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Lake snow as a mercury methylation micro-environment in the oxic water column of a deep peri-alpine lake

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HIGHLIGHTS

• Lake snow is a likely micro-niche for Hg methylation in oxic water columns.
• Highly organic aggregates scavenge Hg²⁺ and release produced MeHg back into the water column.
• THg and MeHg concentrations in lake snow increase with depth in the water column.

ABSTRACT

Hg methylation in the oxic water column of marine environments has been linked to the presence of suspended and settling particles known as marine snow, which acts as a micro-niche for MeHg production. While marine snow has been thoroughly studied, its freshwater counterpart, lake snow, received less attention, even though few works have highlighted its ability to be a micro environment for Hg methylation in freshwater systems. Here we present new data of MeHg and THg concentrations in the lake snow of a deep peri-alpine lake (Lake Geneva, Switzerland-France). Particles were sampled from the lake and from its main tributaries using continuous flow filtration and continuous flow centrifugation, respectively. MeHg concentrations ranged from 0.48 ± 0.09 ng/g to 9.61 ± 0.67 ng/g in the lake particles, and from 0.30 ± 0.08 ng/g to 2.41 ± 0.14 ng/g in tributary particles. Our results suggest that lake snow is a likely micro-niche for Hg methylation, like marine snow, and that this methylation takes place inside the particles with a subsequent diffusion to the water column. Moreover, we propose a conceptual model to explain the MeHg behavior related to the lake snow along Lake Geneva water column and a mass balance model to estimate the time required to reach the steady state of MeHg in the water column. Our calculation indicates that the steady-state is reached after 37 days. This result is compatible with particles residence times from the literature on Lake Geneva. These particles forming the lake snow are probably a major entry point into the lake’s food chain.

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1. Introduction

Mercury (Hg) is a trace metal and a global pollutant of great concern for human and wildlife due to its ability to biomagnify into the food chain under its methylated form CH$_3$Hg$^+$ (methylmercury, MeHg). MeHg is produced in aquatic environments by several microorganisms such as sulfate-reducing bacteria (SRB) (Compeau and Bartha, 1985; King et al., 2000), strains of methanogens (Parks et al., 2013; Pard et al., 2015), iron-reducing bacteria (Fleming et al., 2006; Si et al., 2015; Correia and Guimarães, 2017; Bravo et al., 2018), Firmicutes (Gilmour et al., 2013) and strains of fermenters (Peterson et al., 2020). MeHg biotic production takes place in various settings: bottom sediments, settling particles and hypoxic water layers of marine and lacustrine environments, wetlands, flooded soils (Ullrich et al., 2001; Gallorini and Loizeau, 2021), and reference therein.

In marine and lacustrine systems, bottom sediments are the most studied sites of MeHg production, but a growing number of studies have uncovered the importance of water columns in the methylation of Hg (Topping and Davies, 1981; Mason and Fitzgerald, 1990; Eckley and Hintelmann, 2006, Monperrus et al., 2007a; 2007b, Cossa et al., 2009; Lehnherr et al., 2011; Díez et al., 2016; Soerensen et al., 2018). There is increasing evidence that in oxic water columns of marine and lacustrine environments, permanent redox gradients may exist around and inside micro and macro-aggregates and thus favorable conditions for MeHg production could be present (Glud et al., 2015; Allredge and Cohen, 1987). Marine and estuarine aggregates, known as “marine snow”, are the most studied; however, the scientific community is increasingly focusing its attention toward the lacustrine environments and the “lake snow” (Ortiz et al., 2015; Paranjape and Hall, 2017). Studying lake systems is of importance due to the non-negligible impact that they have on the diet of human communities. The presence of a source of MeHg in the oxic water column of lacustrine systems could represent an important entry point of MeHg in the food chain, increasing the uptake of phyto- and zooplankton. MeHg produced in the water column could be also more readily accessible than the MeHg coming from the bottom sediments, especially in deep lake where the distance between sediments and surface waters is significant. In such setting, MeHg produced in the water column could outcompete the MeHg input from the bottom sediments (Gallorini and Loizeau, 2021).

The present study aims to determine the origin of MeHg in suspended particles along the water column of a deep peri-alpine lake (Lake Geneva, Switzerland-France), and to better understand the role of lake snow in the cycling and transformations of Hg of a deep lake. To extend the work of Díez et al. (2016), we developed a sampling setup to collect suspended particles from the lake with minimal adverse effects (e.g. induction of artificial redox gradient), and to analyze them as closely as possible to their natural conditions. Moreover, we propose a new conceptual model of the interactions between suspended particles and MeHg and THg in the water column of Lake Geneva. A simple mass balance model showed that the potential MeHg production in suspended particles, assuming they have an internal anoxic environment, could explain the MeHg concentrations measured in the water column of this lake.

2. Study area

Lake Geneva (Fig. 1) is a warm monomictic lake of glacial origin situated on the border between Switzerland and France. It is the largest lake in Western Europe with a volume of 89 km$^3$ and a maximum depth of 309 m.

Lake Geneva was chosen as a case study for several reasons: i) Lake Geneva has a deep water column that allows sampling water layers whereas avoiding inputs from bottom sediments (e.g. sediment resuspension); ii) there is a great distance between surface layers and bottom sediments, which allows processes in the water column to better develop; iii) we can compare and extend results between settling particles (Díez et al., 2016) and suspended particles (from this work); iii) the proximity of our laboratories to Lake Geneva allows samples to be to
collect and processed rapidly in order to reduce any bias.

3. Material and methods

3.1. Sampling methods

Two different sampling campaigns were conducted in parallel to obtain samples from the lake and the main tributaries. The sampling point in Lake Geneva was SHL2 (46°27’9.7”N 6°35’19.4”E, Fig. 1), at the maximum lake depth. For the main tributaries we selected four rivers and sampled at: 46°28’15.3’’N 6°24’20.9’’E (Aubonne); 46°31’27.4’’N 6°32’40.4’’E (Venoge); 46°21’28.8’’N 6°31’22.8’’E (Dranse); 46°20’58.2’’N 6°53’18.7’’E (Rhone) (Fig. 1).

The two sampling setups were selected for their capability to collect suspended particles from a water body avoiding any unwanted redox alteration.

Continuous flow filtration was used to collect suspended particles from the lake water column. We used a peristaltic pump to continuously collect the water and send it to the filtration units, which consisted of two stainless steel filter holders (Sterilite, 304 SS) mounting a large diameter glass fiber filter (Stereitec, GC-50 142 mm) each with 0.50 μm pore size. The filters were prepared to avoid sampling bias (weight loss and contamination) by running MilliQ water through them for about 1 h in order to remove loose fibers; then the filters were burned at 500 °C to remove any possible Hg contamination, weighted and placed in aluminum foils to shield them from dust and light.

Before each sampling, a multi-probe (SBE 19plus V2 SeaCAT Profiler CTD) was used to collect vertical profiles of ancillary parameters (pH, DO, conductivity, temperature, turbidity, fluorescence, solar irradiance) along the water column. The concentration of dissolved oxygen (DO) was in turn used to select the first two sampling depths, one in the epilimnion and one in the metalimnion, corresponding to the highest (lake primary production) and lowest (mineralization layer) DO concentrations. The choice to sample according to DO signal is related to the evidence showed by previous works in the ocean (Cossa et al., 2009, 2011) which link the MeHg in water column to in-situ primary production (high DO concentrations) and mineralization of POC (low DO concentrations). The third sampling point was used to investigate the hypolimnion and its depth (100 m) was selected on two factors: i) absence of significant variation in DO values; ii) sufficient distance from the lake bottom to avoid sediment resuspension. Moreover, for each sampling depth water samples were collected to determine the total Hg (THg) and MeHg concentrations in the raw and filtered water.

In order to track the behavior of MeHg concentrations over the year, we sampled Lake Geneva once per season, plus two extra samplings during high production periods for a total of six samplings. After each sampling, we freeze-dried the filters and kept the acidified water samples (5% HCl) at 5 °C. Moreover, to determine the macro-composition of the sampled particles, a microscopic imaging was carried out on the filters using an optical microscope (Olympus BX40, 10x).

Continuous flow centrifugation was used to collect suspended particles from the four main tributaries. A Roxve (U45E) submerged pump fed a Westfalia Separator centrifuge (KA 2-06-075) with a constant water flow for at least 2 h. River sampling was carried out in 2018 and 2019 with 4 samples collected per river (two in high-flow regime and two in low-flow regime), except for the Dranse which has only two sampling periods; it was not possible to travel to France to complete the sampling due to the COVID pandemic.

To measure the particle loads to the lake, three 1-L water samples were filtered on a 0.45 μm glass fiber filter to determine suspended particles concentrations at the beginning, middle and end of the centrifugation. Moreover, river discharge data during the sampling period were obtained for the Swiss tributaries from the Swiss Federal Office for the Environment (FOEN, ), and from the Hydroreel service for the Dranse. After each sampling, the suspended particles were freeze-dried and grinded to obtain a dry powder ready for analysis.

3.2. Laboratory analysis

Samples were analyzed via cold vapor atomic absorption spectrophotometry (CVAAS) (DMA-80 III, MWS GmbH, Switzerland) and cold-vapor atomic fluorescence spectrometry (CVAFS) (Merx Model III, Brooks Rand, USA) using different sample preparation techniques. CVAAS was used to determine THg concentrations on freeze-dried particles from the rivers. All analyses were done in triplicate. The certified reference material SRM 2702 (NIST) gave concentrations inside the acceptance range. CVAFS was used to determine THg and MeHg on rivers (only MeHg) and lake suspended particles and from lake water samples. Depending on the matrix different kinds of extraction and analysis techniques were used.

All the LODs was calculated using a set of method blanks (extraction and distillation blanks) or instrumental blanks (if method blanks were not present), we calculated the mean concentrations and we added three times the standard deviation as described in the 40 CFR. (Appendix B part 136). Regarding the LOQs we calculated them using the procedure explained in all the EPA methods used in our analysis (US-EPA, 2001a, 2002; 2001b).

THg analyses in suspended particles were carried out using aqua regia (HCl/HNO3) extraction following the EPA Method 1631 (US-EPA, 2001a), with a calculated limit of detection (LOD) of 0.28 ng/g and a calculated limit of quantitation (LOQ) of 0.88 ng/g, which are in range with the MDL (Method Detection Limit) (from 0.24 ng/g to 0.48 ng/g) and the ML (minimum level of quantification) (1 ng/g). The method was slightly modified to take into account the small overall quantity of samples and the presence of organic matter.

The filters holding the suspended particles were placed in Teflon reactors for acid digestion. After addition of acids, the samples were placed overnight (around 16 h) in an oven at 60 °C to obtain a full digestion of the suspended particles. The digestates were analyzed following the EPA Method, revision E 1631 (US-EPA, 2002). The certified reference material used in the THg analysis (IAEA - 450) showed between 92.6% ± 2.4%–97.6% ± 4.6% recovery.

THg analysis on water samples were carried out following the EPA Method, revision E 1631 (US-EPA, 2002), with a calculated LOD of 0.27 ng/L and a calculated LOQ of 0.87 ng/L, which are comparable to the MDL of 0.2 ng/L and the ML of 0.5 ng/L. To control the quality of the analysis, two different reference materials were used: ORMS-5 (National Research Council Canada) which gave a recovery percentage of 88.8% ± 1.2%, and a standard solution purchased from Brooks Rand (USA), which is normally used to build the calibration line. The latter was used considering a specific concentration of Hg (25 pg) and repeating it several times during the analysis. The recovery percentage was between 94.1% ± 2.5%–96.3% ± 1.3%.

MeHg analysis on suspended particles were carried out using a HNO3 leaching/CH2Cl2 extraction method (Liu et al., 2012). The solution was analyzed following the EPA Method 1631 (US-EPA, 2002). The certified reference material used in the MeHg analysis (ERM-CC580, European Commission) showed between 91.8% ± 5.2%–98.7% ± 1.6% recovery.

Filter blanks were produced with MQ passing through the filtration setup in the lab and processed as the other samples. Results showed values below the calculated LOD for both THg and MeHg.

MeHg analysis on water samples was carried out after the distillation following the EPA Method 1630 analysis procedure (US-EPA, 2001b), with a calculated LOD of 0.028 ng/L and a calculated LOQ of 0.089 ng/L, which are comparable to the MDL of 0.02 ng/L and the ML of 0.06 ng/L. To check the accuracy of the analysis we used a spiked sample of known concentration that showed a recovery efficiency between 89.5% ± 8.2%–96.6% ± 1.0%.

The percentage of OM in the river particles was determined via loss on ignition (LOI-1) heating 1 g of sample at 550 °C for 1 h (Heiri et al., 2001).
3.3. Statistical analysis

Normality was tested with the Shapiro-Wilk test for all the data collected. THg and MeHg concentrations in lake waters and in the river particles were normally distributed, whereas lake particles showed non-normal distributions. In accordance to the normality test the Mann–Whitney U test was used to determine statistically significant differences for THg and MeHg concentrations between lake suspended particles and the bottom sediments values (Díez et al., 2016, 2018), and between lake suspended particles and river particles. Comparison between THg and MeHg concentrations in lake suspended particles and in settling particles (Díez et al., 2018) was carried out with paired t-tests. Seasonal patterns in THg and MeHg concentrations in suspended particles were tested with a Wilcoxon signed rank test and a paired t-test respectively. Seasonal patterns in THg and MeHg concentrations in water, were tested with one-way ANOVA tests with the Holm-Sidak method for multiple pairs comparison in the case of the THg. MeHg/THg ratios proved to be non-normally distributed for all the investigated matrixes, thus Mann–Whitney U tests were used to determine statistically significant differences between river particles, lake suspended particles and water samples. All the statistical analyses were performed with Sigma Plot 12.5 (Systat Software Inc, USA) with a significance level of 0.05.

4. Results

4.1. Ancillary parameters and particles characterization

The vertical profiles of dissolved oxygen and chlorophyll-a (Fig. S1) allowed to identify production zones (at 5, 7, 15 or 25 m depth, depending on the sampling campaigns) and mineralization zones (at 15, 25, 30 or 40 m) of the lake, where the samplings were carried out. The comparison between the vertical profiles of all months showed that the first sampling depth yield the maximum in DO, with values ranging from 262.1 to 355.8 μmol/L (8.4–11.4 mg/L) with the only exception of May in which both the first two sampling depths showed similar DO concentrations, while the second sampling depth showed the minimum DO concentrations when the stratification is present (June, July and November) with values ranging from 191.8 to 253.8 μmol/L (6.1–8.1 mg/L). When the stratification is not present the hypolimnion show the minimum DO concentrations with values ranging from 196.5 to 272.4 μmol/L (6.3–8.7 mg/L). The chlorophyll-a vertical distributions showed a more variable pattern. While all peak concentrations measured by the multi-probe (range from 1.53 to 6.55 mg/m³) were observed between the first two sampling depths they not always corresponded strictly the DO vertical profile (Fig. S1). The hypolimnion always showed the lower concentrations of chlorophyll-a with values ranging from 0.04 to 0.39 mg/m³.

The particles concentration in the water column of Lake Geneva (Fig. S2) showed an overall very low content of particles, ranging from 0.1 to 1.7 mg/L. In all sampling periods, the 100 m depth is the one showing the lowest suspended particle concentrations, which is probably related to the processes of predation and dissolution that happen from the surface to the bottom of the lake (Miracle, 1974; Rienmann, 1985; Finlay and Berninger, 1984). Microscope observations of the filters from the lake sampling (Fig. S3), highlighted a particle composition mainly made up of diatoms, cyanobacteria and inorganic particles (mainly calcite and iron oxides). This composition did not seem to vary with depth.

Regarding the first two sampling depths (production and mineralization zones), July ‘20 and April ‘21 showed the highest values of particles concentration (Fig. S2). The values showed in April are coherent with results published by CIPEL (“Commission internationale pour la protection des eaux du Leman”) obtained from the same sampling position (SHL2) through the years, which showed an increase in productivity and chlorophyll-a between mid-March and mid-April (Rasconi et al., 2019). On the other hand, the CIPEL reports showed a similar peak in June with a sharp decrease of both primary production and chlorophyll-a in July, which seems in contradiction with the values reported in Fig. S2. This apparent contradiction might be explained by the presence of other sources of particles than the lake primary production such as the glacial tributary of the lake (e.g. Rhone River), which high flow period is in summer, entering the lake with high amounts of particulate matter. Microscope observations were carried out on the river particles showing an absence of diatoms and cyanobacteria, which are linked to the in-situ production in the lake. For inorganic particles, river and lake particles showed no detectable difference under the microscope (Figs. S3 and S4), with presence of calcite and iron oxides as the main components (Fig. S4).

The organic matter content in the particles collected from the rivers ranged from 1.38% to 15.23% with particles from the Rhone River showing the lowest values in OM (Fig. S5). This is linked to the Rhone glacial source, especially during the high flow due to snow and ice melt.

4.2. THg and MeHg concentrations in suspended particles

THg and MeHg concentrations in the suspended particles collected at SHL2 ranged from 32.6 ± 15.9 ng/g to 429.3 ± 25.4 ng/g (Fig. 2-A) and from 0.48 ± 0.09 ng/g to 9.61 ± 0.67 ng/g (Fig. 2-B) respectively. THg concentrations were highest in the deepest sampling point (100 m) at all seasons. MeHg followed THg pattern in late spring and summer, while for the rest of the year, MeHg at 100 m seems more similar to concentrations at the second sampling depth.

4.3. THg and MeHg concentrations in the water column

THg concentrations in water samples collected at SHL2 ranged from 0.32 ± 0.02 ng/L to 1.31 ± 0.07 ng/L in raw water and from 0.41 ± 0.02 ng/L to 1.31 ± 0.3 ng/L in filtered samples (Fig. S6), whereas MeHg concentrations ranged from 0.024 ± 0.008 ng/L to 0.137 ± 0.034 ng/L in raw water and from 0.021 ± 0.006 ng/L to 0.146 ± 0.049 ng/L in filtered samples (Fig. S7).

The very low content of particles in the water column causes the difference in THg and MeHg concentrations between raw and filtered samples to be less than the standard deviation of the analysis, making the estimation of statistical differences between the two populations irrelevant. Overall, THg concentrations in water showed a distinct seasonal pattern. The comparison between May and June, when the lake stratification builds up, vs. July and November, when the lake stratification is well established, showed a significant difference (p < 0.05), the former being higher in concentration than the latter, whereas the other months did not show these differences (p > 0.05). On the other hand, MeHg did not show any statistically significant difference between months (p > 0.05). Both THg and MeHg do not change significantly with depth (p > 0.05), indicating homogeneity along the water column.

THg and MeHg contents in the particles were recalculated as the Hg mass related to the volume of water (from ng/g to ng/L) multiplying THg (and MeHg) concentrations in particles by the particle concentrations in the water, in order to compare Hg attached to the suspended particles with Hg occurring in the dissolved phase of the water column (Fig. 3). The very low particle concentrations in the water column largely reduce the particles contribution in the overall MeHg concentrations of the water. The contribution of the particles to the overall THg and MeHg concentrations in the water column seems to remain comparable between the different depths, in agreement with the increasing THg and MeHg concentrations in particles (Fig. 2) and the decreasing of particles concentrations (Fig. S2) with depth.

4.4. THg and MeHg concentrations in the particles of the main tributaries

THg and MeHg concentrations in particles of the main tributaries...
ranged from 19.0 ± 3.0 ng/g to 111.9 ± 2.4 ng/g (Fig. 4-A) and from 0.30 ± 0.08 ng/g to 2.41 ± 0.14 ng/g respectively (Fig. 4-B).

THg concentrations showed no significant differences between all the rivers (p > 0.05) whereas MeHg concentrations showed significant difference between the Rhone and the Venoge Rivers (p < 0.05). Moreover, MeHg showed increased concentrations during high flow regimes in all rivers except for the Rhone River, a pattern which is not present in the THg concentrations. Overall, the Rhone showed the lowest MeHg concentrations between the main tributaries whereas the Venoge showed the highest concentrations for all periods. Both Aubonne and Venoge showed their highest discharge during the first period of seasonal low regime due to a very intense rain event that happened in the days before the sampling. In Table S1 additional data regarding the flow rate and particles load for the sampled rivers.

Fig. 2. Concentrations of THg (A) and MeHg (B) in the suspended particles of Lake Geneva sampled at SHL2 during different seasons at different depths.

Fig. 3. THg (A) and MeHg (B) concentrations in filtered water samples (green) and attached to the suspended particles (brown). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Fig. 4. THg (A) and MeHg (B) concentrations in the lake tributaries at high (H) and low (L) flow regimes.
4.5. MeHg/THg ratios

MeHg/THg ratios ranged from 0.4% to 35.8%. MeHg/THg ratios of raw and filtered water samples showed no significant differences between them (p > 0.05). On the contrary, statistically significant differences were found between MeHg/THg ratios of raw and filtered water compared to the ratio in both river and lake particles (p < 0.05). The water samples (raw and filter) showed the highest values of MeHg/THg followed by the lake particles and the river particles, which have the lowest ratios.

4.6. Partitioning coefficient calculation

The partitioning coefficient ($K_p$, in $L/kg$) for both THg and MeHg, where $p$ is the solid phase (particles) and $d$ is the dissolved phase, was calculated using the equation of Honeyman and Santschi (1989).

$$K_p = \frac{[Hg_p]}{[Hg_{FW}]} \cdot \frac{1}{C_p} \tag{1}$$

where $[Hg_p]$ is the mass of THg (or MeHg) in the suspended particles related to the volume of water (ng/L), $[Hg_{FW}]$ is the concentrations of THg (or MeHg) in the filtered water (ng/L) and $C_p$ is the particle concentration (kg/L).

The Log $K_p$ values obtained for THg and MeHg during the various field campaigns ranged from 4.59 to 5.77 and from 4.22 to 5.10 with a quadratic mean of 5.17 and 4.75, respectively (Fig. S8) expressing the strong affinity of THg for solids.

5. Discussion

5.1. Hg species behavior in the main tributaries and overall Hg input to the lake

The behavior of Hg species in rivers is strongly related to the flow regimes but more importantly to the characteristics of the watershed from which the rivers take their particles (Babiarz et al., 1998). To explain the Hg species behavior in the three Swiss sampled rivers, the land use in their watersheds was identified based on the thematic maps from the Swiss Federal Office for the Environment (FOEN) (Figs. S9 and S10); the THg and MeHg concentrations were plotted against the OM content (Fig. 5), due to the strong affinity of Hg for OM.

The four considered tributary’s watersheds are mainly divided into two types of land use: agricultural and urban. The Rhone River presents the longest course of all the considered rivers (160 km from the Rhone glacier to Lake Geneva) with a combination of small urban centers and large agricultural sites present along the river course. The Aubonne and the Venoge Rivers are the shorter ones considering the course length (12.2 km and 38.4 km, respectively) and, whereas the two rivers present both agricultural and urban areas, the Venoge River is the most urbanized river among the considered group. The Dranse River presents mainly agricultural areas along its course (data from the French Government (Géopartail, ) (Fig. S11).

The Rhone River is the only river showing a direct linear correlation

![Fig. 5. OM percentages vs THg (A) and MeHg (B) in the particles collected from the rivers. The Rhone is the only river that showed a significant ($r^2 = 0.91$) regression line for THg.](image)
between THg and (R² = 0.907, p-value = 0.05; n = 4 Fig. 5-A), it also shows a slight increase in OM in the low flow. This increase in OM at low flow is in apparent contradiction to other cultivated watersheds where, as the flow increases, more particles and OM are mobilized (Babiarz et al., 1998; Balogh et al., 2003). A possible explanation could be found in the partially glacial nature of the Rhone watershed, which tends to mobilize large amounts of particles during the summer due to ice and snow melt. Due to climate change the ice melt from the glaciers is increasingly mobilizing sediments from the Alpine catchment area (Costa et al., 2018), which are lower in OM than the cultivated watersheds in the Rhone Valley. This enhances a dilution effect on the OM percentage during the high flow period.

The Aubonne River shows a peculiar trend with THg concentrations decreasing as OM contents increase (Fig. 5-A). This behavior is probably the result of two main factors: i) the land use of the Aubonne watershed (Fig. S9): ii) the Aubonne main source of water (precipitations). During high flow periods, the precipitations mobilize particles from the northern part of the catchment area (Jura Mountains) with lower OM content, which dilute the high OM particles from the cultivated section of the watershed. At the same time, mercury from roads, urban and industrial areas are washed into the river, increasing the THg concentrations.

The Venoge River presents most of the highest THg and MeHg concentrations (Fig. 5-A). Data of this river are clustered together showing no significant pattern. This is due to the highly urban and industrial areas present in the river watershed (Babiarz et al., 1998).

The previously mentioned dilution effect determined by heavy precipitations is also highlighted by Fig. 6-A in which the red points (highest discharge for Aubonne and Venoge show similar THg and OM values. The watersheds of Aubonne and Venoge Rivers are similar (Fig. S9), especially in their upstream portions because of their proximity. Therefore, precipitations washing similar catchment areas produce similar THg and OM values.

Regarding MeHg (Fig. 5-B), in the Rhone River the highest MeHg concentration is measured during one of the low flow periods. On the contrary, in the Aubonne and the Venoge Rivers, MeHg presents the lowest concentrations during the rain event (highest flow recorded); this is also probably another effect of the mentioned dilution effect of heavy precipitations, bringing from the watershed particles less rich in MeHg.

The high flow periods presents higher MeHg concentrations than the low flow periods, which may be related to the characteristics of the riverbeds. During low flow periods, both the Aubonne and Venoge Rivers have a large part of their bed exposed to the atmosphere, which decreases the area of production of MeHg due to the oxygenation of non-flooded sediments. After the reinundation of the entire riverbed during high flow periods, the MeHg production area expands and MeHg concentrations increase. Roulet et al. (2001) showed that inundation of semi-aquatic sediments and semi-terrestrial soils have an important effect on the MeHg production yielding methylation up to three times the pre-inundation levels.

The Dranse River has not been included in this discussion due to the limited available data, but it seems to follow the Aubonne River pattern presenting comparable THg concentrations (Fig. 4-A) in both flow regimes, and low MeHg concentrations (Fig. 4-B) during the low flow period. This is probably due to the similarity of their respective watersheds (figre S9–S11).

5.2. Hg in the water column of Lake Geneva

5.2.1. Concentrations of THg and MeHg in the suspended particles

Concentrations of THg and MeHg in the suspended particles of Lake Geneva showed the highest values in the hypolimnion (Fig. 2), except for MeHg in February. Physicochemical parameters collected in parallel with the particles were evaluated to identify the drivers of the THg and MeHg vertical profiles.

As already mentioned, the sampling depths were selected to investigate the primary production zone, the POC mineralization zone and the hypolimnion.

MeHg concentrations showed increasing values with depth in all sampled periods, except in May and July when the mineralization zone presents the lowest values, and February when the hypolimnion has slightly lower MeHg concentrations than the mineralization zone. The MeHg distribution along the water column is not necessarily linked to the presence of layers in which methylation could be enhanced (e.g. POC mineralization zone). This could be explained by the overall high levels of DO, even in the hypolimnion. The maximum DO concentrations in the first two sampling depths ranged from 9.0 to 11.3 mg/L and the minimum from 6.1 to 10.7 mg/L, whereas the hypolimnion showed DO concentrations ranging from 7.7 to 9.1 mg/L. Moreover, the differences in DO concentrations between the first and the second sampling depths are relatively low with a maximum difference of 3.9 mg/L in July and a minimum of 0.7 mg/L in May. Overall, the DO values showed a well oxygenated water column with no hypoxic layer in which methylation could occur.

Hg methylation could be linked to the lake primary production layer due to the production of fresh OM by phytoplankton. Moreover, phytoplankton is known to accumulate Hg and MeHg from the surrounding water (Pickhardt and Fisher, 2007; Quiroga-Flores et al., 2021; Le Faucheur et al., 2014) and to be used by microorganism communities during mineralization processes, including Hg methylation (Le Faucheur et al., 2014). To assess this hypothesis, THg and MeHg concentrations were plotted against chlorophyll-a (Fig. S12). Results showed a negative correlation between the species of Hg and chlorophyll-a highlighting the lack of a direct link between lake primary production and Hg methylation.

DO and chlorophyll-a do not seem to be related to the vertical distribution of THg and MeHg concentrations in the suspended particles, which is probably related to the timing of methylation. The longer the methylation time, the greater the amount of MeHg. This could explain why the particles in the hypolimnion almost always have the highest MeHg values. The fact that we do not see an increase in MeHg concentrations with depth each month can indicate the presence of other processes that decrease MeHg concentrations (demethylation processes). The main demethylation pathways include biotic (microbially mediated), chemical and photochemical processes (Zhang and Planas, 1994; Whalin et al., 2007; Monperrus et al., 2007). All of them could play a role in this environment. Further studies could identify whether some are more or less active at specific times or depths, or if this pattern is the result of co-activity of all of them.

In order to assess the importance of the suspended matter in the limnic Hg cycles, data from this work were compared to a previous work.
on the lake. Díez et al. (2018) deployed sediment traps to collect settling particles at different depths (Fig. S13). To make a meaningful comparison, we used their data from sediment traps at 75 m and 132 m depth to have MeHg concentration values from depths comparable to our data at 100 m depth. MeHg concentrations in the present study are higher in all sampled periods except in November, when they are lower, and June, when they are comparable. This difference is probably linked to the nature of collected particles. Sediment traps collect mainly particles that have such mass and/or density that they settle faster than lighter particles. On the other hand, continuous flow filtration collects all materials coarser than 1.2 μm present in the water, thus incorporating in the samples potential MeHg productive micro-environments such as lake snow that sediment traps strongly undercollect.

Moreover, MeHg showed overall higher concentrations in the suspended particles compared to the bottom sediments (p < 0.05) (Fig. S14), highlighting water column particles as an important component of MeHg cycling in this lacustrine system.

5.2.2. Role of the particle-water interaction in the MeHg budget along the water column

MeHg/THg ratio is commonly used as an indicator of MeHg production in a specific natural compartment (e.g., sediments, particles, etc.) (Sunderland et al., 2006; Ethier et al., 2010; Wu et al., 2011; Guo et al., 2021). The comparison of MeHg/THg ratios between suspended particles and bottom sediments (Fig. 6) shows that suspended particles appear to be an important production site that outcompete bottom sediments.

Fig. 6 shows also that water samples had the highest values in MeHg/THg. Lake Geneva has a fully oxygenated water column which is not suitable for Hg methylation because it is mainly mediated by obligate anaerobes (Gilmour et al., 2013). Abiotic methylation has been studied in laboratory analysis, and found possible in natural environments under specific conditions (Celo et al., 2006), but its relevance is very limited and commonly related to anoxic hypolimnions with high concentration of sulphur and/or OM (Eckley and Hintelmann, 2006). Therefore, the MeHg present in the water column can be the result of: i) diffusion from the bottom sediments (directly underneath or laterally) or from the resuspended bottom particles; ii) input from the tributaries; iii) diffusion from the suspended particles. Diffusion from the bottom sediments is not present at the selected sampling depths because there is no gradient of the THg and MeHg vertical profiles in the dissolved phase. Horizontal diffusion is also improbable due to the far distance of the sampling points from the coastlines and the high oxygenation of the shallow sediment which increases MeHg degradation (D'Inverno et al., 2000). Bottom sediment resuspension induced by wave action is highly important in shallow lake, but for deep lake its importance is negligible (Rosa, 1985; Blais and Kalff, 1995). The tributaries as the origin of the MeHg present in the lake water column is also unlikely. We do not have MeHg concentrations from river water, but Babiarz et al. (1998) found values of unfiltered MeHg concentrations in an urban Wisconsin river (Lincoln) to be around 0.04 ng/L; likewise Hurley et al. (1998) found values of filtered MeHg concentrations in the tributaries of Lake Michigan to be near their MDL of 0.04 ng/L. The previously mentioned values are much lower than our average filtered MeHg concentration (0.63 ng/L).

Therefore, based on the previous considerations, our results suggest that the MeHg concentrations found in the filtered water samples are mainly due to diffusion from the suspended particles.

In addition, the Log $K_d^p$ for both THg and MeHg increases with depth, following the trend of the concentrations of Hg species found in the suspended particles along the water column, except in May and November for THg and in May and July for MeHg. The Log $K_d^p$ values for THg and MeHg are comparable with the results of Hurley et al. (1994) who found in two Wisconsin lakes for THg Log $K_d^p$ values ranging from 4.5 to 5.7, and Mason and Sullivan (1997) who found in Lake Michigan values ranging between 5.5 and 5.8 for THg and a value of 5.7 for MeHg. Our results are also within the range of more than 8000 values of $K_d^p$ of THg from the freshwater environments database given by Tomczak et al. (2019). Moreover, these authors showed that there is a significant difference in Log $K_d^p$ for THg for settling particles ($\approx$5) and for deposited sediments ($\approx$1).

The conceptual model in Fig. 7 illustrates our hypothesis about the role of lake snow in MeHg production in the oxic water column of Lake Geneva. In the surface layers, the primary production supplies the OM necessary to generate the highly organic particles that compose the lake snow. These low density particles, already enriched in THg due to the presence of phytoplankton (Le Faucheur et al., 2014), begin their slow descent, scavenging Hg from the surrounding waters and producing MeHg due to microorganisms inside these particles. A fraction of the produced MeHg diffuses to the surrounding waters ($K_d^p$ MeHg < $K_d^p$ THg). As the particles settle in the water column, both MeHg and THg concentrations in particles increase while concentrations of particles in the water column decrease, probably due to dissolution and predation by anaerobic (Gilmour et al., 2013). Abiotic methylation has been studied in laboratory analysis, and found possible in natural environments under specific conditions (Celo et al., 2006), but its relevance is very limited and commonly related to anoxic hypolimnions with high concentration of sulphur and/or OM (Eckley and Hintelmann, 2006). Therefore, the MeHg present in the water column can be the result of: i) diffusion from the bottom sediments (directly underneath or laterally) or from the resuspended bottom particles; ii) input from the tributaries; iii) diffusion from the suspended particles. Diffusion from the bottom sediments is not present at the selected sampling depths because there is no gradient of the THg and MeHg vertical profiles in the dissolved phase. Horizontal diffusion is also improbable due to the far distance of the sampling points from the coastlines and the high oxygenation of the shallow sediment which increases MeHg degradation (D'Inverno et al., 2000). Bottom sediment resuspension induced by wave action is highly important in shallow lake, but for deep lake its importance is negligible (Rosa, 1985; Blais and Kalff, 1995). The tributaries as the origin of the MeHg present in the lake water column is also unlikely. We do not have MeHg concentrations from river water, but Babiarz et al. (1998) found values of unfiltered MeHg concentrations in an urban Wisconsin river (Lincoln) to be around 0.04 ng/L; likewise Hurley et al. (1998) found values of filtered MeHg concentrations in the tributaries of Lake Michigan to be near their MDL of 0.04 ng/L. The previously mentioned values are much lower than our average filtered MeHg concentration (0.63 ng/L).

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zooplankton and fish (Miracle, 1974; Finlay and Berninger, 1984; Riemann, 1985). The predation of particles is probably the main entry point of MeHg into the lake food chain (Wu et al., 2020; Herrin et al., 1998) and in line with our observation, the deeper the predation happens the higher the amount of MeHg that enters into the food chain (Herrin et al., 1998).

In order to evaluate the plausibility of our hypothesis of lake snow as the principal site of MeHg production in the water column, we calculated the time required for MeHg concentration to reach the steady state in the water column (from the surface to 100 m). As previously mentioned, we assumed that the particles are the only source of MeHg to the water column. Then these results are compared to particle residence time in the first 100 m of the water column, determined in similar environments by other researchers. To calculate this time, we developed a mass balance model (Fig. S15) based on the differential equation:

\[ \frac{d(\text{MeHg})}{dt} = K_m(\text{THg}) - K_d(\text{MeHg}) - k_s(\text{MeHg}) \]

where \(K_m\) and \(K_d\) are the methylation and demethylation rates, respectively; \(k_s\) is the removal rate of MeHg via settling particles; (THg) is the total mercury inventory in the particles; (MeHg) is the methylmercury inventory in the water column. To perform this calculation, we used the \(K_m\) and \(K_d\) ranges available in the work of Díez et al. (2016) that took place in the same environment (Lake Geneva), and only considered the months of May, June and July.. The removal rate \(k_s\) was evaluated from the MeHg settling flux determined by Díez et al. (2018). Moreover, we used from our work the THg concentrations in the suspended particles, the total MeHg, and the particle concentrations in the water column. We averaged May, June and July results from our work (Table S2) to obtain the weighted mean for the water column (0–100 m), using the first sampling depth to characterize the epilimnion, the second one for the metalimnion and the third one for the hypolimnion; we averaged results of the three months in order to avoid overuse of discrete data to simulate a very dynamic system. A calibration step showed that input variables (THg concentrations, particle concentrations, Km and Kd) have to be adjusted by only 20% to correctly simulate, to within 10%, the measured MeHg inventory in the water column.

The simulation result showed that the time needed to reach the 95% of the steady state was 37 days. This result is based on the assumption that methylation in the particles has already started, i.e. an anoxic substratum was already present at the beginning of the simulation. Porvari and Verta (1995) found in their laboratory analysis that submerged peat showed a rapid increase in MeHg concentrations (linked to MeHg production) after \(\approx 4\) days from the addition of the overlying water. Peat is a highly organic compound, as the particles that we are considering here, we can therefore use this data as an indicator of the time needed to reach anoxia in an aquatic environment.

The time derived from our model is compatible with particles settling velocities and residence time. In Lake Geneva, Dominik et al. (1989) found that the settling time of particles to reach the bottom at 310 m depth was between 60 and 270 days, and Stabel (1987) found in Lake Constance residence time of the bulk of settling particles to be around 28 days. It is important to stress that the mentioned researches have studied particles as they move down through the entire water column, whereas we only consider the first 100 m of the water column. Moreover, difference in the dynamics of the different kinds of particles (suspended vs settling) may play an important role in the descent velocity and in the overall residence time. This difference is evident in the work of Stabel (1987), who found a settling velocity of 0.4 m/d during clear water phases when particles are mainly phytoplankton and organic debris, whereas for denser particles velocities are much higher (2.6–7.5 m/d).

In our work we sampled all the particles present at a certain depth, so we have a mixture of particles of different sizes and densities and the actual settling velocities may well be in the range shown by Stabel (1987). In order to confirm the conceptual model illustrated in Fig. 7, additional investigations are needed, such as methylation and demethylation rates to assess the actual net methylation for the lake snow; further study on the chemistry of the particles to confirm the presence of anoxic layers and the quality of the OM, which can increase or decrease the time needed for the particles to reach anoxia; identification of the microorganism’s populations and productivity rates to assess the kind of microorganisms which are actually methylating, and to identify other populations which may be involved in other processes (OM mineralization, demethylation); and identification of the amount of the MeHg input from the rivers water, to confirm the small contribution of the tributaries to the total MeHg budget of the water column.

6. Conclusions

MeHg concentrations measured in the suspended particles of Lake Geneva were comparable to the previous results on settling particles from the same lake (Díez et al., 2016, 2018). MeHg concentrations in tributary particles and in bottom sediments of Lake Geneva were lower than the suspended particles of the lake, highlighting the fact that the MeHg found in the lake particles is most likely produced \(in situ\) and not derived from the lake catchment or the bottom sediments. Comparison of MeHg concentrations in the three distinct parts of the lake water column showed that the hypolimnion is the compartment in which the particles are the richest in MeHg for all the sampled periods, except in February when the metalimnion slightly exceeds the MeHg concentrations of the hypolimnion. This shows that “micro-conditions” (micro-environments in the particles) can be of great importance in terms of Hg methylation, creating a niche for MeHg production in an environment in which normally Hg methylation could not occur (oxic conditions). The observed decrease in particles concentration with depth could be linked to predation of the bacterial communities present in the particles, which could represent an important entry point of MeHg into the lake food chain. Overall, lake snow has the potentiality to be an important environmental micro-niche in the Hg methylation process, and also a compartment of great importance in the inclusion of MeHg into the food chain.

Further research is needed to examine and better understand this environmental micro-niche. A quantification of the impact that the main tributaries have on a lake Hg budget is essential to determine the full Hg cycle in a lacustrine environment. Moreover, it is of utmost importance: i) to determine microorganism production through experiments with Hg isotopes, to better characterize the methylation and demethylation rates in the particles; ii) to characterize the microorganism populations in lake snow by DNA and RNA extraction, to detect the presence of the gene cluster hgcAB and to identify the microorganism populations responsible for the Hg methylation.

Credit author statement

Andrea Gallorini: Study conception and design, Samples collection, Analysis, Interpretation of results, Manuscript preparation, All authors reviewed the results and approved the final version of the manuscript. Jean-Luc Loizeau: Study conception and design, Interpretation of results, Manuscript preparation, All authors reviewed the results and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.134306.