Gulf of Trieste may be considered as an important source of DHg species for the water column. On the contrary, re-oxygenation of the bottom compartment mitigates Hg and MeHg release from the sediment, thus acting as a natural "defence" from possible interaction between the metal and the aquatic organisms.

Keywords Mercury · Incubation · Anoxia · Coastal sediments · Benthic chamber · SSE

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Introduction

In coastal marine areas, degradation of sedimentary organic matter (OM) is dependent on the oscillating redox conditions of surface sediments (Bouchet et al. 2011). Organic carbon remineralization drives the biogeochemical cycling of several elements (Canfield et al. 2005); among them, mercury (Hg) is characterized by a complex cycle (Merritt and Amirbahman 2009) which involves its transformation into methylmercury (MeHg), the most toxic and potentially bioaccumulable form of Hg to humans and wildlife (Clarkson 1998).

Under controlled laboratory conditions, it is possible to observe the effects of oxygen (O_2) depletion on the biogeochemical behavior of the benthic compartment (Belias et al. 2007) and to assess both Hg mobility and the release of MeHg from sediments to the upper water column (Covelli et al. 2008; Emili et al. 2011; Koron and Faganeli 2012).

A suitable test area for these kinds of experiments is represented by the Gulf of Trieste (Northern Adriatic Sea), one of the most Hg-contaminated coastal areas in the world, where cinnabar (HgS)-rich material from the Idrija (Slovenia) mining district have been (Covelli et al. 2001) and are still being

delivered (Horvat et al. 1999; Faganeli et al. 2003; Covelli et al. 2006, 2007) by the Isonzo River, the largest contributor of this metal into the area since the 16th century.

The Isonzo River is the main freshwater input in the Gulf, with an estimated annual flow rate ranging between 1.1–666 $m^3 s^{-1}$ (1998–2007 period; Comici and Bussani 2007) and fluvial inputs appear to control primary production in the Gulf, as the highest inputs of land-born nutrients, in particular, nitrate leaching from cropping areas of the Venezia Giulia plain (Cantoni et al. 2003) are associated with the highest river discharges (Malej et al. 1995). The Gulf is a shallow coastal basin with a maximum water depth of 25 m. Water circulation is anticlockwise and wind-driven superficial currents mostly influence the first 5 m of the water body (Stravisi 1983). Periodical hypoxic/anoxic conditions have been observed in the Gulf, as a consequence of high loadings of nutrients and OM and strong late summer water stratification (Faganeli et al. 1985, 1991; Kemp et al. 1999). This latter represents one of the key factors controlling in situ MeHg production at the sediment–water interface (SWI, Fitzgerald et al. 2007; Merritt and Amirbahman 2009).

Within this context, the aim of this study was to evaluate Hg and MeHg cycling at the SWI in the Gulf of Trieste under anoxic conditions, simulating an oxic/anoxic redox transition in a dark incubated benthic chamber, as previously reported for heavily Hg-contaminated coastal sites (Covelli et al. 2008; Emili et al. 2011). To this purpose, a volume of undisturbed marine sediment was sampled right in front of the Isonzo River mouth, where on the basis of previous research (Covelli et al. 2001; Hines et al. 2006), the highest Hg contents in the Gulf (up to about 20–30 $\mu g\ g^{-1}$) were expected.

In addition, in order to investigate the potential Hg mobility, the speciation in the sampled sediment was determined by means of a selective sequential chemical extraction (Bloom et al. 2003) and the results from the sediment incubation were compared to similar investigations previously conducted in the area (Emili et al. 2011; Koron and Faganeli 2012).

Materials and methods

Sampling

Bottom sediment and the overlying water were collected by a SCUBA diver in June 2010 at station D6 (Fig. 1), using a cylindrical Plexiglas chamber as a sampler (h = 25 cm, i.d. = 24 cm). The chamber was carefully transported to the laboratory, in order to prevent disturbance of the SWI. Here, the water overlying the sediment was drained off and the chamber was filled with bottom seawater collected at the same location.

The chamber was sealed and placed in a dark room at in situ temperature for incubation (Fig. 2). After 27 days, the system was reoxygenated by opening the chamber and leaving the water surface in contact with the atmosphere for 7 days. The water temperature throughout this period was kept constant at 23.0±1 °C, which was the water temperature at the sampling day.

During the incubation experiment, water samples were periodically collected from the chamber using a plastic syringe and the withdrawn volume of water was compensated by in situ collected bottom seawater. Prior to each sampling, the system was homogenized for 15 min by means of a stirrer driven by a stepper electric motor housed in a waterproof case. Coupling between the stirrer and the motor is provided by rare earth magnets. Rotation of the stirrer is kept to 5 rpm to prevent sediment resuspension while at the same time providing constant and effective mixing of the water column.

Analyses of the solid phase

Total mercury (THg) was determined by CV-AAS (Perkin-Elmer, AAnalyst 100-FIAS 100) employing $NaBH_4$ 3 % in NaOH 1 % for the reduction step, after a total decomposition of the sample with a mixture of HF + aqua regia (HNO_3:HCl = 1:3 v.v.) in a closed microwave system (Milestone, MLS 1200). Accuracy was tested using a Certified Reference Material (PACS-2 marine sediment, NRCC). Results obtained (3.03±0.30 mg kg^{-1} Hg; n = 3) were in good agreement with the certified value (3.04±0.20 mg kg^{-1} Hg). The detection limit of the method was 0.13 $\mu g\ g^{-1}$. The precision expressed as relative standard deviation of at least three determinations was at least <7 %.

For Hg speciation, a five-step selective sequential extraction (SSE) procedure described by Bloom et al. (2003) and subsequently adapted for sediments by Shi et al. (2005) was followed. Extractions were carried out using about 0.4 g of lyophilized and fine-ground surface sediment samples placed into 50 ml pretreated borosilicate glass centrifuge tubes. After the addition of each selective extractant (V = 20 ml), the sediment was subject to end-over-end shaking (t = 18±4 h; at room temperature). The tubes were then centrifuged (3,000 rpm; t = 15 min) and the supernatant liquid was decanted and filtered through 0.45 μm pore size membrane filters (Millipore Millex-HA). Finally, 1 ml of HNO_3 was added and the extract was placed in a clear borosilicate bottle and oxidized by adding 500 μl of BrCl. The solid residue was washed with the related extractant and the rinse was discarded. The four extractants used were: (1) Milli-Q water for "water-soluble Hg" (Hg-w); (2) 0.1 mol l^{-1} CH_3COOH + 0.01 mol l^{-1} HCl for "human stomach acid soluble Hg" (Hg-h); (3) 1 mol l^{-1} KOH for "organo-chelated Hg" (Hg-O); (4) 12 mol l^{-1} HNO_3 for "elemental/strongly complexed Hg" (Hg-e). In the last step (5), the residue was air dried and total digestion with aqua regia was performed at room temperature for the "mercuric sulfide" (Hg-s) fraction which comprises Hg sulfides and Hg immobilized by pyrite (FeS_2). Filtered extractant solutions were

Fig. 1 Location of sampling site D6, in front of the Isonzo River mouth in the Gulf of Trieste, selected for the incubated benthic chamber experiment. Location of sites of former incubation experiments compared with this study (CZ, Emili et al. 2011; E and F1, Koron and Faganeli 2012) are also indicated

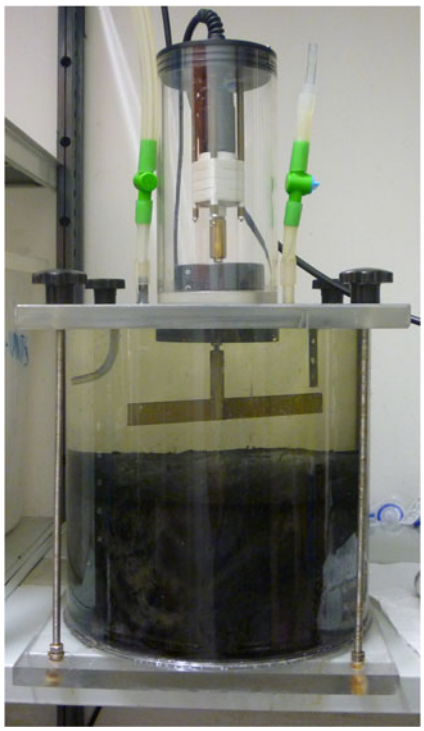

Fig. 2 Chamber used for the incubation experiment. The plexiglas cylinder is watertight and it is equipped with a magnetic stirrer which mixes water inside the chamber before each sampling. Water samples were periodically collected using a plastic syringe through a stopcock at the top. The average depth of sediments inside the chamber was 15 cm with about 10 cm of overlying water

employed as analytical blanks. Total dissolved Hg (DHg) in each extract was determined by CV-AFS (Brooks Rand, Model III) after a prereduction step with $SnCl_2$ and single gold trap amalgamation. The obtained values were corrected for blanks. The method was verified for all samples by summing the ratio of extracted Hg in each phase (Hg_x) to THg in the sample ($\sum Hg_x$/THg). The average extraction budget obtained from these ratios was about 80 %.

Analyses of the dissolved phase

Dissolved oxygen (O_2) was determined by the Winkler method (Grasshoff et al. 1983) using an automated titration system (Mettler DL21). Determination of sulfides (H_2S) was performed spectrophotometrically after trapping with Zn acetate (Grasshoff et al. 1983). Other parameters in the dissolved phase were determined on samples filtered through a Millipore Millex HA 0.45 μm filter. Dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) determinations were performed using a Shimadzu TOC 5000A analyzer. Analysis showed a variation coefficient < 2 %. The reproducibility of the method was between 1.5 % and 3 %. Dissolved manganese (Mn^{2+}) was determined by GF-AAS (Perkin-Elmer AAnalyst 100-HGA 850) using $Mg(NO_3)_2$ as a matrix modifier. Analyses showed a variation coefficient < 4 %. Nutrients (NO_2^-, NO_3^-, NH_4^+ and PO_4^{3-}) were determined

according to Grasshoff et al. (1983) using a continuous flow segmented system (Bran-Luebbe, AAQuattro). Detection limits for nutrients were, respectively: 0.5 μmol l^{-1} for NO_3^- + NO_2^- as N, 0.4 μmol l^{-1} for NH_4^+ as N, 0.01 μmol l^{-1} for PO_4^{3-} as P.

The determination of total DHg in the overlying water column was performed after an initial step of oxidation with BrCl (500 μl/100 ml sample). A prereduction using NH_2OH-HCl (30 %, 250 μl), until the yellow color disappeared, was followed by reduction with $SnCl_2$ and Au trapping. Analyses were performed by means of a Brooks Rand CV-AFS (Horvat et al. 1991).

For dissolved methylmercury (DMeHg) analyses all samples were distilled to separate MeHg from the water matrix (Horvat et al. 1993). Briefly, after an ethylating agent was added to each sample to form a volatile methyl-ethylmercury derivative, the volatile organomercury species were purged onto graphite carbon traps as a means of preconcentration and interference removal. The sample was then isothermally chromatographed, pyrolitically broken down to elemental Hg, and detected by CV-AFS (Bloom and Fitzgerald 1989). Sample results were corrected for distillation efficiency. Samples were analyzed by EPA Method 1630 (EPA 1998). The achieved detection limit was 0.0188 ng l^{-1}, while recoveries ranged from 70 % to 112 %.

Results and discussion

Mercury content and speciation in sediments

The average total Hg (THg) concentration in the surficial sediment (0–3.5 cm) was about 13 μg g^{-1}, which is lower than the levels previously reported for the same location (23.3 μg g^{-1} Covelli et al. 2001; >30 μg g^{-1} Hines et al. 2006). This result can be justified by the fact that this site is directly affected by riverine discharge which can vary with time, thus changing the accumulation rate and the associated Hg content in sediments. In the Gulf of Trieste, the highest Hg concentrations were found within the Isonzo River mouth and in near-shore sediments of the Italian sector, particularly alongside both sides of the river delta (Covelli et al. 2001). Moreover, the highest Hg concentrations were found to be associated with near-shore coarse sediments, whereas Hg concentrations sharply decrease with distance from the river mouth to offshore areas, where fine particles (mostly <16 μm) settle down.

Results from the SSE showed that by large, the predominant fraction in the D6 sediment was Hg-s, representing 76 % of the extracted Hg. Hg-s comprises mostly the poorly soluble cinnabar (α-$HgS_{(s)}$; $K_{sp} = 10^{-36.8}$) and metacinnabar (β-$HgS_{(s)}$; $K_{sp} = 10^{-36.4}$), but also HgSe and HgAu complexed forms (Bloom et al. 2003).

Recent studies suggest that in addition to methylating bacteria activity and the pore water speciation of DHg, the solid-phase speciation of Hg in sediments is a key factor controlling methylation rates (Jonsson et al. 2012). The authors reported a wide range of Hg methylation rate constants determined in estuarine sediments, depending on the chemical form of the added isotopic tracer. They concluded that the incorporation of Hg(II) in less soluble phases, such as cinnabar and metacinnabar, decreases the availability of Hg(II) for methylation processes and, consequently, the formation of MeHg in estuarine sediments. However, the same authors recognized that in reducing sediments, both cinnabar and metacinnabar could be dissolved to a significant rate, thus providing Hg(II) to methylating bacteria and enhancing MeHg formation.

Cinnabar from the Idrija Hg mine, inflowing from the Isonzo River and deposited at the Isonzo River mouth, is responsible for the predominance of the Hg-s fraction in the D6 surface sediment. Separation of Hg phases performed by means of a solid-phase Hg thermodesorption technique to quantify cinnabar (HgS) and non-cinnabar Hg compounds in sediments of the Isonzo River and the Gulf showed the predominance of microcrystalline red cinnabar, prevalently bound to the coarse fraction, which was higher than 90 % at D6 (Biester et al. 2000). Although sampling and analyses were conducted on different samples and in different times, the results seem to be quite concordant with this study, taking into account minor discrepancies due to the different analytical approaches.

The second most abundant fraction in the D6 site was Hg-e, which accounts for 23 % of the extracted Hg. This fraction can also be considered as poorly mobile. However, it is often considered as a good estimate of the free Hg(0) present in the sediment matrix, although some interferences with Hg(I), amorphous organo-sulfur and crystalline Fe/Mn oxide phases could lead to an overestimation (Bloom et al. 2003).

The remaining 1 % is constituted by the sum of Hg-w (0.08 %), Hg-h (0.03 %) and Hg-o (0.77 %). Regarding Hg mobility and bioavailability, the most concerning fractions are the first two (Hg-w, Hg-h) which comprise highly soluble and easily exchangeable compounds (i.e. $HgCl_2$, $HgSO_4$ and HgO). The sum of these fractions in D6 accounts for just 0.11 % of the extracted Hg, which can be considered negligible. The organo-chelated Hg fraction (Hg-o), which has a moderate mobility, is also extremely low in terms of percentage, and it is not expected to give a significant contribution to Hg mobility in this sediment. This fraction includes Hg complexed with humic, fulvic and amino acids, living and dead biota and the relative small fraction of methylated species. Bloom et al. (2003) reported a positive correlation between the Hg-o fraction in sediments and MeHg content, but emphasized how each sediment type has a different methylation potential. If redox conditions change, the slow dissolution of

Hg-binding solid phases would occur, followed by the consequent release of Hg into porewaters, where it can reprecipitate in other phases (such as complexes with OM and Fe/Mn oxyhydroxides) and, thus, contribute to the Hg-o and Hg-e fractions.

Variability of chemical parameters in the incubated chamber

An O_2 concentration < 32 μmol l^{-1} (1 mg l^{-1}) was considered the limit for the hypoxic/anoxic transition. In this experiment, after 7 days of incubation, O_2 concentration in the benthic chamber was no more detectable (Fig. 3). Oxygen was resupplied to the system by opening the benthic chamber after 27 days of incubation. As a consequence, O_2 concentration quickly rose to 142 μmol l^{-1}, reaching a maximum of 291 μmol l^{-1}, which was only 25 % lower than the concentration value observed at the beginning of the experiment.

Oxic conditions at t_0 were associated to a positive Eh value (125 mV). From t_3, Eh appeared negative (-14 mV), and it rapidly fell down to -286 mV (t_6) just before the disappearance of oxygen. In anoxic conditions, Eh remained constant (about -360 mV). From t_{16} on, when the system started to recover oxic conditions, Eh values turned back to positive values in a couple of days.

The pH value decreased from 8.27 at t_0 to 7.75, when anoxic conditions were established. During anoxia (t_7–t_{14}), pH decreased only slightly, falling to 7.66 before reoxygenation. When the system shifted back to oxic conditions, pH sharply increased up to 8.39, slightly higher than at the beginning of the experiment.

During the oxic/anoxic transition, O_2 is consumed near the SWI due to early diagenetic processes involving OM. The remineralization follows the well-established sequence of reactions controlled by the preferential use of the electron acceptor that yields the highest amount of free energy for the bacterially mediated oxidation of OM (Froelich et al. 1979). These processes were clearly evident by following nitrogen (N) species behavior during incubation.

At the beginning of the experiment, nitrate (NO_3^-) was, as expected, the predominant N inorganic form present in the water column (≈66 %). As incubation proceeded, NO_2^- and NO_3^- were rapidly consumed, in parallel with O_2 depletion, as alternative electron acceptors, falling from a starting concentration of 7.25 μmol l^{-1} to <0.5 μmol l^{-1} after 5 days. Following reoxygenation, these redox-sensitive species were effectively regenerated, quickly rising in concentration to a maximum of 15.6 μmol l^{-1} at the end of the experiment.

Ammonium (NH_4^+) was promptly released into the water column, rising from the initial concentration of 3.55 μmol l^{-1} to around 300 μmol l^{-1} during anoxia, and showed a slow decrease after O_2 resupply. The simultaneous decrease of NO_3^- concentration and increase of NH_4^+ is consistent with the bacterial or enzyme reduction of N species in the system. Throughout the experiment, NH_4^+ was by far the dominant species in the system, being its concentration at the end of incubation one order of magnitude greater than the (NO_3^- + NO_2^-) concentration. However, nitrites and nitrates conversion alone does not justify the high NH_4^+ concentration measured in the water column, thus suggesting that the rapid degradation of N-rich soluble organic compounds in the sediment was the primary process responsible for the relevant release of NH_4^+ into the water column (Emili et al. 2011).

Phosphate (PO_4^{3-}) recycling from the sediment to the water column was also related to the redox transition. Phosphate concentration was extremely low, increasing from 0.01 μmol l^{-1} at t_0 to a maximum of about 30 μmol l^{-1} before reoxygenation. Following oxygen depletion, the progressive shift to anoxic conditions leads to reduction of Fe(III) to Fe(II), thus determining the transformation of insoluble $FePO_4$ into more soluble $Fe_3(PO_4)_2$, releasing in turn PO_4^{3-} into the overlying water. When oxygen was resupplied, PO_4^{3-} scavenging from the water column was quick, and at the end of the experiment, PO_4^{3-} concentration was 3.82 μmol l^{-1}, higher than t_0 but one order of magnitude lower than the average concentration during anoxia. Removal from the water column probably proceeded with the formation of Fe(III) oxyhydroxides, as previously suggested (Ogrinc and Faganeli 2006). The significant positive correlation (r =0.820, p <0.0001, n =21) found between NH_4^+ and PO_4^{3-} suggests a common origin of these species from sedimentary OM degradation.

Reduction of sulfate produced dissolved sulfide (H_2S), which rose from 0.36 μmol l^{-1} at t_0 to a maximum of 75 μmol l^{-1} after 20 days of incubation. After that, H_2S concentration decreased, reaching undetectable levels after reoxygenation. As expected, sulfide was significantly correlated with PO_4^{3-} (r =0.850, p <0.0001, n =15) and NH_4^+ (r = 0.724, p <0.003, n =15), given that sulfate reduction is one of the main processes of microbial degradation of OM in sediments. Sulfide was also significantly correlated with both pH (r =-0.756, p <0.002, n =15) and Eh (r =-0.898, p <0.0001, n =15). Sulfide scavenging from the water column before reoxygenation suggests the precipitation of insoluble sulfides (Benoit et al. 1999; Mason et al. 2006) and the consequent removal of S-binding species from solution, such as Hg, due to its high degree of pyritization (Huerta-Diaz and Morse 1992).

DIC steadily increased during the experiment (up to 5.78 mmol l^{-1}), as a consequence of OM respiration by the benthic compartment, showing a slight decrease following reoxygenation. Throughout the experiment, DOC exhibited low concentration (0.6 mmol l^{-1} on average) and did not follow a clear trend. DOC represents a fraction of dissolved organic matter (DOM) and inorganic Hg can form stable complexes in aquatic media with DOM (Benoit et al. 2001). The interaction between DOC and Hg prevails in systems where its concentration is higher than 5 mg l^{-1} (0.42 mmol l^{-1};

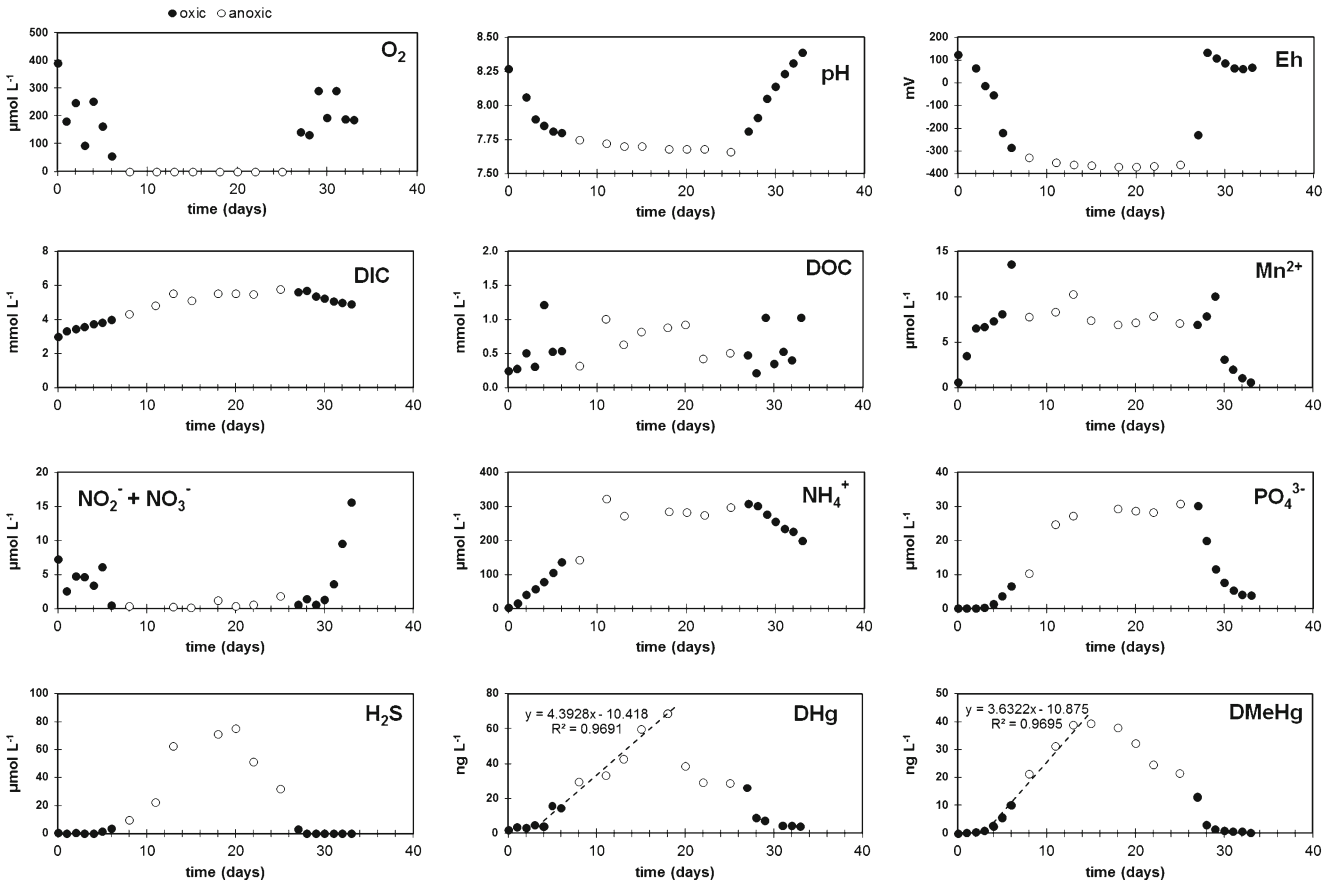

Fig. 3 Evolution of dissolved O_2 concentration, pH, Eh, DIC and DOC, nutrients (NO_2^-+NO_3^-, NH_4^+ and PO_4^{3-}), Mn^{2+}, H_2S, DHg and DMeHg in the benthic chamber during the incubation experiment at D6 (oxic, anoxic and reoxigenation phases)

Hissler et al. 2006). While the average concentration of DOC in the benthic chamber was above this threshold, the lack of correlation between DOC and Hg suggests that OM was not the primary control on Hg availability for methylation in the water column, as expected in sulfidic environments (Benoit et al. 2001).

Manganese was already released from the sediment at the beginning of the experiment, when O_2 depletion was slowly ongoing. The quick drop in concentration following reoxygenation suggests that Mn oxides were formed, possibly removing other solutes from solution, MeHg included, by coprecipitation and/or adsorption.

Behavior of mercury species in the benthic chamber

Total DHg was present in the water column at t_0 (2.1 ng l^{-1}), and its concentration in the benthic chamber steadily increased with the progressive O_2 depletion (Fig. 3). After 5 days of incubation, DHg concentration rose to 15.6 ng l^{-1}, further increasing during the anoxic phase, up to 68.6 ng l^{-1} at t_{11}. After peaking, the concentration of DHg rapidly decreased to about 30 ng l^{-1} before reoxygenation. Dissolved MeHg (DMeHg), which was almost undetectable (0.03 ng l^{-1}) at the beginning of the experiment, paralleled DHg, with a smoother behavior in the intermediate phase of anoxia, when its concentration hit a plateau of about 39 ng l^{-1}. After 20 days of incubation, still under anoxic conditions, DMeHg concentration decreased, falling to about 22 ng l^{-1} before reoxygenation

A positive correlation was found between DHg, H_2S ($r=0.898$, $p<0.0001$, $n=15$) and DMeHg ($r=0.935$, $p<0.00001$, $n=21$). DMeHg was also positively correlated with H_2S ($r=0.786$, $p<0.001$, $n=14$), suggesting that Hg methylation by sulfate-reducing bacteria (Compeau and Bartha 1985; Gilmour and Henry 1991) was active in the incubated sediment. Hypoxic/anoxic conditions favored methylation processes, as long as H_2S concentration in the water column was below 80 µmol l^{-1}. After reaching the optimum value in sulfate reduction and the corresponding methylation processes, the formation of insoluble sulfides buffered H_2S and Hg, removing them from solution. The corresponding disappearance of DMeHg suggests coprecipitation with sulfides and possible demethylation by reductive pathway (Hines et al. 2006).

When O_2 was resupplied to the system, DHg quickly dropped below 10 ng l^{-1}, finally returning to concentration

values (3.8 ng l^{-1}) similar to the starting concentration at t_0. DMeHg quickly disappeared from the water column, decreasing to 0.3 ng l^{-1} at the end of the experiment.

The decrease of DHg and DMeHg in reoxigenated conditions could be due to the coprecipitation with newly formed authigenic Fe and Mn oxides, which are scavengers of soluble metal compounds in oxic surface sediments (Gagnon et al. 1997; Muresan et al. 2007). The decreasing percentage of DMeHg to DHg (from 76 % at t_{14} to 7.5 % at t_f), following reoxygenation, also suggests an important role played by demethylation in oxic conditions (Hines et al. 2006).

Comparison with previous incubation experiments in the Gulf of Trieste

These results were compared to previous incubation and reoxygenation experiments (Emili et al. 2011; Koron and Faganeli 2012) conducted in the Gulf of Trieste along the southward Isonzo River mouth–Piran transect (Fig. 1), which is characterized by a decrease of Hg concentration in sediments, related to the progressive distance from the source of contamination (Covelli et al. 2001). The CZ station (Emili et al. 2011) is roughly located 12 km away from the river mouth, with an average water depth of 21 m. Stations E and F1 (Koron and Faganeli 2012) are located in the southern part of the Gulf, in Slovenian waters: F1 is approximately located 2.5 km off the coastal city of Piran at an average water depth of 22 m; E is located in front of the Marine Biological Station of Piran, at a water depth of 8 m.

Mercury concentration in CZ was determined by Hines et al. (2000) to range between 0.8 and 0.9 μg g^{-1}, whereas it accounted for 0.6 μg g^{-1} at station E and 0.2 μg g^{-1} at station F1 (Covelli et al. 2001), far less than the average 13 μg g^{-1} determined in this study for D6 (0–3.5 cm). Methylmercury concentration varied between 0.21 and 0.48 ng g^{-1} at sites E and F1 (Koron and Faganeli 2012), similar to what observed in CZ (<0.5 ng g^{-1}; Hines et al. 2006) but lower than D6 (about 2 ng g^{-1}; Hines et al. 2006). Overall, MeHg accounted for a minimal percentage of total Hg in sediments (<0.1 %).

O_2 consumption in the benthic chamber followed different dynamics in the selected sites. At station E (May 2009; 20 °C), O_2 (t_0 = 469 μmol l^{-1}) was consumed in only 4 days, whereas at CZ (November 2007; 20 °C; t_0 = 393 μmol l^{-1} O_2), it was depleted in 7 days, similar to D6 (t_0 = 469 μmol l^{-1} O_2). Conversely, at F1 (May 2010; 20 °C), O_2 consumption ($t_0 \approx$ 500 μmol l^{-1}) took about 15 days. In all cases, O_2 was resupplied to the system after 16 days of incubation, by opening the chamber lid and letting O_2 diffuse into water. Since differences in the initial O_2 concentration among the three sites do not seem to be significant, it is suggested that mineralization of OM progressed more slowly at F1.

Patterns of H_2S, DHg and DMeHg (Fig. 4) proceeded in parallel at CZ and F1, peaking during the anoxic phase of the experiment and quickly disappearing following reoxygenation, similarly to D6. At station E, on the other hand, the coupling of DHg and DMeHg was not well correlated with H_2S.

At all three sites, DHg increased during anoxia, peaking at 95 ng l^{-1} at F1, 44 ng l^{-1} at E, and 11 ng l^{-1} at CZ, compared to 69 ng l^{-1} at D6. DMeHg peaked at about 100 ng l^{-1} at F1, 21 ng l^{-1} at E, and 12 ng l^{-1} at CZ, compared to 39 ng l^{-1} at D6. The percentage of DHg as DMeHg in the benthic chamber reached a maximum of 100 % during anoxia in CZ (Emili et al. 2011) and F1 (Koron and Faganeli 2012), 94 % in D6, and 50 % in E (Koron and Faganeli 2012). Thus, DMeHg was the dominant form of Hg in the water column during the anoxic phase of the incubation experiments. This evidence poses a major concern regarding the possible transfer of Hg to the aquatic trophic chain. However, such observations were made under laboratory conditions, simulating a persistent and extreme anoxic event and not the normal environmental conditions.

Scavenging of Hg species was evident at D6 and E during anoxia, while it only occurred after reoxygenation at both CZ and F1. At D6, such scavenging ran in parallel with H_2S removal, while at station E, such relation was less evident.

For stations E and F1, Koron and Faganeli (2012) identified the dissolution of Fe and Mn oxyhydroxides due to sediment anoxia as a major control in MeHg release from sediments. At the same time, co-precipitation of Hg species with Fe and Mn and intensive demethylation were suggested as factors explaining the rapid decrease of DHg and MeHg following reoxygenation.

Benthic fluxes of mercury species and factors influencing mercury cycling in the incubation experiments

Benthic fluxes for D6 were calculated from linear regression of the variation of solutes concentration with incubation time, according to Covelli et al. (2008) and Emili et al. (2011), and compared to CZ (Emili et al. 2011), E and F1 (Koron and Faganeli 2012).

As shown in Table 1, DHg fluxes under anoxic conditions were quite similar among D6, E and F1 (about 665 ng m^{-2} day^{-1}), despite a much higher THg content in D6. At CZ, DHg flux was one order of magnitude lower than at the other sites (71 ng m^{-2} day^{-1}). DMeHg fluxes were highest in E (577 ng m^{-2} day^{-1}) and D6 (550 ng m^{-2} day^{-1}), followed by F1 (361 ng m^{-2} day^{-1}) and CZ (238 ng m^{-2} day^{-1}).

It appears that THg content in sediments alone cannot predict the flux of DHg and DMeHg from the solid phase to the upper water column during an oxic–anoxic transition. DHg and DMeHg fluxes are for the most part comparable

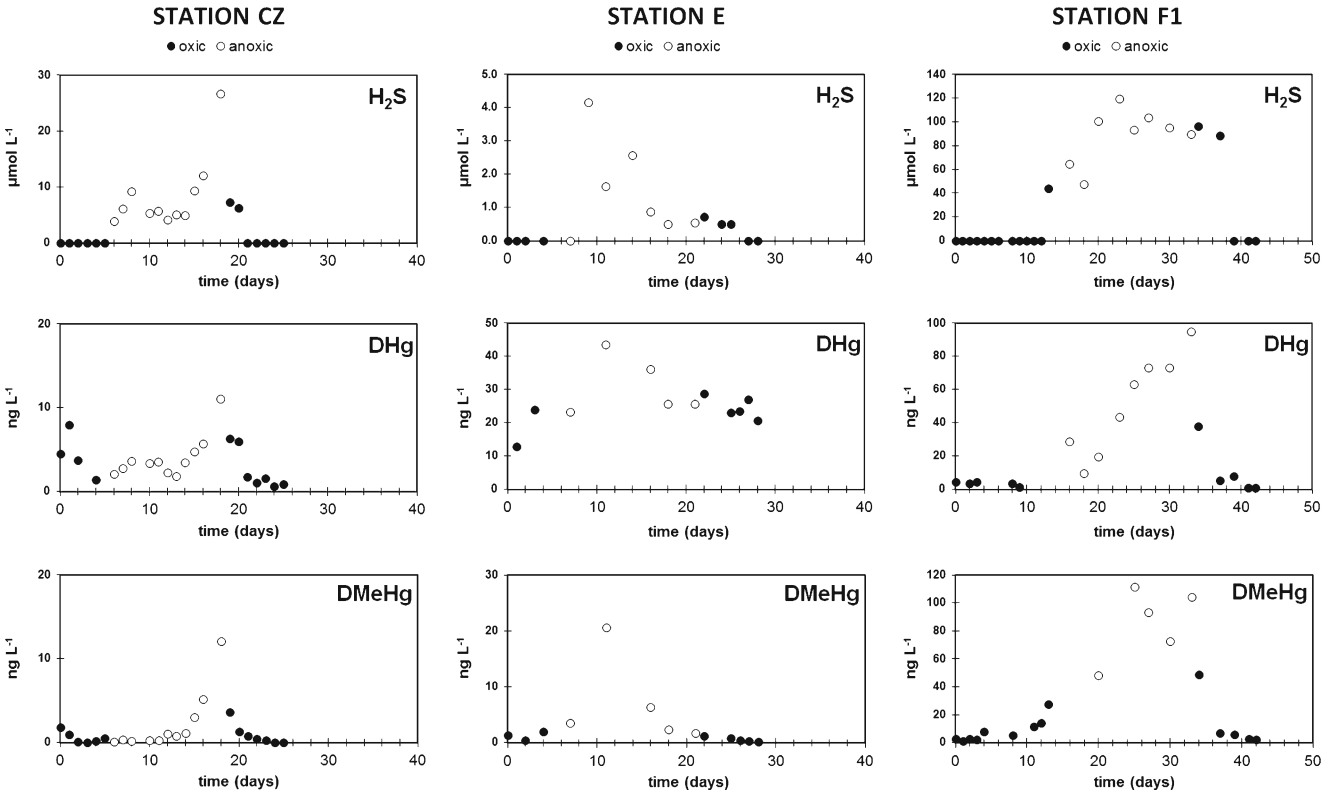

Fig. 4 Evolution of H_2S, DHg and DMeHg in the benthic chamber during previous incubation experiments performed at CZ, E and F1 (oxic, anoxic and reoxigenation phases) stations in the Gulf of Trieste. Data for CZ are from Emili et al. (2011), while data for E and F1 are from Koron and Faganeli (2012)

throughout the Gulf transect, despite a wide range of Hg concentration. (0.2–13 μg g^{-1}) in sediments.

Speciation analysis by SSE showed that most of sedimentary Hg in D6 is present in highly insoluble forms. Such information is lacking for the other sites but, based on their location, further away from the Isonzo River mouth where cinnabar-rich coarser particulate matter tends to settle (Biester et al. 2000), it is probable that more labile forms should be present (Covelli et al. 2001).

As previously noted (Emili et al. 2011), sediment incubation and the persistence of anoxic conditions favor Hg and MeHg release from sediments to the water column. In spite of the high percentage of the cinnabar component, sediments of D6 become a source of MeHg to the water column if anoxic conditions occur.

Besides, in accordance with the SSE results, THg in the D6 sediments exhibited a strong binding to the solid phase, limiting its dissolution into porewaters and, such, its availability.

Table 1 Total mercury content in sediments and benthic fluxes of DHg and DMeHg (ng m^{-2} day^{-1}) calculated from incubation experiments performed at sites D6, CZ, E and F1 in the Gulf of Trieste

Site	Period	*T* (°C)	Depth (m)	THg content (μg g^{-1})	Anoxic fluxes	
					DHg	DMeHg
Gulf of Trieste (D6)	June 2010	24	3.5	12.6	665	550
Gulf of Trieste (CZ)[a]	November 2007	20	21	0.77–0.89[c]	71	238
Gulf of Trieste (E)[b]	May 2009	20	8	0.6[d]	685	577
Gulf of Trieste (F1)[b]	May 2010	20	22	0.2[d]	646	361

[a] Emili et al. (2011)

[b] Koron and Faganeli (2012)

[c] Hines et al. (2000)

[d] Covelli et al. (2001)

The partitioning of Hg between the solid and the dissolved phase can be described in terms of the log K_D value (Bloom et al. 1999), where K_D is expressed in l kg^{-1}:

$$K_D = [Hg]_s / [Hg]_{pw}$$

$[Hg]_s$ is the solid-phase concentration of Hg (in ng kg^{-1}), whereas $[Hg]_{pw}$ is the pore–water concentration of Hg (in ng l^{-1}).

Low K_D values are associated with an enhanced release into porewaters from the solid phase, while higher values are indicative of a stronger binding of the species to the sedimentary matrix. The log K_D for THg in D6 (0–3.5 cm) accounted for 6.12 at t_0 and 6.27 at t_f. Such values are higher than those reported by Covelli et al. (1999) for the central part of the Gulf of Trieste (station AA1) and by Hines et al. (2006) for the lower Isonzo River.

Conclusions

Hypoxic and anoxic conditions play a significant role in the remobilization of Hg from sediments at the Isonzo River mouth in the Gulf of Trieste. The increasing sulfide concentration in the benthic chamber is positively correlated with the parallel increase in MeHg levels, in accordance with methylation being related to sulfate reduction in anoxic sediments.

However, total Hg concentration in sediments is not the primary factor determining Hg mobility and transformation, such as methylation. In sediments, mercury speciation seems to play a key role, especially where mining activity was the main source of Hg into the environment.

The higher concentration of Hg in sediments of the Isonzo River mouth, compared to other sites in the Gulf where Hg content was up to two orders of magnitude lower, did not result in a higher methylmercury flux, in spite of significant anoxic conditions established in the incubated chamber.

Although extreme anoxic events are unlikely at the Isonzo River mouth, due to shallowness and water circulation, if bottom sediments were dredged and discharged in environments more inclined to such extreme oxygen depletion, we should not expect significantly higher MeHg release into the water column.

Besides, the observed effects of sediment reoxygenation on the solutes concentration suggest that reversion to oxic conditions removes Hg species from the water column, most probably through precipitation and coprecipitation with Fe and Mn oxyhydroxides. These findings are of paramount importance to understand Hg cycling at the SWI during hypoxic/anoxic events in this highly contaminated environment and to foresee the fate of Hg species following the restoration of the normal oxygenated conditions of bottom waters. However, field observations generally lead to a more correct quantification of the production and mobility of mercury species in natural settings. This kind of research should therefore be strongly encouraged and pursued in conjunction with laboratory experiments.

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