Seasonal dynamics of microbial mercury transformations in sediments and water column of Gulf of Trieste (northern Adriatic Sea)

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Abstract. A series of relatively simple incubation experiments to assess and evaluate microbial mercury (Hg) transformations in water column and sediments were performed monthly or bimonthly in a period from March 2011 to December 2011. Sampling campaigns were performed at station F in the southeastern part of Gulf of Trieste, northern Adriatic Sea. Gulf of Trieste has been for the last 500 year continuously impacted with inflow of Hg, originating from the Idrija Hg mine. The microbial mercury transformations were assessed using a short-lived ($t_{1/2}=64.12$ h) radioisotope ^{197}Hg . Calculated activities of microbial Hg reduction and methylation were correlated with other environmental factors, such as temperature, nutrient availability, oxygenation, organic matter, substrate (Hg in bioavailable chemical form) availability, structure of microbial community and presence of *mer* operon. Preliminary results show that in water column a substantial Hg reduction occurs, whereas methylation was not observed. On the other hand, long-term incubation experiments with sediments show that methylation process is active however preliminary results also suggest a significant MeHg degradation.

Key words: mercury, microbial transformation, water column, sediments, radiotracer

Introduction

Mercury (Hg) and its species have been recognized as one of the most potent environmental pollutants. Hg, especially monomethylmercury (MeHg) has a tendency to accumulate in aquatic food webs and can through fish consumption pose a risk also to human population.

Biogeochemistry of Hg is very complex and has been thoroughly studied for the last three decades. During this time main actors, biotic as well as abiotic, that drive transformations of Hg have been mainly recognized (Compeau and Bartha, 1985; Kerin et al., 2006), however it has yet to be accurately assessed how and to what extent do microbial transformations in water column

contribute to global Hg budget.

Gulf of Trieste (northern Adriatic Sea) is one of shallow marine environments that have been greatly subjected to continuous input of Hg. For the last 500 years it is being carried from the second largest Hg mine in the world (Idrija Hg mine) to the Gulf by the river Soča /Isonzo.

At a research station F in the Southeastern part of the Gulf in a period from March 2011 to November 2011 monthly and bimonthly sampling campaigns were performed. Sediments and water samples were used in rather long-term incubation experiments using radioisotope ^{197}Hg to assess microbial methylation and reduction activities. It is well known that different factors

influence these processes, so the importance of most of them (temperature, nutrient availability, oxygenation, organic matter, Hg bioavailability, structure of microbial community and presence of *mer* operon) was also investigated in this study.

Materials and Methods

The study site was located 2.3 km off Cape Madona (Piran, Slovenia) in the southeastern part of Gulf of Trieste where also Slovenian hydrographic buoy Vida is located. Water column depth in this part of the Gulf does not exceed 22 m.

For each experiment the water samples were collected from three different depths using a rosette of Niskin bottles. Two depths were constant, 1 m below surface (1 m) and 1 m above sediment (21 m), whereas intermediate depth was chosen after an examination of CTD profile. Based on the hypothesis of this work, the depth of choice displayed the highest values of chlorophyll *a* (Chl *a*) or when this criterion was not met (there was no maximum), the samples were collected in the middle of thermocline. Sediment cores were collected at the same site using gravity corer.

In the laboratory, cores used for conventional Hg speciation analyses were extruded and cut into 1 cm thick slices in a N₂-filled glove bag and afterward centrifuged to extract pore waters. These were then filtered, acidified and frozen until analyses. Sediments devoid of pore were also frozen.

Sediments for incubation experiments were sectioned in only two slices (0-2 cm and 2-4 cm) and transferred into acid pre-cleaned and autoclaved 100 ml glass bottles (Schott Duran).

To accurately follow the dynamics of microbial transformations in water and sediment samples, radiotracer ¹⁹⁷Hg was used. Its applicability has already been described (Ribeiro Guevara et al., 2007; Žižek et al., 2008) and highlighted for these kind of experiments (Koron et al., 2012).

This short-lived radioisotope ($t_{1/2}=64.12$ h) was produced from enriched (52 %) ¹⁹⁶Hg stable isotope in TRIGA Mark II (250 kW) research nuclear reactor of the 'Jozef Stefan' Institute in Ljubljana, Slovenia (Snoj et al., 2011).

Water samples for tracer experiments were transferred to acid pre-cleaned glass bottles and amended with radiotracer to a final concentration of 8 ng L⁻¹. ¹⁹⁷Hg⁰ produced in water column was lead to traps containing KMnO₄. The amount of ¹⁹⁷Hg⁰ collected was determined on a daily basis (app. every 24 h) using a well type HPGe (High Purity Germanium) detector (Canberra Industries Inc., USA). Total ¹⁹⁷Hg values were also determined on a daily basis.

Since the methylation activity in water column is expected to be low, the determination of Me¹⁹⁷Hg was conducted every other day. The extraction of Me¹⁹⁷Hg from samples was done by an organic solvent extraction (Liang et al., 1996) with an additional step using

DOWEX ion exchange resin to remove any residual ¹⁹⁷Hg(II). All values obtained were corrected for nuclear decay (Ribeiro Guevara et al., 2007) and finally the activities were calculated.

Sediment samples were only measured for Me¹⁹⁷Hg production. Extraction was done by solvent extraction (Liang et al., 1996) also using additional step with DOWEX.

To determine not only the activities of microbial transformations but also to identify the key environmental factors that influence the ongoing transformations, samples for conventional Hg analyses and all other ancillary analyses, i.e. for nutrient concentrations in water column, C_{org}/N_{tot} in sediments and samples for determination of microbial community and presence of *mer* operon were also collected and analysed.

Results and Discussion

Sediments

A series of analyses were performed in the sediments sampled, including C_{org} and N_{tot} analyses (data not shown), THg and MeHg and THg and MeHg in pore waters (data not shown). Figure 1 and Figure 2 show the concentrations of THg and MeHg, respectively, in sediments of the Gulf during the period of study. It can be seen that THg values range from 150 to 220 ng g⁻¹_{dw}, with upper layers exhibiting the lowest values in summer and autumn months.

Fig. 1. THg values in sediments of Gulf of Trieste at station F in period from June to December, 2011

MeHg values (Figure 2) range from 0.2 to 0.7 ng g⁻¹_{dw} with the lowest values coinciding with THg values. Values observed might be a consequence of a significant

benthic outflow from the sediment into the overlying water due to enhanced solubility of Hg species in these circumstances (Hines et al., 2012).

Fig. 2. MeHg concentrations in sediments of Gulf of Trieste at station F in a period from June to December, 2011

Fig. 3. Methylation activities in sediment in summer months (note the scale)

Activities reported here are first rate constants ($\% \text{ d}^{-1}$) and represent a percentage of radioisotope ^{197}Hg transformed to observed species during a period of 24 hours. Measured methylation activities show a significant 'flash' methylation, assessed 30 min after spiking of sediments. Since incubation experiments altogether lasted from 3 to 9 days, they are considered as long-term incubation experiments, showing a net Hg methylation. Measured Me^{197}Hg concentrations are thus a result of two

simultaneously occurring opposing processes, methylation and demethylation. On the other hand 'flash' methylation is a measure for gross methylation.

Our results are consistent with results published by Hines et al. (2000, 2012) who determined net methylation rates of $0.5 - 1 \text{ \% d}^{-1}$ for the Gulf. Demethylation activities in sediments of the Gulf and nearby Grado and Marano Lagoons exceed methylation activities up to 6 times, which explains why the calculated methylation activities drop during the experiment.

Water column

The aim of this part of the study was to assess the activities of microbial transformations in water column. Hg methylation was not observed, whereas reduction activities proved to be quite high. Here only the results gained from samples taken at intermediate depth are shown, since this depth was of primary interest.

Fig. 4. Reduction activity in water column at intermediate depth

Since the experiments were conducted in glass bottles, the photoreduction of Hg can be excluded therefore the observed reduction is a result of activity of heterotrophic bacteria and phytoplankton.

The results show increasing reduction activity in intermediate depth from beginning to late summer. In the second half of September activity drops and remains relatively constant also in October and November, with maximum at day 3 in October experiment. It must be emphasized that this intermediate depth was not fixed, but varied from 6 m to 18 m, depending on current environmental factors. All the samples except two in September and one in October were chosen based on the highest Chl *a* contents in water.

The other important difference between these samples is the temperature (the incubation temperature was the same as temperature of the sea at the time of

sampling), which has important influence on microbial metabolism.

During the period from May to late September the biomass of heterotrophic bacteria, which actually play the vital role in Hg transformations in water column (Barkay et al., 2003) is also increasing, and remain high even in October and November and also later, when an autumn phytoplankton bloom occurs (data not shown). Currently any other definite conclusions are impossible since the samples for determination of microbial community and presence of *mer* operon are yet to be analyzed.

Conclusion

Methylation activities in sediments have proven to be quite high, however these rather long-term incubation experiments indirectly point to even more active demethylation process, occurring simultaneously.

Methylation in water column was not detected, however the reduction activity seems to be quite high and comparable to activities found by Monperrus et al. (2007). Any definite conclusions are currently avoided, but preliminary data show very actively reducing microbial population.

It was again shown that radioisotope ^{197}Hg is a valuable tool for assessing the rates of Hg transformations in different environmental samples. Nevertheless, the usage of this isotope is more convenient for short-term experiment since the need for constant correction for nuclear decay is this way omitted.

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