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UNIVERSITÉ DE GENÈVE **FACULTÉ DES SCIENCES**

Département F.-A. Forel
des sciences de l'environnement et de l'eau

Docteur Jean-Luc Loizeau

Role of *Lake Snow* in the Methylmercury cycle of a Deep Lake

THESE

présentée à la Faculté des Sciences de l'Université de Genève
pour obtenir le grade de Docteur ès Sciences, mention Sciences de l'Environnement

par

Andrea Gallorini

De

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intitulée :

«Role of Lake Snow in the Methylmercury Cycle of a Deep Lake»

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Résumé étendu

Le mercure est un métal trace connu pour sa neurotoxicité, en particulier ses espèces organiques, collectivement connues sous le nom de méthylmercure (MeHg). L'Hg est émis dans l'environnement à partir de sources naturelles et anthropiques et son cycle biogéochimique comprend tous les compartiments environnementaux (sol, eau et atmosphère). Depuis l'industrialisation, du Hg a été émis dans l'atmosphère à partir de diverses sources anthropiques, notamment: des centrales électriques alimentées par des combustibles fossiles, des mines d'or artisanales et à petite échelle, des industries de métaux ferreux et non ferreux, des usines de production de soude caustique, des installations de traitement du minerai, des incinérateurs de déchets urbains, médicaux et industriels, cimenteries et des usines de production de produits chimiques. De l'atmosphère, l'Hg se dépose après oxydation sur les systèmes terrestres et aquatiques par déposition humide (par les précipitations) et sèche, où il subit différentes transformations biogéochimiques, y compris la méthylation. Le MeHg n'est généralement pas rejeté dans l'environnement mais produit par des processus biotiques pilotés par plusieurs groupes de micro-organismes (bactéries sulfato-réductrices (SRB), certaines souches de méthanogènes, bactéries réductrices de fer, Firmicutes et certaines souches de fermenteurs), présents dans le milieu anoxique, tels les sédiments de fond des écosystèmes aquatiques. Cependant, ces dernières années, de nombreux chercheurs ont montré que cette méthylation se produisait également le long de la colonne d'eau oxique en relation avec une forte teneur en matière organique particulaire et une raréfaction localisée en oxygène dissous. Tous les méthylateurs connus sont des anaérobies obligatoires, donc pour qu'ils puissent méthyler l'Hg dans des environnements globalement oxiques, des micro-niches anoxiques doivent être présentes. La plupart de la littérature a montré que les particules présentes dans la colonne d'eau des environnements marins et d'eau douce, connues sous le nom de neige marine et lacustre (taille > 300 µm) et d'agrégats marins et lacustres (<300 µm), présentent les caractéristiques du microenvironnement dans laquelle la méthylation du Hg pourrait se produire à travers les colonnes d'eau oxiques, car elles ont été reconnues comme un site de minéralisation de la matière organique et comme présentant des gradients d'oxygène autour et à l'intérieur d'elles. A ce jour, la majorité de ces études concernent les milieux marins, soulignant la nécessité de davantage d'études dans les milieux d'eau douce, en particulier les systèmes lacustres. La présente thèse vise à étudier la neige lacustre en tant que micro-niche pour la production de MeHg le long de la colonne d'eau d'un lac périalpin profond (Lac Léman – Suisse/France). La filtration en flux continu a été utilisée pour échantillonner les particules de la colonne d'eau du lac afin d'analyser le THg et le MeHg. Les concentrations de MeHg variaient de 0,48 ± 0,09 ng/g à 9,61 ± 0,67 ng/g dans les particules du lac. Les concentrations de MeHg mesurées dans la neige lacustre du lac Léman ont montré des valeurs plus élevées que celles mesurés par Diez et al. (2016) dans les sédiments de

fond, avec des concentrations croissantes avec la profondeur le long de la colonne d'eau. De plus, les concentrations de matières particulaires ont montré une diminution avec la profondeur, probablement en raison de la prédation par le zooplancton et les poissons. Afin d'étudier la possibilité pour la neige de lac d'héberger des micro-environnements anoxiques, des particules de sédimentation ont été échantillonnées avec des pièges à sédiments couplés à un film de gel de polyacrylamide. Ce gel s'est avéré utile pour retarder les processus biologiques et pour éviter les changements redox dans les échantillons. Des analyses d'oxygène dissous ont été réalisées dans les agrégats de particules se trouvant à l'intérieur des gels avec une micro-sonde à oxygène couplée à un microscope et un micro-manipulateur manuel, afin d'être le plus précis possible dans l'insertion de la sonde. Les résultats ont montré des concentrations d'OD allant de 0,22 mg/L à 8,61 mg/L. Micro-environnements hypoxiques et anoxiques ont été trouvés dans les agrégats de décantation mettant en évidence la possibilité pour la neige de lac d'agir comme un substrat anoxique pour la méthylation du Hg, et, grâce à l'analyse SEM, des bactéries ont été observées sur les agrégats intégrés dans des films de matière organique, qui pourraient mettre en évidence la présence d'un processus de minéralisation de la matière organique et, à terme, la possible formation d'un milieu anoxique. Les résultats présentés dans cette thèse montrent que la neige lacustre, comme la neige marine, a la capacité d'être une micro-niche importante pour la production de MeHg en milieu oxique. La neige de lac a une grande importance dans le cycle du mercure du milieu limnique en raison de sa présence dans la colonne d'eau, qui est l'habitat de la majorité du biote limnique. L'effet de prédation qui concourt à la diminution des particules avec la profondeur est très probablement la principale entrée de MeHg dans la chaîne alimentaire limnique, déterminant l'insertion de MeHg dans les poissons consommés par la population humaine, soulignant l'importance de la neige lacustre dans l’écologie d'un système lacustre.

Abstract

Mercury is a trace metal known for its neurotoxicity, especially its organic species, collectively known as methylmercury (MeHg). Hg is emitted into the environment from both natural and anthropogenic sources and its biogeochemical cycle comprises all environmental compartments (soil, water and atmosphere). Since the industrialization, Hg had been emitted in the atmosphere from various anthropogenic sources including: fossil-fuel fired power plants, artisanal and small-scale gold mining, ferrous and non-ferrous metals manufacturing facilities, caustic soda production plants, ore processing facilities, incinerators for urban, medical and industrial wastes, cement plants and chemicals production facilities. From the atmosphere Hg is deposited after oxidation on terrestrial and aquatic systems via both wet (through precipitation) and dry deposition, where it undergoes different biogeochemical transformations including methylation. MeHg is not usually released in the environment but produced by biotic processes driven by several groups of microorganisms (sulfate-reducing bacteria (SRB), some strains of methanogens, iron-reducing bacteria, Firmicutes and some strains of fermenters), present in the anoxic bottom sediments of aquatic ecosystems. However, in recent years, many researchers have shown that this methylation also occurs along the oxic water column in relation to a high content of particulate organic matter and localized depletion of dissolved oxygen. All known methylators are obligate anaerobes, so for them to be able to methylate Hg in globally oxic environments, anoxic micro-niches must be present. Most of the literature showed that the particulate matter present in the water column of both marine and freshwater environments, known as marine and *lake snow* (>300 μm) and marine and lake aggregates (<300 μm), present the characteristics to be the microenvironment in which Hg methylation could occur across oxic water columns, because they have been recognized as a site of organic matter mineralization and as presenting oxygen gradients around and inside them. To date, the majority of these studies concern marine environments, highlighting the need for more studies in freshwater environments, particularly lacustrine systems. The present thesis aimed to investigate the *lake snow* as a micro-niche for MeHg production along the water column of a deep peri-alpine lake (Lake Geneva – Switzerland/France). Sampling and analytical technique were devised to collect particles and aggregates from Lake Geneva water column in order to analyze THg, MeHg and dissolved oxygen concentrations. MeHg concentrations found in Lake Geneva *lake snow* showed higher values than in bottom sediment (Diez *et al*, 2016), with increasing concentrations with depth along the water column. Furthermore, the concentrations of particulate matter showed a decrease with depth, probably due to predation by zooplankton and fish. Moreover, hypoxic and anoxic micro-environments were found in the settling aggregates highlighting the possibility for *lake snow* to act as an anoxic substratum for Hg methylation, and, thanks to SEM analysis, bacteria were found on the aggregates embedded in films of OM which could highlighting the presence of a mineralization process of OM and, in time, the

possible formation of an anoxic environment. The findings presented in this thesis show that *lake snow*, as *marine snow*, has the ability to be an important micro-niche for MeHg production in oxic environments. *Lake snow* has a great importance in the Hg cycle of limnic environment due to its presence in the water column, which is the habitat of the majority of limnic biota. The predation effect that concur in the decrease of particulate with depth is most likely one of the main entry point of MeHg into the limnic food chain, determining the insertion of MeHg into the fish that are consumed by the human population, highlighting the importance of *lake snow* in the ecology of a lake system.

Foreword

Introduction of the project and framework of the thesis

This thesis takes place in the context of a larger project that aims to investigate the ensemble of particles and aggregates, known as *lake snow*, presents in the water column of Lake Geneva (Switzerland-France) to determine its role in the Hg cycle of a freshwater environment, and to produce evidence about the possibility of *lake snow* to be a micro-niche for Hg methylation in the water column of a limnic environment.

The mentioned project comprises three major modules:

Module 1: Determination and testing of a specific setup designed to sample the particulate matter in Lake Geneva water column, avoiding as much as possible unwanted redox perturbation (e.g. induced anoxic conditions) and analysis of THg and MeHg concentrations on the sampled particles to determine the presence of methylmercury in the *lake snow*.

Module 2: Development and testing of a specific setup designed to collect physically and chemically undisturbed settling particles of Lake Geneva, avoiding as much as possible unwanted redox perturbation (e.g. induced anoxic conditions) in order to determine the dissolved oxygen concentrations inside the settling particles to identify oxygen gradient in the *lake snow*.

Module 3: DNA and RNA analysis on the *lake snow* of Lake Geneva to determine the microbial population present and to identify a specific gene cluster (*hgcAB*) considered as an indicator of microbial strains capable of methylating Hg. This module has been covered by the work of Capo et al. 2022 currently under revision.

The present thesis aims to reach two main objectives in Lake Geneva: i) the quantification of THg and MeHg concentrations attached to *lake snow*, and ii) the identification of possible anoxic micro-niches inside *lake snow*. These two objectives are related to the first two modules of the project, which are described in chapter II and III respectively, while the first chapter encompasses the present state of the art regarding MeHg in oxic aquatic compartments. Furthermore, the fourth chapter will place the main results of this thesis in the overall context of the cycling of MeHg in freshwater aquatic environments and where future research should focus. Chapter I to III are organized as papers (I and II published and III under revision).

The references are organized by chapter. At the end of each chapter references are given under the homonym sub title. The same goes for any supplementary material.

Chapter I

In chapter I are summarized the current understanding of Hg methylation in water columns of both marine and freshwater environments, as well the knowledge gaps and future research needs. Most of the literature showed that suspended particles (known as *marine* and *lake snow*) could be the microenvironment in which Hg methylation could occur across oxic water columns, because they have been recognized as a site of organic matter mineralization and as presenting oxygen gradients around and inside them. To date, the majority of these studies concern marine environments, highlighting the need for more studies in freshwater environments, particularly lacustrine systems. Investigating this new methylmercury production environment is essential for a better understanding of methylmercury incorporation into the trophic chain.

This chapter is published in the "Journal of Limnology" as a review article (Gallorini and Loizeau, 2021, 80/2, doi.org/10.4081/jlimnol.2021.2007).

Mercury methylation in oxic aquatic macro-environments: a review

Abstract

Mercury methylation in aquatic environments is a key process that incorporates this neurotoxin into the food chain and ultimately the human diet. Mercury methylation is considered to be essentially biotic and mainly driven by sulfate-reducing bacteria present in the bottom sediments in aquatic systems. However, in recent decades, many researchers have shown that this methylation also occurs in oxic layers in conjunction with a high content of particulate organic matter and localized depletion of dissolved oxygen. The goals of this review are to summarize our current understanding of Hg methylation in water columns of both marine and freshwater environments, as well as to highlight knowledge gaps and future research needs. Most of the literature showed that suspended particles (known as *marine* and *lake snow*) could be the microenvironment in which Hg methylation could occur across oxic water columns, because they have been recognized as a site of organic matter mineralization and as presenting oxygen gradients around and inside them. To date, the majority of

these studies concern marine environments, highlighting the need for more studies in freshwater environments, particularly lacustrine systems. Investigating this new methylmercury production environment is essential for a better understanding of methylmercury incorporation into the trophic chain. In this review, we also propose a model which attempts to highlight the relative importance of a MeHg epilimnetic path over a MeHg benthic-hypolimnetic path, especially in deep lakes. We believe that this model could help to better focus future scientific efforts in limnic environments regarding the MeHg cycle.

I.1. Introduction

Mercury is one of the most hazardous trace elements in the environment because of its neurotoxicity and its ability to bioaccumulate and biomagnify in food webs, under its methylated form CH_3Hg^+ (monomethylmercury, MMHg), which poses a direct threat to humans and wildlife (AMAP/UNEP, 2013, 2015).

In an attempt to assess how the expected Hg emission reduction—following the entry into force of the Minamata Convention—will reflect on Hg concentrations in the human food chain, Wang et al. (2019) investigated the Hg concentrations in biota from an oceanic dataset. They found that in most cases, the evolution of Hg concentration in biota did not follow Hg atmospheric deposition trends, with a divergence more evident in the last two decades. Two factors appear to be at the origin of this lack of correlation: 1) the legacy Hg present in aquatic environments, which allows Hg to remain bioavailable for a very long time; and 2) local processes are responsible for Hg speciation conversion and, in turn, its bioavailability. Together, they produce a substantial lag in the response of Hg in biota to external Hg input (e.g., atmospheric emission and deposition). This evidence highlights the importance of legacy Hg in aquatic systems over the expected decrease of new Hg emissions into the atmosphere (Selin, 2014). The Minamata Convention may only have long-term effects; legacy Hg pollution present in the environment could affect humans and wildlife for centuries to millennia because of the long timescales of mercury cycling (Sunderland and Selin, 2013).

The organic forms of mercury (MMHg and dimethylmercury [DMHg]) are usually not released into the environment, but are produced in aquatic environments, mostly by biological processes taking place in a variety of settings, such as bottom sediments, flooded soils, wetlands, oxygen deficient zones of water columns, and settling particles (Benoit et al., 2003; Bravo and Cosio, 2019; Ullrich et al., 2001), and carried out by a variety of microorganisms (Pak and Bartha, 1998; Paranjape and Hall, 2017; Regnell and Watras, 2019).

Despite the importance of sediments in the production of MeHg, several studies (Cossa et al., 2009; Díez et al., 2016; Eckley and Hintelmann, 2006; Lehnherr et al., 2011; Mason and Fitzgerald, 1990;

Monperrus et al., 2007a; Monperrus et al., 2007b; Soerensen et al., 2018; Topping and Davies, 1981) have shown that a non-negligible fraction of Hg is methylated within the water column of both marine and freshwater systems, environmental compartments in which this transformation has been underestimated so far.

Growing evidence shows that conditions for the methylation of mercury could exist inside and around micro- and macroaggregates in the water column under both oxic and anoxic conditions (Alldredge and Cohen, 1987; Glud et al., 2015). The marine and estuarine aggregates known as *marine snow* are the most studied so far; however, the scientific focus is slowly turning toward lake environments and *lake snow* (Ortiz et al., 2015; Paranjape and Hall, 2017).

Fish consumption is the primary pathway for human exposure to MeHg, which is a major health concern (Fitzgerald and Lamborg, 2014). Of the global fish production in 2016, 90.9 million tons came from captured wildlife and a non-negligible part of it (12.8%) is represented by freshwater fish (FAO, 2018). The presence of a MeHg production zone in the oxic layers of limnic environments is likely to increase the MeHg uptake by phyto- and zooplankton, which represent the base of the trophic chain, and in turn, MeHg becomes available to higher trophic-level organisms.

This endogenic source (production within the water column) of MeHg is an entry point into the trophic chain, which may be particularly important in deep lakes because of the great distance between the surface water and the sediments. Using a conceptual transport model, we highlighted the relative importance between the transfer paths created by the presence of these two sources of MeHg (i.e., bottom sediments and settling particles), underlining their effect on the biota MeHg uptake.

I.2. Mercury methylation and demethylation in aquatic systems

Mercury methylation in aquatic environments is recognized to be mainly related to a biological pathway, in particular to the activity of sulfate-reducing bacteria (SRB) belonging to the class of δ-proteobacteria (Compeau and Bartha, 1985; King et al., 2000). Other bacteria are known to play roles in the methylation process, including some strains of methanogens (Parks et al., 2013; Podar et al., 2015), iron-reducing bacteria (Bravo et al., 2018; Correia and Guimarães, 2017; Fleming et al., 2006; Si et al., 2015), and Firmicutes (Gilmour et al., 2013). A particular gene cluster that is proposed to be essential for Hg methylation has been found in every known methylator. The two-gene cluster hgcAB is currently the primary indicator used to detect bacteria capable of methylation (Bravo and Cosio, 2019; Parks et al., 2013; Podar et al., 2015; Regnell and Watras, 2019; Schaefer et al., 2011). Peterson et al. (2020) investigated an anoxic sulfidic hypolimnion lake with shotgun metagenomics to determine the presence of the gene cluster hgcAB in the microorganism's population. Surprisingly they found that the well-studied sulfate-reducing bacteria only account for the 22% of all the genome

coverage, whereas fermenters were the most abundant accounting for more than half of the genome coverage.

The reason why microbes methylate Hg is still unclear. Methylation of Hg facilitates the detoxification of the cell (Regnell and Watras, 2019); however no strong correlation between the concentrations of Hg and MeHg and presence of the hgcAB gene cluster has been found (Christensen et al., 2019; Regnell and Watras, 2019), in contrast to the mer operon which is a multi-proteins detoxification systems in which clustered genes in an operon produce the proteins needed to reduce Hg^{2+} to volatile Hg^0 and expel it from the cell (Nascimento and Chartone-Souza, 2003). This evidence could imply that while hgcAB is used by cells to remove Hg, the primary function of these genes may not be Hg detoxification but could be related to one-carbon metabolism and metal homeostasis, as suggested by Qian et al. (2018). Additionally, the hgcAB gene cluster is not essential for the survival of the microorganisms in the environment (Parks et al., 2013).

The methylation rate depends mainly on two parameters: the concentration of bioavailable Hg and the activity of methylating bacteria. Microbial populations need specific conditions to live and subsequently to produce MeHg. Several parameters have to be taken into account when assessing the productivity of a bacterial population, such as temperature, pH, redox potential, organic matter (OM), and sulfide (Bravo and Cosio, 2019; Paranjape and Hall, 2017).

In contrast, both biotic and abiotic degradations of MeHg are important processes in natural systems that regulate the concentration of MeHg in sediments and waters. Demethylation in natural environments occurs through biotic (mediated by numerous strains of aerobes and anaerobes) (Lu et al., 2017; Lu et al., 2016; Matilainen and Verta, 1995; Paranjape and Hall, 2017; Zhang and Planas, 1994) and abiotic processes such as photodegradation of MeHg, which is considered to be the main process of abiotic demethylation, and chemical degradation of MeHg, which is linked mainly to the selenoamino assisted degradation (Du et al., 2019; Paranjape and Hall, 2017).

I.3. Mercury methylation in the water column

All known microorganisms carrying the hgcAB gene cluster are obligate anaerobes (Gilmour et al., 2013), an observation that suggests an incompatibility between Hg methylation and water columns (in which anoxic conditions are rare), limiting the MeHg production zone of aquatic systems to anoxic bottom sediments. Early studies (Mason and Fitzgerald, 1990; Topping and Davies, 1981) showed the presence of MeHg in the marine water column. Many other studies - discussed in the next paragraph - have confirmed these findings both in marine and freshwater water columns. This apparent paradox has three possible explanations: 1) the presence of anoxic microenvironments along the water column that can sustain Hg methylation by obligate anaerobes; 2) different pathways for

Hg methylation beside the hgcAB pathway; and 3) the presence of the hgcAB methylation pathway inside microorganisms that are not obligate anaerobes (Bowman et al., 2019). The last two hypotheses remain to be tested, although some indications in their favor already exist. Gionfriddo et al. (2016) identified the microaerophilic bacterium Nitrospina as a potential methylator in Antarctic sea ice, which was supported by Villar et al. (2019) who identified Nitrospina as a likely key player in Hg methylation in the oxic subsurface of all oceans. Podar et al. (2015) found little to no evidence for the presence of the hgcAB gene cluster in the pelagic marine water column; interestingly, they found hgcA-like sequences in several metagenomes from the mesopelagic equatorial Pacific Ocean and the Southern Atlantic Ocean, which were also identified in Artic seawater by Bowman et al. (2019). Using polymerase chain reaction amplification and shotgun metagenomics, they did not find hgcAB in the Artic water column; instead, they identified Hg-cycling genes from the mer operon and hgcA-like paralogs. Munson et al. (2018) proposed that non-cellular or extracellular methylation and demethylation mechanisms, such as Hg-ligands, competing metals and particle-driven demethylation, could be of major importance in understanding the concentrations of MeHg in oligotrophic marine waters.

The hypothesis of a specific microenvironment in which Hg methylation could occur along the water column is supported by an increasing number of reports in both marine (Bianchi et al., 2018; Blum et al., 2013; Cossa et al., 2011; Lamborg et al., 2016; Lehnherr et al., 2011; Monperrus et al., 2007a; Monperrus et al., 2007b; Wang et al., 2012) and freshwater environments (Díez et al., 2016; Eckley and Hintelmann, 2006). As the literature agrees that particulate organic carbon (POC) is the main candidate for this ecological niche of methylating obligate anaerobes, we present a comprehensive review of the role of POC in Hg methylation in the following paragraphs.

I.3.1. Evidence from macro-environment observations

Evidence for the presence MeHg in oceanic water columns has been collected by several authors from every ocean and the Mediterranean Sea (Bowman et al., 2015; Bowman et al., 2016; Cossa et al., 2009; Cossa et al., 2011; Cossa et al., 2018; Kirk et al., 2008; Lehnherr et al., 2011; Mason and Fitzgerald, 1990; Mason and Fitzgerald, 1993; Sunderland et al., 2009; Topping and Davies, 1981; Wang et al., 2012; Wang et al., 2018), except for the Indian Ocean, which remains untested. The vast majority of the literature agrees on the importance of remineralization of OM in driving Hg methylation along water columns. Many pieces of evidence were collected and experiments have been carried out to test this hypothesis, e.g., Monperrus et al. (2007a); (2007b) conducted experiments in the marine and coastal waters of the Mediterranean Sea. They carried out several incubation experiments using isotopically enriched spikes of Hg and MeHg species (199Hg[II] and Me201Hg) to assess the relative importance of photochemical versus biological processes in Hg transformation

mechanisms. Their results show that Hg methylation takes place in the oxic surface seawater, especially in the lower euphotic zone where the photochemical processes (i.e., photodemethylation) are attenuated and the biomass concentration is at a maximum.

Similarly, Lehnherr et al. (2011) conducted several incubations in polar marine water, with samples from across the Canadian Artic Archipelago. They reported Hg methylation in the oxic waters, which was strongly related to POC abundance and microbial decomposition.

Cossa et al. (2009) produced a high-resolution vertical profile of MeHg concentrations in the Mediterranean Sea. Their results showed, for the first time, that within the most biologically active zone, the MeHg vertical profile followed a nutrient-like pattern. Following this evidence, the authors suggest that the in situ methylation of inorganic Hg is associated with the mineralization of OM.

Cossa et al. (2011) found some of the highest concentrations of MeHg among open ocean waters in Antarctic waters. They found that MeHg concentrations increased with depth at the 27 stations located between Tasmania and Antarctica. They suggested that Hg methylation results from phytoplankton blooms, which produce particles able to scavenge Hg from the subsurface waters and are subsequently used as substrate for methylating microorganisms in hypoxic zones along the water column. The link between the oxygen depleted zone (ODZ) and mineralization of OM as the main parameters that promote Hg methylation in marine water columns has been suggested by several authors in other parts of the world (Blum et al., 2013; Bowman et al., 2015; Bowman et al., 2016; Cossa et al., 2017; Cossa et al., 2018; Kim et al., 2017; Lamborg et al., 2016; Lamborg et al., 2008; Soerensen et al., 2018; Wang et al., 2012). Recently, Soerensen et al. (2018) found MeHg in hypoxic and anoxic waters at 2–6 and 30–55 times higher concentrations than in oxic water in the Baltic Sea, respectively. Their results suggest that concentrations of elemental Hg can be associated with redox conditions in the water column and are linked to the cycles of Fe and S, which in turn cause a highly dynamic speciation and bioavailability of Hg in the hypoxic zone. While phytoplankton mostly thrive in the oxic zone, Soerensen et al. (2018) also found that zooplankton are exposed to two to six times higher MeHg concentrations in hypoxic than in oxic water during summer, creating a dangerous input of MeHg into the food chain.

In contrast, other authors suggest that only the bacterial decomposition of POC has a real impact on Hg methylation in the marine water column. Malcolm et al. (2010) found no evidence correlating the oceanic oxygen deficient zone with increased Hg methylation, but did observe a strong link with the rate of biological decomposition of OM, an observation supported by the work of Sunderland et al. (2009) who found decreasing MeHg concentrations with increasing apparent oxygen utilization in the Eastern North Pacific. Moreover, Lamborg et al. (2008) showed that the highest MeHg concentration in the Black Sea was recorded at the top of the low oxygen zone, in contrast with the

study of Rosati et al. (2018), which identified the maximum MeHg peak in the permanently anoxic water of the Black Sea, suggesting that this layer is the major source of MeHg for the entire basin. Eckley and Hintelmann (2006) found increasing concentrations of MeHg along the water column of several Canadian lakes, where the seasonal variations in the oxycline create anoxic conditions. Their data showed a doubling of the concentration of MeHg at 80 cm above the sediment/water interface, which was oxic in July and became anoxic in September, and the occurrence of active methylation. They propose two possible explanations for this finding: 1) diffusion from the epilimnetic sediment via particles that resettle in other parts of the lake, and 2) methylation in the anoxic portion of the water column. The second hypothesis is consistent with the work of Rosati et al. (2018) in the permanent anoxic layer of the Black Sea. These authors conclude from their findings that in water bodies where oxygen depletion or absence at the bottom of the water column is induced by summer stratification, hypoxia-anoxia conditions could enhance MeHg production over areas much larger than originally thought, producing a significant amount of MeHg.

Despite some contradictory evidence regarding the importance of the ODZ in Hg methylation along marine water columns, POC mineralization appears to be a driving factor regulating MeHg production in the water column. POC, especially sinking particles, has been the leading candidate in determining the microenvironment in which Hg methylation takes place in the water column.

I.3.2. Evidence from microenvironment observations

In order for the settling particles to sustain Hg methylation, there is the need for an anoxic microenvironment inside the particle or the entire particle to be anoxic. In both cases, an Eh gradient should exist between the particle and external environment (i.e., the water column) resulting from the different redox conditions.

Alldredge and Cohen (1987) found a persistent oxygen and pH gradient in the microenvironment around marine snow. They used a calibrated oxygen microelectrode with a sensing tip of 2.5 μm on particles ranging from 1 to 4 mm. The oxygen was partially, but continuously, depleted within and around marine snow in the dark and at times completely depleted within large fecal pellets, creating anoxic microenvironments at the core of these particles where oxygen-free related processes could occur. Moreover, Glud et al. (2015) found evidence of existing anoxic microenvironments with high microbial activity inside copepod carcasses. Even in oxygen-saturated water, carcasses of *Calanus finmarchicus* had an anoxic interior that gradually expanded with decreasing ambient O2 levels. Following this evidence, Ortiz et al. (2015) designed an experiment in which marine settling particles were produced in a controlled microcosm using sieved estuarine sediment. The size of these particles was heterogeneous and spanned 0.2 μm to > 300 μm. Using isotopically enriched Hg spikes, these

authors measured the Hg methylation rates comparable to those measured in sediments, highlighting the possibility of methylation in marine snow and small particles in open ocean and coastal waters.

In a previous study in Lake Constance, Grossart and Simon (1993) studied the macroaggregates—named *lake snow*—and showed the similarities and differences between this and its marine equivalent. In terms of its abundance, chemical composition, settling velocity, microbial colonization, and bacterial production, *lake snow* is fairly similar to marine snow. In contrast, the formation of *lake snow* aggregates is mostly dependent on wind induced turbulences and presents differences in particle composition because of the differences between marine and freshwater plankton communities. Regardless, all of these results show that *lake snow*, similar to marine snow, represents an important site for OM mineralization, nutrient regeneration, and, potentially, Hg methylation.

As discussed above, settling particles are of great importance in the aquatic environment. Inputs of new particulate material via rivers, the resuspension of sediments from the lake (or sea) basin, and contributions originating from the in situ production of fresh OM from plants, algae, phyto- and zooplankton, and inorganic particles (silica from diatom frustules and carbonates from precipitation in hard water lakes) represent the main source of settling particles in aquatic environments (Blais and Kalff, 1995; Gardner et al., 1985). The presence of micro- and macroaggregates in the water column plays an important role in the cycles of nutrients and pollutants (Grossart and Simon, 1993; Ortiz et al., 2015; Wieland et al., 2001), owing to the mainly organic composition and high concentration of bacteria in these particles (Grossart and Simon, 1993; Simon et al., 2002).

All of these studies suggest the presence of a source of MeHg aside from sediment in freshwater/estuarine environments. Presently, there is no direct observation of Hg methylation in the settling particles in a lake water column. However, Díez et al. (2016), via sediment traps in Lake Geneva, found high MeHg concentrations in settling particles, likely as a result of SRB methylation activity within the oxic water column.

I.3.3. Evidence from biota

Finally, indirect evidence of the possible role of methylation within the water column include studies in freshwater environments highlighting the unrelated concentrations of MeHg in lake biota compared to the concentrations in sediment. Hammerschmidt and Fitzgerald (2006) compared a large dataset of MeHg concentrations in fish across the US. They found a weak correlation between MeHg in fish and parameters such as surface water pH, temperature, and wet atmospheric precipitations of sulfate; however, the levels of atmospheric Hg account for about two-thirds of the MeHg variation in fish. Moreover, Zhou et al. (2017) found declining temporal trends in MeHg concentrations in top predator fish in several US lakes from 2004 to 2015. Those trends were related to a decreasing regional atmospheric Hg emission rather than a lower concentration of MeHg in the sediment. Hodson et al.

(2014) found the highest MeHg concentrations in biota near the Canadian Saint Francis Lake tributaries and not near the most contaminated industrial site, suggesting that legacy Hg in surficial sediments is not bioavailable to aquatic biota.

Other important evidence was collected in both marine and freshwater environments in studies on the base of the trophic chain (i.e., phyto and zooplankton), coupling MeHg concentrations with parameters such as feeding ecology, size, species, etc. Kainz and Mazumder (2005) studied the zooplankton efficiency in retaining MeHg in several lakes on Vancouver Island, Canada. Using dietary lipid biomarkers, they found that MeHg concentrations were not significantly related to zooplankton taxonomy, but did have a strong direct correlation with zooplankton size. Their results suggest that macrozooplankton (>500 μm), the preferred size for planktivorous fish, are the most efficient at retaining and accumulating MeHg, and eventually incorporating it into the trophic chain.

Chiang et al. (2021) studied bio-magnification of MeHg in coastal food webs of Patagonian fjords and Antarctic Peninsula. They found that nearshore food webs show an increased MeHg bio-magnification compared to the off-shore ones. They conclude that this is probably due to the supply of freshwater, which increases the bioavailability of Hg for the base of the food web.

Wu et al. (2019) reviewed several works from different aquatic ecosystem on the bio-concentration and the bio-magnification of MeHg at the base of the food chain (phyto and zoo-plankton), to determine which process better predicts MeHg concentration in fish. They found that bio-concentration of MeHg in phytoplankton predicts 63% of the variability of MeHg concentrations in fish, while zooplanktivory diet did not appear to have a significant correlation with MeHg in fish.

Phytoplankton represents the entry point of Hg species into the food chain because of its faculty to scavenge Hg from the subsurface waters and transfer it deeper by settling after the cell death. Pickhardt and Fisher (2007) used 203Hg to compare inorganic Hg and MeHg uptake in five phytoplankton species (three eukaryotic and two prokaryotic) in two water bodies characterized by low and high dissolved organic carbon (DOC). For MeHg, they found concentrations 2 to 2.6 times higher in high DOC water than in low DOC water for eukaryotic cells, while the concentrations were similar in prokaryote cells. This increase can be explained by a difference in the Hg speciation between high and low DOC; the authors suggest that due to the greater abundance of the lipid-soluble, neutral methylmercury chloride complex CH3HgCl in high DOC waters, MeHg uptake could be enhanced. They also found a positive correlation between MeHg concentrations and the ratio of the surface area over volume of the cell, a correlation that is not present with inorganic mercury.

These results are consistent with the work of Zhang et al. (2020) who developed a global 3D simulation of MeHg in seawater and phyto and zooplankton based on the Massachusetts Institute of Technology global circulation model. Their model suggests that diatoms and Synechococcus spp. (a

picocyanobacteria) are the most important phytoplankton categories for the transfer of MeHg from seawater to herbivorous zooplankton, contributing 35% and 25%, respectively.

Moreover, a dietary analysis was carried out by Wu (2019) using stable isotopes and fatty acids to assess the role of the food source in Hg and MeHg biomagnification in six Swedish lakes. They found that terrestrial and algae diets together predicted more than 66% of the Hg variability in meso zooplankton (100–500 μm) and macro-zooplankton (>500 μm). Moreover, physicochemical parameters like pH and DOC were also correlated with Hg bioaccumulation, suggesting an influence of such parameters on mediating the impact of consuming different dietary sources.

Poste et al. (2019) conducted a comparative research between two contrasted boreal lakes in Norway characterized one by low amount of terrestrial OM (clear-water) and the other with high amount of terrestrial OM (brown-water). They determined zooplankton MeHg accumulation and dietary preferencess in both lakes. They found that high amount of terrestrial OM results in high concentrations of MeHg in water and zooplankton and reduces zooplankton dietary dependence on phytoplankton. This in turn reduces the quality of zooplankton as a feeding source (i.e. high MeHg concentrations) with effects on all trophic-levels.

Phytoplankton and zooplankton represent the first step in the pelagic trophic chain and are important vectors for MeHg biomagnification across the food web. The localization and characterization of their source of exposure to MeHg is an important step to understanding how MeHg enters the food web and is an essential information for every remediation project in a given environment.

I.3.4. Conceptual model of the short path of the food chain

The role of Hg methylation in settling particles in a limnic environment is schematized in figure I.1. Microorganism colonies in settling particles will begin to methylate Hg thanks to the redox gradient present inside the particles and their prevalent organic composition. The two production sites, represented by sediment (brown) and settling particles (green), create two different entry points into the trophic chain for MeHg, which in turn creates two different paths of MeHg biomagnification, represented in Figure 1 by the short and long path.

The importance of the short path can be seen in marine environments in which the greater depths make the transfer of MeHg from the sediment into the pelagic trophic chain less likely, making MeHg production from suspended and settling particles more important because it takes place where low-trophic-level biota, such as zooplankton, thrive, increasing their MeHg exposure and entering into the pelagic food web (Soerensen et al., 2018; Wang et al., 2018). While the bottom sediments of a shallow lake probably represent the main MeHg source of exposure for all the biota, a deep lake could behave similarly to an ocean in terms of separation between the long and short transfer paths, giving more importance to Hg methylation in the water column with respect to the exposure of biota. Nevertheless,

several different site-specific variables must be taken into account to adjust this model. Depth can function as a separator between the pelagic short path and the benthic long path, but sediment resuspension can transport MeHg produced in the bottom sediment to the upper layers of the lake via particles, mixing the MeHg contribution from epilimnetic production with that from the sediment (figure I.1). This resuspension from the bottom sediment can occur following several dynamics, the presence and effectiveness of which are site-specific: wind, tributaries, and density currents. Moreover, biotic and abiotic demethylation inside and around resuspended particles could occur, reducing the amount of MeHg that reaches the epilimnetic production zone. Finally, photoreduction of Hg to volatile Hg0 is an important reaction in removing bioavailable Hg before it reaches the MeHg production microenvironments. In natural waters, Hg photoreduction is known to increase with increasing content of DOC (Costa and Liss, 1999). This trend peaks at a Hg/DOC ratio of 1134 ng mg^{-1}, according to Wang et al. (2020), corresponding to the maximum photoreduction rate; further increase or decrease of this ratio will in turn decrease the Hg reduction rate. Studies on these dynamics and how they affect the system are needed to determine the relative importance between the short and long path in a given environment.

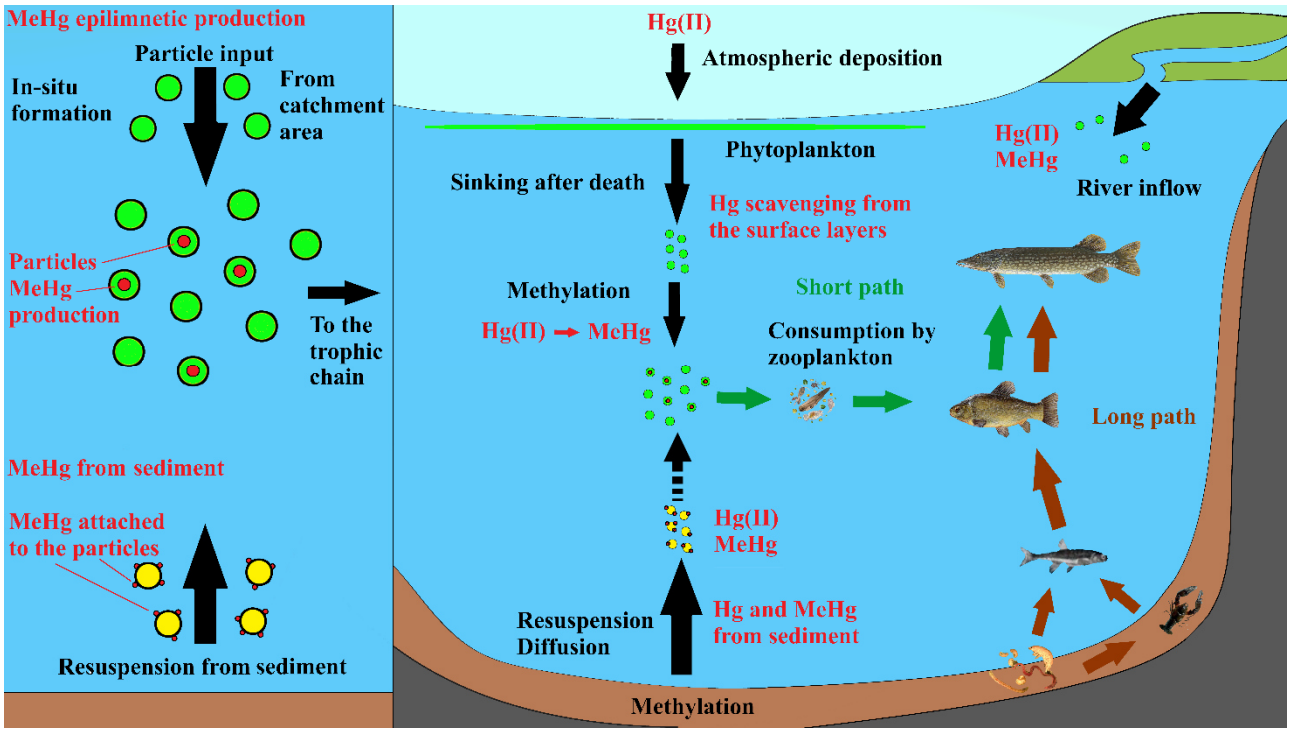

Figure I.1: Schematic summary of the role of suspended particles for Hg incorporation into the food chain in a freshwater environment. Settling particles (green circles) produce MeHg (red dots) at a shallower depth in respect to the classic source in the bottom sediments (brown), determining two different transfer paths in the trophic chain: short (green) and long (dark brown) paths. On the left, the epilimnetic production zone (settling particles) is represented in detail. Resuspended particles (yellow circles) can transport MeHg from the bottom sediment zone of methylation to the epilimnetic one, making it very difficult to quantify the relative importance of the two sources. However, the deeper a water body is, the less important becomes the effect of the sediment resuspension due to distance between the primary production zone to the bottom sediments, so depth can play a major role in separating these two sources of MeHg. Moreover, depth could affect the amount of MeHg that arrives into the surface layers from the long-path trophic chain, in turn giving more importance to epilimnetic methylation.

I.4. Summary and perspectives

This review highlights the oxic water column as another important site of MeHg production in deep lakes. The presence of settling particles in aquatic systems (lake and marine snow) is the key factor which permits Hg methylation in an environment otherwise incompatible with anoxic bacterial activity. The microenvironments that form around and inside these micro- and macroaggregates, coupled with their composition rich in OM, create the perfect substrate for methylators to proliferate. The existence of a source of MeHg inside the ecosystem in which the majority of macroinvertebrates and fish live is of great importance and should be further investigated and elucidated. While marine environments present a large and increasing literature on the subject, the same cannot be said of freshwater systems.

To address this knowledge gap, it is of great importance to identify and standardize sampling and analysis setups to create comparable data about THg and MeHg concentrations. Specifically, there is a need to design a sampling setup capable of recovering suspended and settling particles without creating any unwanted effects (e.g., change in redox conditions, increased temperature) that could interfere with the actual MeHg concentrations. An ideal sampling setup should consist of a system capable of sampling particles from the water column (e.g. an in-situ pump) and quickly transfer them onto a collecting device on which the particles can accumulate (e.g. filters). Afterward, the sample must be stored (possibly frozen to block biotic processes) and freeze-dried as soon as possible. In order to facilitate analysis, a colleting device from which the particles can be isolated could be very useful. Moreover, the collected samples should be used to determine the presence of the gene cluster hgcAB and to identify the microbial community present in the samples through shotgun or high-throughput sequencing metagenomics. Once the analytical setup has been identified, it should be applied to different systems in order to obtain a set of relevant data needed to elucidate this step in Hg cycling in the aquatic environment. Another knowledge gap that needs to be addressed is the quantification of the MeHg flux that goes from settling particles to low-trophic-level biota (e.g., zooplankton). This step is of major importance to quantify the real threat of this pelagic source of MeHg and to assess biomagnification rates. This could be realized in a macrocosm experiment with isotopic tracers to track MeHg inside various biota species.

I.5. References

Alldredge, A. L., and Cohen, Y., 1987, Can Microscale Chemical Patches Persist in the Sea? Microelectrode Study of Marine Snow, Fecal Pellets: Science, v. 235, no. 4789, p. 689-691.

AMAP/UNEP, 2013, Technical Background Report for the Global Mercury Assessment 2013. Arctic Monitoring and Assessment Programme, Oslo, Norway/UNEP Chemicals Branch, Geneva, Switzerland. vi + 263 pp.

-, 2015, Global Mercury Modelling: Update of Modelling Results in the Global Mercury Assessment 2013. Arctic Monitoring and Assessment Programme, Oslo, Norway/UNEP Chemicals Branch, Geneva, Switzerland. iv + 32 pp.

Benoit, J. M., Gilmour, C. C., Heyes, A., Mason, R. P., and Miller, C. L., 2003, Geochemical and biological controls over Methylmercury production and degradation in aquatic ecosystems, Biogeochemistry of Environmentally Important Trace Elements.

Bianchi, D., Weber, T. S., Kiko, R., and Deutsch, C., 2018, Global niche of marine anaerobic metabolisms expanded by particle microenvironments: Nature Geoscience, v. 11, no. 4, p. 263-268.

Blais, J. M., and Kalff, J., 1995, The influence of lake morphometry on sediment focusing: Limnology and Oceanography, v. 40, no. 3, p. 582-588.

Blum, J. D., Popp, B. N., Drazen, J. C., Anela Choy, C., and Johnson, M. W., 2013, Methylmercury production below the mixed layer in the North Pacific Ocean: Nature Geoscience, v. 6, no. 10, p. 879-884.

Bowman, K. L., Collins, R. E., Agather, A. M., Lamborg, C. H., Hammerschmidt, C. R., Kaul, D., Dupont, C. L., Christensen, G. A., and Elias, D. A., 2019, Distribution of mercury-cycling genes in the Arctic and equatorial Pacific Oceans and their relationship to mercury speciation: Limnology and Oceanography, p. lno.11310.

Bowman, K. L., Hammerschmidt, C. R., Lamborg, C. H., and Swarr, G., 2015, Mercury in the North Atlantic Ocean: The U.S. GEOTRACES zonal and meridional sections: Deep Sea Research Part II: Topical Studies in Oceanography, v. 116, p. 251-261.

Bowman, K. L., Hammerschmidt, C. R., Lamborg, C. H., Swarr, G. J., and Agather, A. M., 2016, Distribution of mercury species across a zonal section of the eastern tropical South Pacific Ocean (U.S. GEOTRACES GP16): Marine Chemistry, v. 186, p. 156-166.

Bravo, A. G., and Cosio, C., 2019, Biotic formation of methylmercury: A bio–physico–chemical conundrum: Limnology and Oceanography, p. lno.11366.

Bravo, A. G., Zopfi, J., Buck, M., Xu, J., Bertilsson, S., Schaefer, J. K., Poté, J., and Cosio, C., 2018, Geobacteraceae are important members of mercury-methylating microbial communities of sediments impacted by waste water releases: The ISME Journal, v. 12, no. 3, p. 802-812.

Chiang, G., Kidd, K. A., Díaz-Jaramillo, M., Espejo, W., Bahamonde, P., O'Driscoll, N. J., and Munkittrick, K. R., 2021, Methylmercury biomagnification in coastal aquatic food webs from western Patagonia and western Antarctic Peninsula: Chemosphere, v. 262.

Christensen, G. A., Gionfriddo, C. M., King, A. J., Moberly, J. G., Miller, C. L., Somenahally, A. C., Callister, S. J., Brewer, H., Podar, M., Brown, S. D., Palumbo, A. V., Brandt, C. C., Wymore, A. M., Brooks, S. C., Hwang, C., Fields, M. W., Wall, J. D., Gilmour, C. C., and Elias, D. A., 2019, Determining the Reliability of Measuring Mercury Cycling Gene Abundance with Correlations with Mercury and Methylmercury Concentrations: Environmental Science & Technology, v. 53, no. 15, p. 8649-8663.

Compeau, G. C., and Bartha, R., 1985, Sulfate-Reducing Bacteria: Principal Methylators of Mercury in Anoxic Estuarine Sedimentt: APPL. ENVIRON. MICROBIOL., v. 50, no. 2, p. 498-501.

Correia, R. R. S., and Guimarães, J. R. D., 2017, Mercury methylation and sulfate reduction rates in mangrove sediments, Rio de Janeiro, Brazil: The role of different microorganism consortia: Chemosphere, v. 167, p. 438-443.

Cossa, D., Averty, B., and Pirrone, N., 2009, The origin of methylmercury in open Mediterranean waters: Limnology and Oceanography, v. 54, no. 3, p. 837-844.

Cossa, D., Durrieu de Madron, X., Schäfer, J., Lanceleur, L., Guédron, S., Buscail, R., Thomas, B., Castelle, S., and Naudin, J.-J., 2017, The open sea as the main source of methylmercury in the water column of the Gulf of Lions (Northwestern Mediterranean margin): Geochimica et Cosmochimica Acta, v. 199, p. 222-237.

Cossa, D., Heimbürger, L.-E., Lannuzel, D., Rintoul, S. R., Butler, E. C. V., Bowie, A. R., Averty, B., Watson, R. J., and Remenyi, T., 2011, Mercury in the Southern Ocean: Geochimica et Cosmochimica Acta, v. 75, no. 14, p. 4037-4052.

Cossa, D., Heimbürger, L.-E., Pérez, F. F., García-Ibáñez, M. I., Sonke, J. E., Planquette, H., Lherminier, P., Boutorh, J., Cheize, M., Menzel Barraqueta, J. L., Shelley, R., and Sarthou, G., 2018, Mercury distribution and transport in the North Atlantic Ocean along the GEOTRACES-GA01 transect: Biogeosciences, v. 15, no. 8, p. 2309-2323.

Costa, M., and Liss, P. S., 1999, Photoreduction of mercury in sea water and its possible
implications for Hg0 air–sea fluxes: Marine Chemistry, v. 68, p. 87-95.

Díez, E. G., Loizeau, J.-L., Cosio, C., Bouchet, S., Adatte, T., Amouroux, D., and Bravo, A. G., 2016, Role of Settling Particles on Mercury Methylation in the Oxic Water Column of Freshwater Systems: Environmental Science & Technology, v. 50, no. 21, p. 11672-11679.

Du, H., Ma, M., Igarashi, Y., and Wang, D., 2019, Biotic and Abiotic Degradation of Methylmercury in Aquatic Ecosystems: A Review: Bulletin of Environmental Contamination and Toxicology, v. 102, no. 5, p. 605-611.

Eckley, C. S., and Hintelmann, H., 2006, Determination of mercury methylation potentials in the water column of lakes across Canada: Science of The Total Environment, v. 368, no. 1, p. 111-125.

FAO, 2018, Meeting the sustainable development goals.

Fitzgerald, W. F., and Lamborg, C. H., 2014, Geochemistry of Mercury in the Environment, In: Treatise on Geochemistry (Second Edition): Oxford, Elsevier,, p. 91-129.

Fleming, E. J., Mack, E. E., Green, P. G., and Nelson, D. C., 2006, Mercury Methylation from Unexpected Sources: Molybdate-Inhibited Freshwater Sediments and an Iron-Reducing Bacterium: Applied and Environmental Microbiology, v. 72, no. 1, p. 457-464.

Gallorini, A., and Loizeau, J.-L., 2021, Mercury methylation in oxic aquatic macro-environments: a review: Journal of Limnology.

Gardner, W. D., Southard, J. B., and Hollister, C. D., 1985, Sedimentation, resuspension and chemistry of particles in the northwest Atlantic: Marine Geology, v. 65, no. 3-4, p. 199-242.

Gilmour, C. C., Podar, M., Bullock, A. L., Graham, A. M., Brown, S. D., Somenahally, A. C., Johs, A., Hurt, R. A., Bailey, K. L., and Elias, D. A., 2013, Mercury Methylation by Novel Microorganisms from New Environments: Environmental Science & Technology, v. 47, no. 20, p. 11810-11820.

Gionfriddo, C. M., Tate, M. T., Wick, R. R., Schultz, M. B., Zemla, A., Thelen, M. P., Schofield, R., Krabbenhoft, D. P., Holt, K. E., and Moreau, J. W., 2016, Microbial mercury methylation in Antarctic sea ice: Nature Microbiology, v. 1, no. 10, p. 16127.

Glud, R. N., Grossart, H.-P., Larsen, M., Tang, K. W., Arendt, K. E., Rysgaard, S., Thamdrup, B., and Gissel Nielsen, T., 2015, Copepod carcasses as microbial hot spots for pelagic denitrification: Copepod carcasses and denitrification: Limnology and Oceanography, v. 60, no. 6, p. 2026-2036.

Grossart, H.-P., and Simon, M., 1993, Limnetic macroscopic organic aggregates (lake snow): Occurrence, characteristics, and microbial dynamics in Lake Constance: Limnology and Oceanography, v. 38, no. 3, p. 532-546.

Hammerschmidt, C. R., and Fitzgerald, W. F., 2006, Methylmercury in Freshwater Fish Linked to Atmospheric Mercury Deposition: Environmental Science & Technology, v. 40, no. 24, p. 7764-7770.

Hodson, P. V., Norris, K., Berquist, M., Campbell, L. M., and Ridal, J. J., 2014, Mercury concentrations in amphipods and fish of the Saint Lawrence River (Canada) are unrelated to concentrations of legacy mercury in sediments: Science of The Total Environment, v. 494-495, p. 218-228.

Kainz, M., and Mazumder, A., 2005, Effect of Algal and Bacterial Diet on Methyl Mercury Concentrations in Zooplankton: Environmental Science & Technology, v. 39, no. 6, p. 1666-1672.

Kim, H., Soerensen, A. L., Hur, J., Heimbürger, L.-E., Hahm, D., Rhee, T. S., Noh, S., and Han, S., 2017, Methylmercury Mass Budgets and Distribution Characteristics in the Western Pacific Ocean: Environmental Science & Technology, v. 51, no. 3, p. 1186-1194.

King, J. K., Kostka, J. E., Frisher, M. E., and Saunders, F. M., 2000, Sulfate-Reducing Bacteria Methylate Mercury at Variable Rates in Pure Culture and in Marine Sediments: Applied and Environmental Microbiology, v. 66, no. 6, p. 2430-2437.

Kirk, J. L., St. Louis, V. L., Hintelmann, H., Lehnherr, I., Else, B., and Poissant, L., 2008, Methylated Mercury Species in Marine Waters of the Canadian High and Sub Arctic: Environmental Science & Technology, v. 42, no. 22, p. 8367-8373.

Lamborg, C. H., Hammerschmidt, C. R., and Bowman, K. L., 2016, An examination of the role of particles in oceanic mercury cycling: Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, v. 374, no. 2081, p. 20150297.

Lamborg, C. H., Yiğiterhan, O., Fitzgerald, W. F., Balcom, P. H., Hammerschmidt, C. R., and Murray, J., 2008, Vertical distribution of mercury species at two sites in the Western Black Sea: Marine Chemistry, v. 111, no. 1-2, p. 77-89.

Lehnherr, I., St. Louis, V. L., Hintelmann, H., and Kirk, J. L., 2011, Methylation of inorganic mercury in polar marine waters: Nature Geoscience, v. 4, no. 5, p. 298-302.

Lu, X., Gu, W., Zhao, L., Haque, M. F. U., DiSpirito, A. A., Semrau, J. D., and Gu, B., 2017, Methylmercury uptake and degradation by methanotrophs: SCIENCE ADVANCES, p. 6.

Lu, X., Liu, Y., Johs, A., Zhao, L., Wang, T., Yang, Z., Lin, H., Elias, D. A., Pierce, E. M., Liang, L., Barkay, T., and Gu, B., 2016, Anaerobic Mercury Methylation and Demethylation by Geobacter bemidjiensis Bem: Environmental Science & Technology, v. 50, no. 8, p. 4366-4373.

Malcolm, E. G., Schaefer, J. K., Ekstrom, E. B., Tuit, C. B., Jayakumar, A., Park, H., Ward, B. B., and Morel, F. M. M., 2010, Mercury methylation in oxygen deficient zones of the oceans: No evidence for the predominance of anaerobes: Marine Chemistry, v. 122, no. 1-4, p. 11-19.

Mason, R. P., and Fitzgerald, W. F., 1990, Alkylmercury species in the equatorial Pacific: Nature, v. 347, no. 6292, p. 457-459.

Mason, R. P., and Fitzgerald, W. F., 1993, The distribution and biogeochemical cycling of mercury in the equatorial Pacific Ocean: Deep Sea Research Part I: Oceanographic Research Papers, v. 40, no. 9, p. 1897-1924.

Matilainen, T., and Verta, M., 1995, Mercury methylation and demethylation in aerobic surface waters: Canadian Journal of Fisheries and Aquatic Sciences, v. 52, no. 8, p. 1597-1608.

Monperrus, M., Tessier, E., Amouroux, D., Leynaert, A., Huonnic, P., and Donard, O. F. X., 2007a, Mercury methylation, demethylation and reduction rates in coastal and marine surface waters of the Mediterranean Sea: Marine Chemistry, v. 107, no. 1, p. 49-63.

Monperrus, M., Tessier, E., Point, D., Vidimova, K., Amouroux, D., Guyoneaud, R., Leynaert, A., Grall, J., Chauvaud, L., Thouzeau, G., and Donard, O. F. X., 2007b, The biogeochemistry of mercury at the sediment–water interface in the Thau Lagoon. 2. Evaluation of mercury methylation potential in both surface sediment and the water column: Estuarine, Coastal and Shelf Science, v. 72, no. 3, p. 485-496.

Munson, K. M., Lamborg, C. H., Boiteau, R. M., and Saito, M. A., 2018, Dynamic mercury methylation and demethylation in oligotrophic marine water: Biogeosciences, v. 15, no. 21, p. 6451-6460.

Nascimento, A. M. A., and Chartone-Souza, E., 2003, Operon mer: Bacterial resistance to mercury and potential for bioremediation of contaminated environments: Genetics and Molecular Research, p. 10.

Ortiz, V. L., Mason, R. P., and Evan Ward, J., 2015, An examination of the factors influencing mercury and methylmercury particulate distributions, methylation and demethylation rates in laboratory-generated marine snow: Marine Chemistry, v. 177, p. 753-762.

Pak, K.-R., and Bartha, R., 1998, Mercury Methylation and Demethylation in Anoxic Lake Sediments and by Strictly Anaerobic Bacteria, v. 64, no. 3, p. 1013-1017.

Paranjape, A. R., and Hall, B. D., 2017, Recent advances in the study of mercury methylation in aquatic systems: FACETS, v. 2, no. 1, p. 85-119.

Parks, J. M., Johs, A., Podar, M., Bridou, R., Hurt, R. A., Smith, S. D., Tomanicek, S. J., Qian, Y., Brown, S. D., Brandt, C. C., Palumbo, A. V., Smith, J. C., Wall, J. D., Elias, D. A., and Liang, L., 2013, The Genetic Basis for Bacterial Mercury Methylation: Science, v. 339, no. 6125, p. 1332-1335.

Peterson, B. D., McDaniel, E. A., Schmidt, A. G., Lepak, R. F., Janssen, S. E., Tran, P. Q., Marick, R. A., Ogorek, J. M., DeWild, J. F., Krabbenhoft, D. P., and McMahon, K. D., 2020, Mercury Methylation Genes Identified across Diverse Anaerobic Microbial Guilds in a Eutrophic Sulfate-Enriched Lake: Environmental Science & Technology, v. 54, no. 24, p. 15840-15851.

Pickhardt, P. C., and Fisher, N. S., 2007, Accumulation of Inorganic and Methylmercury by Freshwater Phytoplankton in Two Contrasting Water Bodies: Environmental Science & Technology, v. 41, no. 1, p. 125-131.

Podar, M., Gilmour, C. C., Brandt, C. C., Soren, A., Brown, S. D., Crable, B. R., Palumbo, A. V., Somenahally, A. C., and Elias, D. A., 2015, Global prevalence and distribution of genes and microorganisms involved in mercury methylation: Science Advances, v. 1, no. 9, p. e1500675-e1500675.

Poste, A. E., Skaar Hoel, C., Andersen, T., Arts, M. T., Færøvig, P.-J., and Borgå, K., 2019, Terrestrial organic matter increases zooplankton methylmercury accumulation in a brown-water boreal lake: Science of the Total Environment, p. 10.

Qian, C., Chen, H., Johs, A., Lu, X., An, J., Pierce, E. M., Parks, J. M., Elias, D. A., Hettich, R. L., and Gu, B., 2018, Quantitative Proteomic Analysis of Biological Processes and Responses of the Bacterium Desulfovibrio desulfuricans ND132 upon Deletion of Its Mercury Methylation Genes: PROTEOMICS, v. 18, no. 17, p. 1700479.

Regnell, O., and Watras, C. J., 2019, Microbial Mercury Methylation in Aquatic Environments: A Critical Review of Published Field and Laboratory Studies: Environmental Science & Technology, v. 53, no. 1, p. 4-19.

Rosati, G., Heimbürger, L. E., Melaku Canu, D., Lagane, C., Laffont, L., Rijkenberg, M. J. A., Gerringa, L. J. A., Solidoro, C., Gencarelli, C. N., Hedgecock, I. M., De Baar, H. J. W., and Sonke, J. E., 2018, Mercury in the Black Sea: New Insights From Measurements and Numerical Modeling: Global Biogeochemical Cycles, v. 32, no. 4, p. 529-550.

Schaefer, J. K., Rocks, S. S., Zheng, W., Liang, L., Gu, B., and Morel, F. M. M., 2011, Active transport, substrate specificity, and methylation of Hg(II) in anaerobic bacteria: Proceedings of the National Academy of Sciences, v. 108, no. 21, p. 8714-8719.

Selin, N. E., 2014, Global change and mercury cycling: Challenges for implementing a global mercury treaty: Global change and mercury cycling: Environmental Toxicology and Chemistry, v. 33, no. 6, p. 1202-1210.

Si, Y., Zou, Y., Liu, X., Si, X., and Mao, J., 2015, Mercury methylation coupled to iron reduction by dissimilatory iron-reducing bacteria: Chemosphere, v. 122, p. 206-212.

Simon, M., Grossart, H., Schweitzer, B., and Ploug, H., 2002, Microbial ecology of organic aggregates in aquatic ecosystems: Aquatic Microbial Ecology, v. 28, p. 175-211.

Soerensen, A. L., Schartup, A. T., Skrobonja, A., Bouchet, S., Amouroux, D., Liem-Nguyen, V., and Björn, E., 2018, Deciphering the Role of Water Column Redoxclines on Methylmercury Cycling Using Speciation Modeling and Observations From the Baltic Sea: Global Biogeochemical Cycles, v. 32, no. 10, p. 1498-1513.

Sunderland, E. M., Krabbenhoft, D. P., Moreau, J. W., Strode, S. A., and Landing, W. M., 2009, Mercury sources, distribution, and bioavailability in the North Pacific Ocean: Insights from data and models: MERCURY IN THE NORTH PACIFIC OCEAN: Global Biogeochemical Cycles, v. 23, no. 2, p. n/a-n/a.

Sunderland, E. M., and Selin, N. E., 2013, Future trends in environmental mercury concentrations: implications for prevention strategies: Environmental Health, v. 12, no. 1.

Topping, G., and Davies, I. M., 1981, Methylmercury production in the marine water column: Nature, v. 290, no. 5803, p. 243-244.

Ullrich, S. M., Tanton, T. W., and Abdrashitova, S. A., 2001, Mercury in the Aquatic Environment: A Review of Factors Affecting Methylation: Critical Reviews in Environmental Science and Technology, v. 31, no. 3, p. 241-293.

Villar, E., Cabrol, L., and Heimbürger-Boavida, L.-E., 2019, Widespread microbial mercury methylation genes in the global ocean: Genomics.

Wang, F., Macdonald, R. W., Armstrong, D. A., and Stern, G. A., 2012, Total and Methylated Mercury in the Beaufort Sea: The Role of Local and Recent Organic Remineralization: Environmental Science & Technology, v. 46, no. 21, p. 11821-11828.

Wang, F., Outridge, P. M., Feng, X., Meng, B., Heimbürger-Boavida, L.-E., and Mason, R. P., 2019, How closely do mercury trends in fish and other aquatic wildlife track those in the atmosphere? – Implications for evaluating the effectiveness of the Minamata Convention: Science of The Total Environment, v. 674, p. 58-70.

Wang, K., Munson, K. M., Beaupré-Laperrière, A., Mucci, A., Macdonald, R. W., and Wang, F., 2018, Subsurface seawater methylmercury maximum explains biotic mercury concentrations in the Canadian Arctic: Scientific Reports, v. 8, no. 1, p. 14465.

Wang, Z., Fei, Z., Wu, Q., and Yin, R., 2020, Evaluation of the effects of Hg/DOC ratios on the reduction of Hg(II) in lake water: Chemosphere, v. 253, p. 126634.

Wieland, E., Lienemann, P., Bollhalder, S., Lück, A., and Santschi, P. H., 2001, Composition and transport of settling particles in Lake Zurich: relative importance of vertical and lateral pathways:: Aquatic Sciences, v. 63, no. 2, p. 123-149.

Wu, P., 2019, The importance of bioconcentration into the pelagic food web base for methylmercury biomagnification: A meta-analysis: Science of the Total Environment, p. 11.

Wu, P., Kainz, M., Åkerblom, S., Bravo, A. G., Sonesten, L., Branfireun, B., Deininger, A., Bergström, A.-K., and Bishop, K., 2019, Terrestrial diet influences mercury bioaccumulation in zooplankton and macroinvertebrates in lakes with differing dissolved organic carbon concentrations: Science of The Total Environment, v. 669, p. 821-832.

Zhang, L., and Planas, D., 1994, Biotic and abiotic mercury methylation and demethylation in sediments: Bulletin of Environmental Contamination and Toxicology, v. 52, no. 5.

Zhang, Y., Soerensen, A. L., Schartup, A. T., and Sunderland, E. M., 2020, A global model for methylmercury formation and uptake at the base of marine food webs: Global Biogeochemical Cycles.

Zhou, C., Cohen, M. D., Crimmins, B. A., Zhou, H., Johnson, T. A., Hopke, P. K., and Holsen, T. M., 2017, Mercury Temporal Trends in Top Predator Fish of the Laurentian Great Lakes from 2004 to 2015: Are Concentrations Still Decreasing?: Environmental Science & Technology, v. 51, no. 13, p. 7386-7394.

Chapter II

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. Chapter II will develop the first module of the project where this thesis took place. It is also the main core of the discussion of MeHg in the oxic water column of limnic environments presenting new data of MeHg and THg concentrations in the *lake snow* of a deep peri-alpine lake (Lake Geneva, Switzerland-France).

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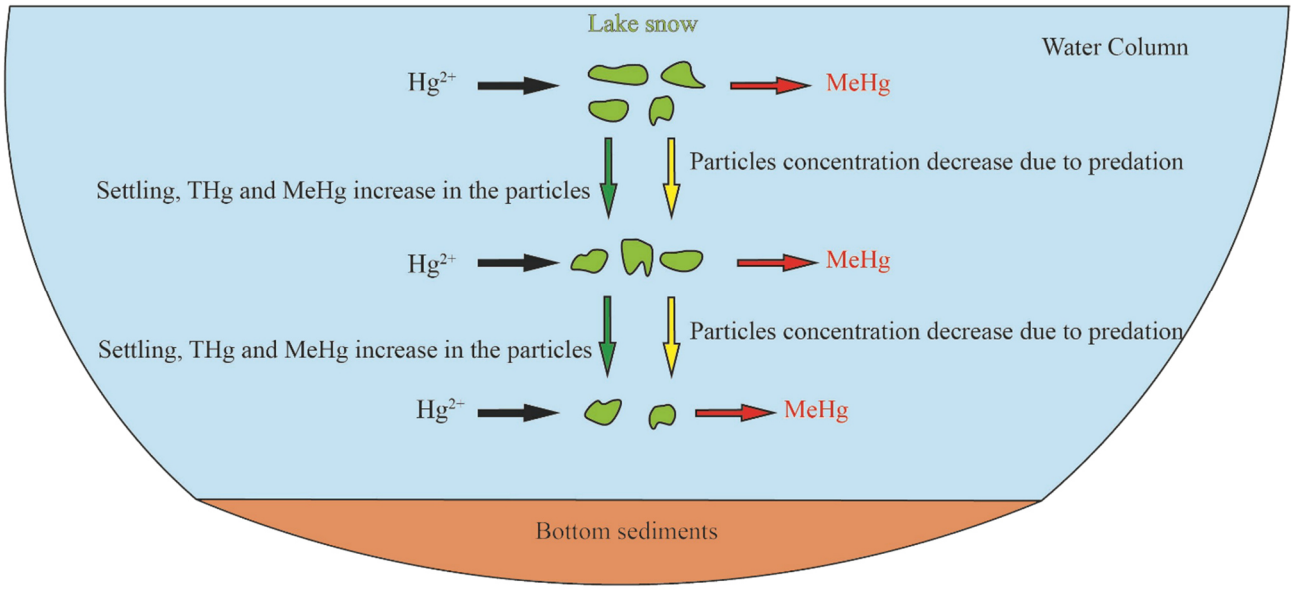
Lake snow as a mercury methylation micro-environment in the oxic water column of Lake Geneva

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.



II.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 CH3Hg+ (methylmercury, MeHg). MeHg is produced in aquatic environments by several microorganisms such as sulfate-reducing bacteria (SRB) (Compeau & Bartha, 1985, King et al., 2000), strains of methanogens (Parks et al., 2013, Podar et al., 2015), iron-reducing bacteria (Fleming et al., 2006, Si et al., 2015, Correia & 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 & Loizeau, 2021) and references 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 & Davies, 1981, Mason & Fitzgerald, 1990, Eckley & Hintelmann, 2006, Monperrus, Tessier, Amouroux, et al., 2007, Monperrus, Tessier, Point, et al., 2007, 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, Alldredge & 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 & 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 & 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.

II.2. Study area

Lake Geneva (figure II.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 km3 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 collected and processed rapidly in order to reduce any bias.

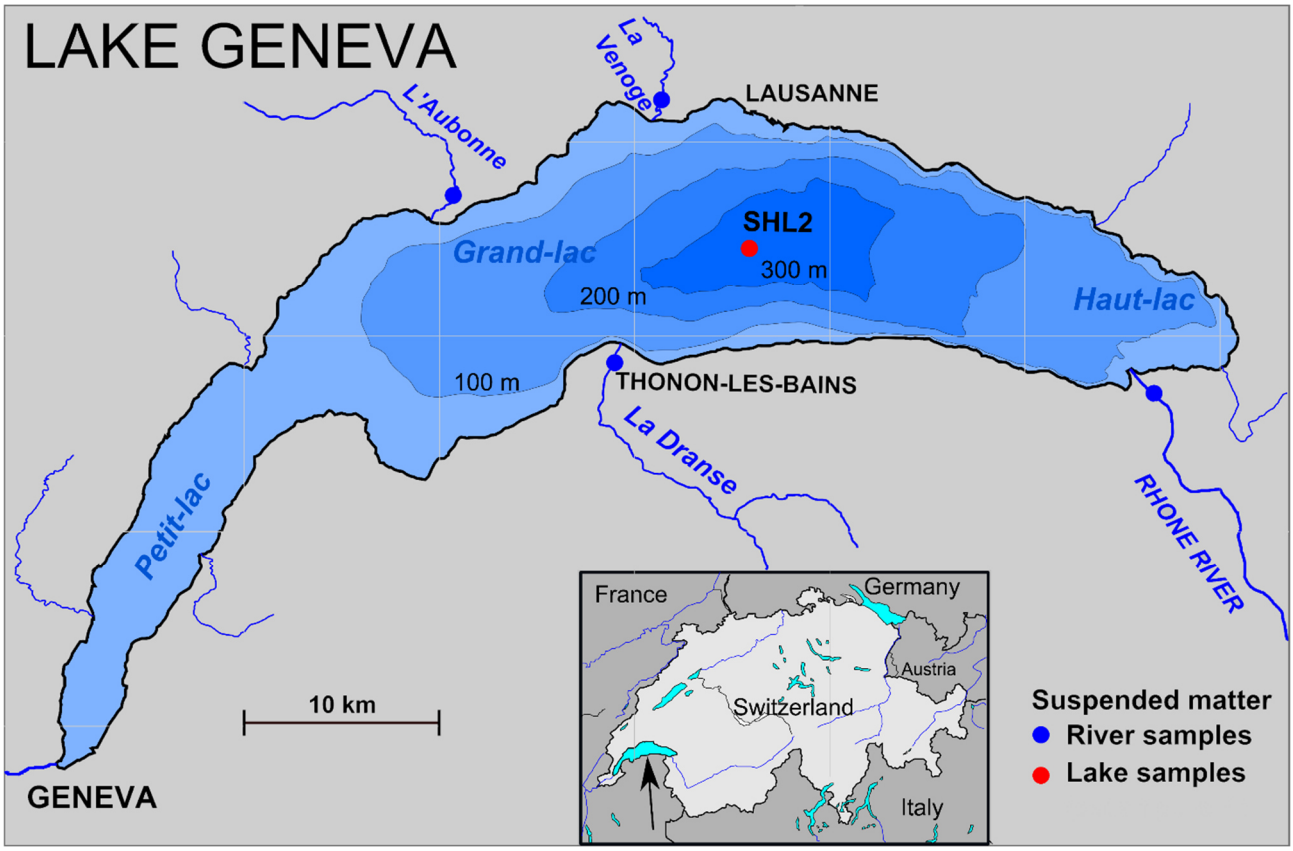


Figure II.1: Map of Lake Geneva with the positions of the sampling points. Inset. Map of Switzerland with Lake Geneva location (arrow).

II.3. Material and Methods

II.3.1. Sampling setups

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, figure II.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) (figure II.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 (Sterlitech, 304 SS) mounting a large diameter glass fiber filter (Sterlitech, 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

one hour 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 Seabird 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, Cossa et al., 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 (100m) 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 Rovex (U45E) submerged pump fed a Westfalia Separator centrifuge (KA 2-06-075) with a constant water flow for at least two hours. 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 concentration 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.

II.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 references 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/HNO_3) 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 hours) 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 references material used in the THg analysis (IAEA - 450) showed between 92.6% ± 2.4% to 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 references 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% to 96.3% ± 1.3%.

MeHg analysis on suspended particles were carried out using a HNO_3 leaching/CH_2Cl_2 extraction method (Liu et al., 2012). The solution was analyzed following the EPA Method 1631 (US-EPA, 2002). The certified references material used in the MeHg analysis (ERM-CC580, European Commission) showed between 91.8% ± 5.2% to 98.7% ± 1.6% recovery.

Filtrations 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% to 96.6% ± 1.0%.

The percentage of OM in the river particles was determined via loss on ignition (L.O.I.) heating 1 g of sample at 550 °C for one hour (Heiri et al., 2001).

II.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., 2018, Díez et al., 2016), 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.

II.4. Results

II.4.1. Ancillary parameters and particles characterization

The vertical profiles of dissolved oxygen and chlorophyll-a (figure II.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 to 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 to 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 to 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 (figure II.S1). The hypolimnion always showed the lower concentrations of chloropyll-a with values ranging from 0.04 to 0.39 mg/m3.

The particles concentration in the water column of Lake Geneva (figure II.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 values of 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 & Berninger, 1984). Microscope observations of the filters from the lake sampling (figure II.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 (figure II.S2). The values showed in April are coherent with results published by CIPEL (“Commission internationale pour la protection des eaux du Léman”) 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 figure II.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 (figure II.S3 and II.S4), with presence of calcite and iron oxides as the main components (figure II.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 (figure II.S5). This is linked to the Rhone glacial source, especially during the high flow due to snow and ice melt.

II.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 428.3 ± 25.4 ng/g (figure II.2-A) and from 0.48 ± 0.09 ng/g to 9.61 ± 0.67 ng/g (figure II.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.

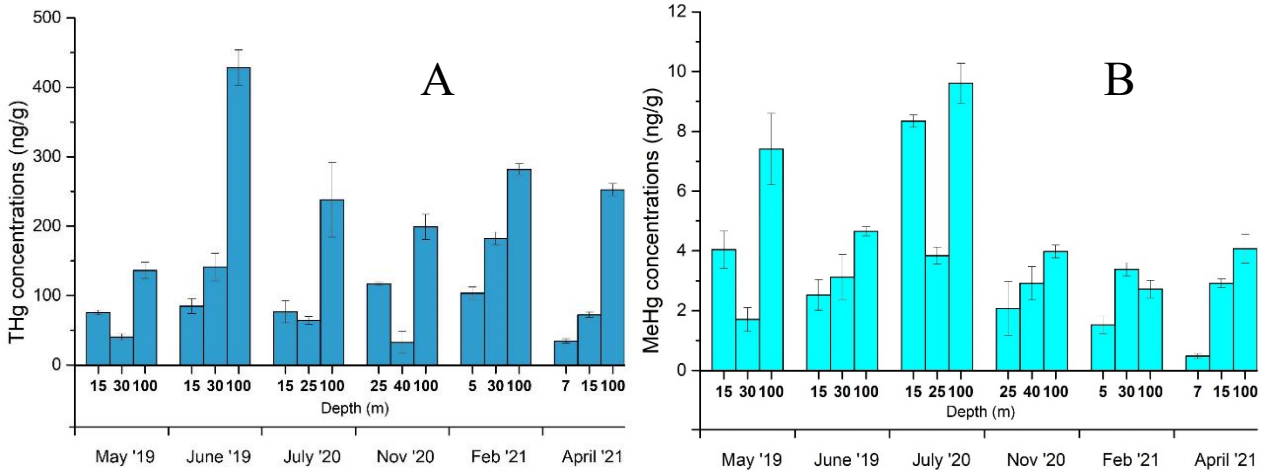


Figure II.2: Concentrations of THg (A) and MeHg (B) in the suspended particles of Lake Geneva sampled at SHL2 during different seasons at different depths.

II.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 (figure II.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 (figure II.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 (figure II.3).

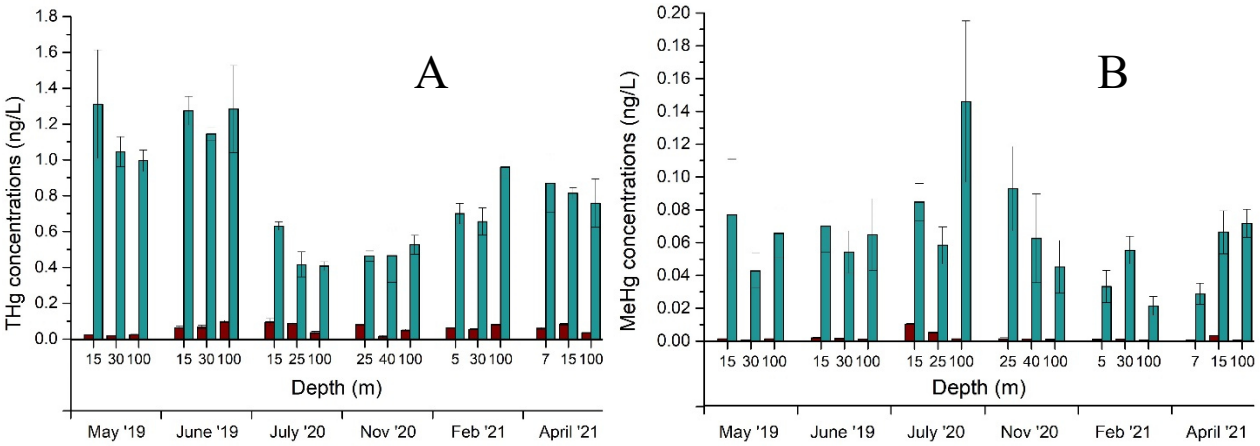


Figure II.3: THg (A) and MeHg (B) concentrations in filtered water samples (green) and attached to the suspended particles (brown).

The very low particles 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 (figure II.2) and the decreasing of particles concentrations (figure II.S2) with depth.

II.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 (figure II.4-A) and from 0.30 ± 0.08 ng/g to 2.41 ± 0.14 ng/g respectively (figure II.4-B).

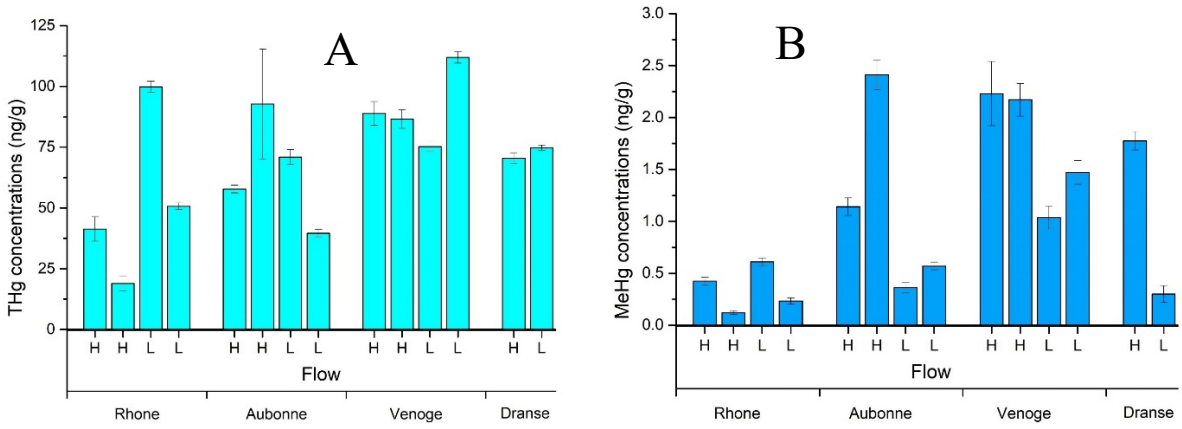


Figure II.4: THg (A) and MeHg (B) concentrations in the lake tributaries at high (H) and low (L) flow regimes.

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 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 II.S1 additional data regarding the flow rate and particles load for the sampled rivers.

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the sampling. In table II.S1 additional data regarding the flow rate and particles load for the sampled rivers.

II.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.

II.4.6. Partitioning coefficient calculation

The partitioning coefficient (K_d^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).

$$K_d^p = \frac{[Hg]_P}{[Hg]_{FW}} * \frac{1}{Cp} \quad (1)$$

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 Cp is the particle concentration (kg/L).

The $Log\ K_d^p$ 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 (figure II.S8) expressing the strong affinity of THg for solids.

II.5. Discussion

II.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) (figure II.S9 and II.S10); the THg and MeHg concentrations were plotted against the OM content (figure II.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 (Géoportail) (figure II.S11).

The Rhone River is the only river showing a direct linear correlation between THg and OM (R^2 = 0.907, p-value = 0.05; n = 4 Figure II.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 (Figure II.5-A). This behavior is probably the result of two main factors: i) the land use of the Aubonne watershed (figure II.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 (figure II.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 figure II.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 (figure II.S9), especially in their upstream portions because of their proximity. Therefore, precipitations washing similar catchment areas produce similar THg and OM values.

Regarding MeHg (figure II.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 (figure II.4-A) in both flow regimes, and low MeHg concentrations (figure II.4-B) during the low flow period. This is probably due to the similarity of their respective watersheds (figure II.S9- II.S11).

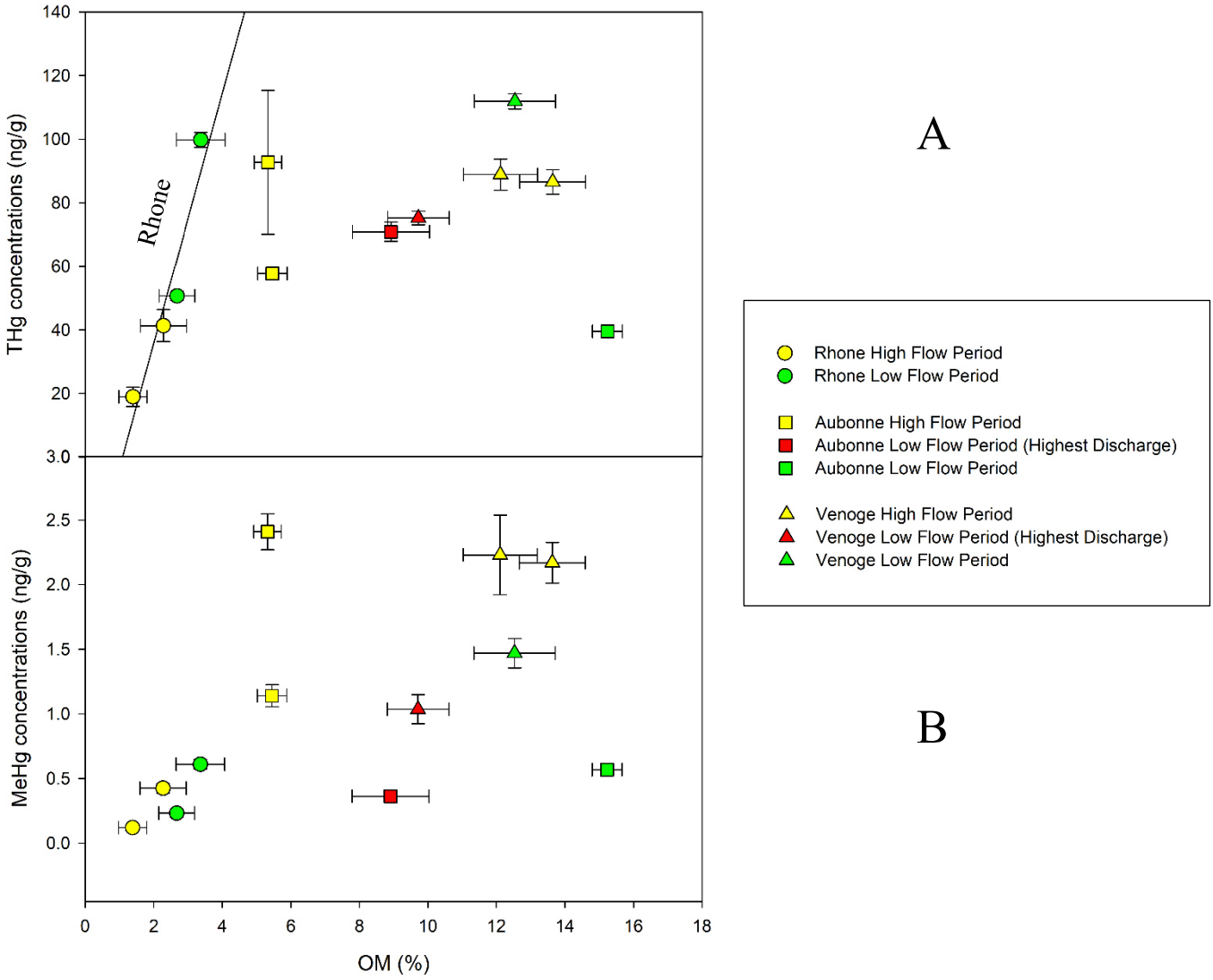


Figure II.5: OM percentages vs THg (A) and MeHg (B) in the particles collected from the rivers. The Rhone is the only river that showed a significant (r^2=0.91) regression line for THg.

II.5.2. Hg in the water column of Lake Geneva

II.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 (figure II.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 & 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 (figure II.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 could 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 & 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 (figure II.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 0.5 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 ($p < 0.05$) (figure II.S14), highlighting water column particles as an important component of MeHg cycling in this lacustrine system.

II.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 (figure II.6) shows that suspended particles appear to be an important production site that outcompete bottom sediments.

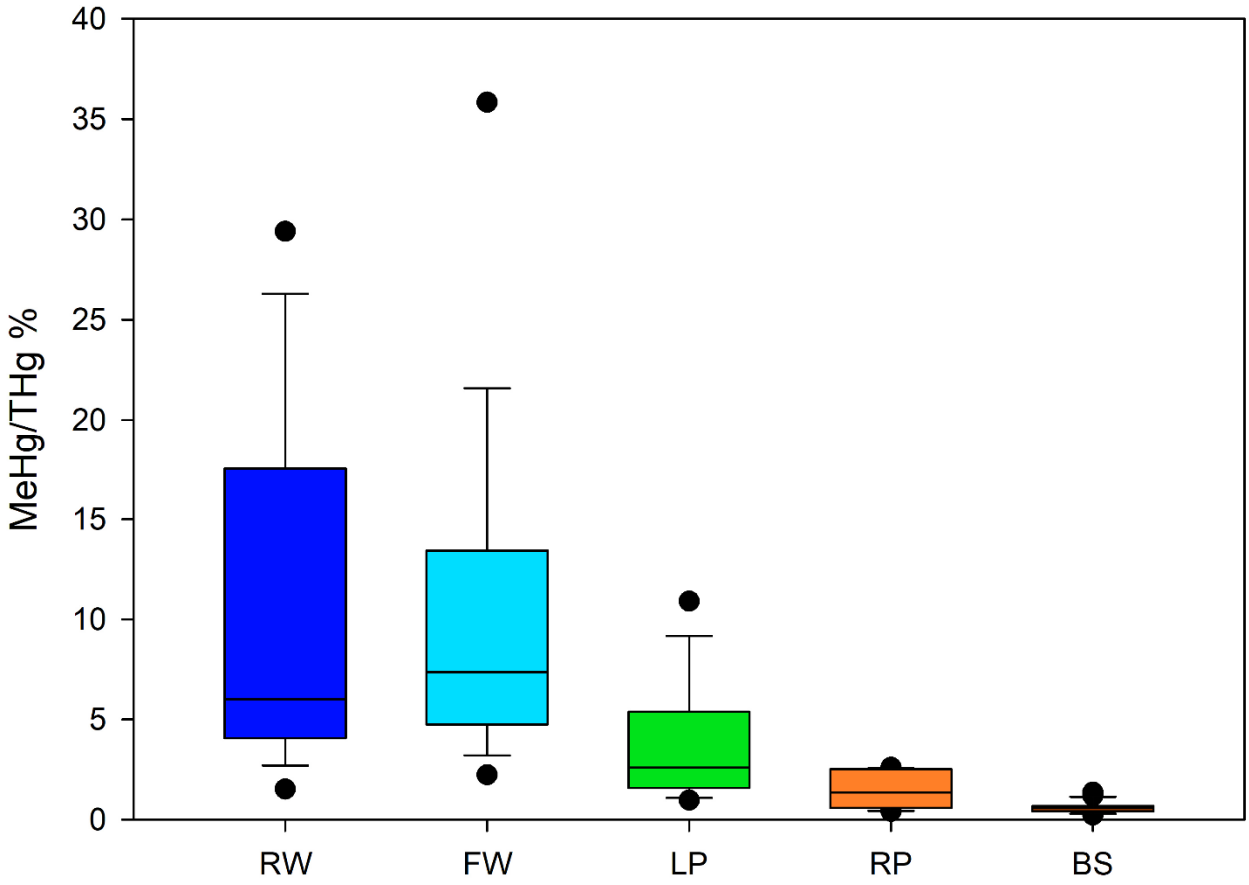


Figure II.6: Box plots of MeHg/THg ratios for several compartments. Raw (RW) and filtered (FW) water of the lake water column, lake (LP) and rivers (RP) particles and bottom sediments (BS) from Díez et al. (2016).

Figure II.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 & 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 (Dipasquale 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 & 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 lower than our average filtered MeHg concentration (0.063 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 $Log\ K_d^p$ THg for settling particles (≈ 5) and for deposited sediments (≈1).

The conceptual model in figure II.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 zooplankton and fish (Miracle, 1974, Finlay & Berninger, 1984, Rienmann, 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).

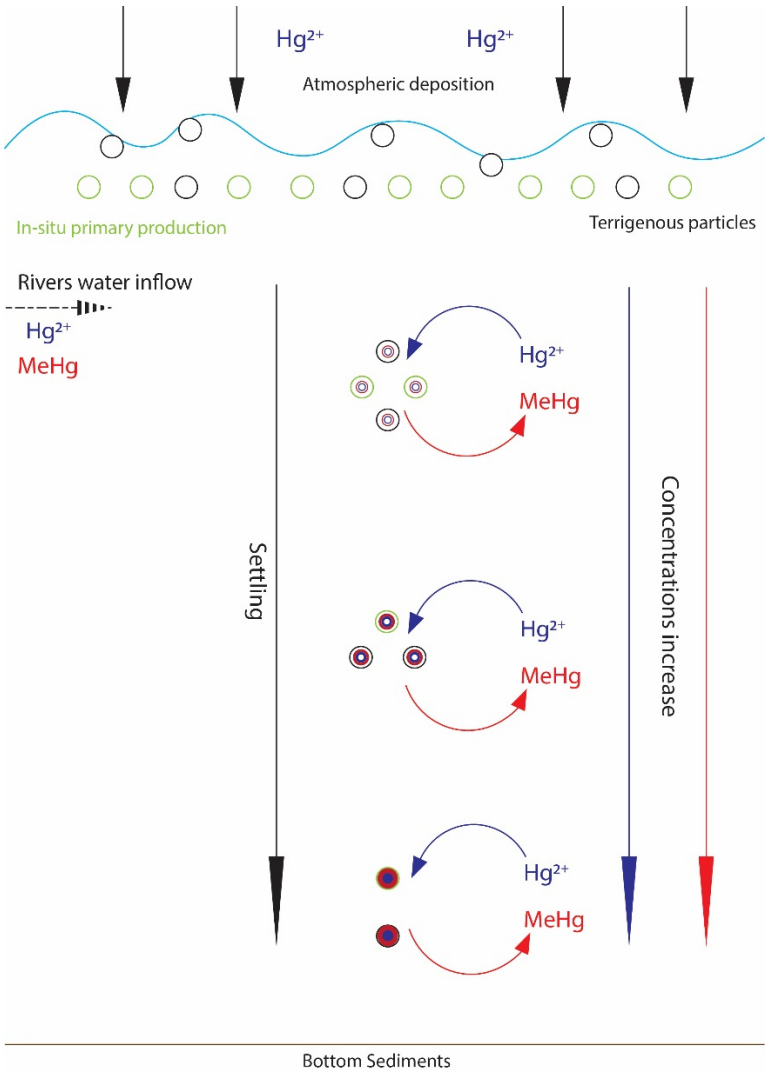


Figure II.7: Schematic of the particle descent in the water column of Lake Geneva. Particles are formed with both inorganic and organic matter coming mainly from tributaries input and in-situ production (black and green circles). As the particles begin to settle (black downward arrow) the microorganisms inside the particles begin to methylate and the particles scavenge mercury from the surrounding water. With increasing depth the concentrations of THg and MeHg bonded to the particles increase (red and blue arrows) and the particles concentration decreases. The importance of the main tributaries input needs to be better evaluated, but considering the THg and MeHg concentrations found in the rivers particles, their impact is probably of little relevance in the Hg-particles interaction in the water column.

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 (figure II.S15) based on the differential equation:

$$\frac{d(MeHg)_i}{dt} = K_m(THg)_i - K_d\,(MeHg)_i - k_s(MeHg)_i \qquad (2)$$

Where Km and Kd are the methylation and demethylation rates, respectively; ks is the removal rate of MeHg via settling particles; (THg)i is the total mercury inventory in the particles; (MeHg)i is the methylmercury inventory in the water column. To perform this calculation, we used the Km and Kd 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 (ks) 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 II.S2) to obtain the weighted mean for the water column (0 to 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, particles 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 ≈4 days from the addition of the overlaying 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 to 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 figure II.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.

II.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., 2018, Díez et al., 2016). 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 upmost 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.

II.7. References

Alldredge, A. L., and Cohen, Y., 1987, Can Microscale Chemical Patches Persist in the Sea? Microelectrode Study of Marine Snow, Fecal Pellets: Science, v. 235, no. 4789, p. 689-691.

Babiarz, C. L., Hurley, J. P., Benoit, J. M., Shafer, M. M., Andren, A. W., and Webb, D. A., 1998, Seasonal influences on partitioning and transport of total and methylmercury in rivers from contrasting watersheds: Biogeochemistry, v. 41, p. 237–257.

Balogh, S. J., Huang, Y., Offerman, H. J., Meyer, M. L., and Johnson, D. K., 2003, Methylmercury in rivers draining cultivated watersheds: Science of The Total Environment, v. 304, no. 1-3, p. 305-313.

Blais, J. M., and Kalff, J., 1995, The influence of lake morphometry on sediment focusing: Limnology and Oceanography, v. 40, no. 3, p. 582-588.

Bravo, A. G., Zopfi, J., Buck, M., Xu, J., Bertilsson, S., Schaefer, J. K., Poté, J., and Cosio, C., 2018, Geobacteraceae are important members of mercury-methylating microbial communities of sediments impacted by waste water releases: The ISME Journal, v. 12, no. 3, p. 802-812.

Celo, V., Lean, D. R. S., and Scott, S. L., 2006, Abiotic methylation of mercury in the aquatic environment: Science of The Total Environment, v. 368, no. 1, p. 126-137.

CFR, Definition and Procedure for the Determination of the Method Detection Limit - Revision 2.

Compeau, G. C., and Bartha, R., 1985, Sulfate-Reducing Bacteria: Principal Methylators of Mercury in Anoxic Estuarine Sedimentt: APPL. ENVIRON. MICROBIOL., v. 50, no. 2, p. 498-501.

Correia, R. R. S., and Guimarães, J. R. D., 2017, Mercury methylation and sulfate reduction rates in mangrove sediments, Rio de Janeiro, Brazil: The role of different microorganism consortia: Chemosphere, v. 167, p. 438-443.

Cossa, D., Averty, B., and Pirrone, N., 2009, The origin of methylmercury in open Mediterranean waters: Limnology and Oceanography, v. 54, no. 3, p. 837-844.

Cossa, D., Heimbürger, L.-E., Lannuzel, D., Rintoul, S. R., Butler, E. C. V., Bowie, A. R., Averty, B., Watson, R. J., and Remenyi, T., 2011, Mercury in the Southern Ocean: Geochimica et Cosmochimica Acta, v. 75, no. 14, p. 4037-4052.

Costa, A., Molnar, P., Stutenbecker, L., Bakker, M., Silva, T. A., Schlunegger, F., Lane, S. N., Loizeau, J.-L., and Girardclos, S., 2018, Temperature signal in suspended sediment export from an Alpine catchment: Hydrology and Earth System Sciences, v. 22, no. 1, p. 509-528.

Díez, E. G., Graham, N. D., and Loizeau, J.-L., 2018, Total and methyl-mercury seasonal particulate fluxes in the water column of a large lake (Lake Geneva, Switzerland): Environmental Science and Pollution Research, v. 25, no. 21, p. 21086-21096.

Díez, E. G., Loizeau, J.-L., Cosio, C., Bouchet, S., Adatte, T., Amouroux, D., and Bravo, A. G., 2016, Role of Settling Particles on Mercury Methylation in the Oxic Water Column of Freshwater Systems: Environmental Science & Technology, v. 50, no. 21, p. 11672-11679.

Dipasquale, M. M., Agee, J., McGowan, C., Oremland, R. S., Thomas, M., Krabbenhoft, D., and Gilmour, C. C., 2000, Methyl-Mercury Degradation Pathways: A Comparison among Three Mercury-Impacted Ecosystems: Environ Sci Technol, v. 34, p. 4908-4916.

Dominik, J., Schuler, C., and Santschi, P. H., 1989, Residence times of 234 Th and 7 Be in Lake Geneva: Earth and Planetary Science Letters, v. 93, p. 345-358.

Eckley, C. S., and Hintelmann, H., 2006, Determination of mercury methylation potentials in the water column of lakes across Canada: Science of The Total Environment, v. 368, no. 1, p. 111-125.

Ethier, A. L., Scheuhammer, A. M., Blais, J. M., Paterson, A. M., Mierle, G., Ingram, R., and Lean, D. R., 2010, Mercury empirical relationships in sediments from three Ontario lakes: Sci Total Environ, v. 408, no. 9, p. 2087-2095.

Finlay, B. J., and Berninger, U.-G., 1984, Coexistence of congeneric ciliates (karyorelictida: loxodes) in relation to food resources in two freshwater lakes: Journal of Animal Ecology, v. 53, p. 929-943.

Fleming, E. J., Mack, E. E., Green, P. G., and Nelson, D. C., 2006, Mercury Methylation from Unexpected Sources: Molybdate-Inhibited Freshwater Sediments and an Iron-Reducing Bacterium: Applied and Environmental Microbiology, v. 72, no. 1, p. 457-464.

FOEN.

Gallorini, A., and Loizeau, J.-L., 2021, Mercury methylation in oxic aquatic macro-environments: a review: Journal of Limnology.

Géoportail.

Gilmour, C. C., Podar, M., Bullock, A. L., Graham, A. M., Brown, S. D., Somenahally, A. C., Johs, A., Hurt, R. A., Bailey, K. L., and Elias, D. A., 2013, Mercury Methylation by Novel Microorganisms from New Environments: Environmental Science & Technology, v. 47, no. 20, p. 11810-11820.

Glud, R. N., Grossart, H.-P., Larsen, M., Tang, K. W., Arendt, K. E., Rysgaard, S., Thamdrup, B., and Gissel Nielsen, T., 2015, Copepod carcasses as microbial hot spots for pelagic denitrification: Copepod carcasses and denitrification: Limnology and Oceanography, v. 60, no. 6, p. 2026-2036.

Guo, P., Du, H., Wang, D., and Ma, M., 2021, Effects of mercury stress on methylmercury production in rice rhizosphere, methylmercury uptake in rice and physiological changes of leaves: Sci Total Environ, v. 765, p. 142682.

Heiri, O., Lotter, A. F., and Lemcke, G., 2001, Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results: Journal of Paleolimnology, v. 25, p. 101-110.

Herrin, R. T., Lathrop, R. C., Gorski, P. R., and Andren, A. W., 1998, Hypolimnetic methylmercury and its uptake by plankton during fall destratification: A key entry point of mercury into lake food chains?: Limnology and Oceanography, v. 43, p. 1476-1486.

Honeyman, B. D., and Santschi, P. H., 1989, A Brownian-pumping model for oceanic trace metal scavenging: Evidence from Th isotopes: Journal of Marine Research, v. 47, p. 951-992.

Hurley, J. P., Cowell, S. E., Shafer, M. M., and Hughes, P. E., 1998, Partitioning and Transport of Total and Methyl Mercury in the Lower Fox River, Wisconsin: Environ Sci Technol, v. 32, p. 1424-1432.

Hurley, J. P., Krabbenhoft, D. P., Babiarz, C. L., and Andren, A. W., 1994, Cycling of Mercury across the Sediment-Water Interface in Seepage Lakes, Environmental Chemistry of Lakes and Reservoirs, p. 425-449.

King, J. K., Kostka, J. E., Frisher, M. E., and Saunders, F. M., 2000, Sulfate-Reducing Bacteria Methylate Mercury at Variable Rates in Pure Culture and in Marine Sediments: Applied and Environmental Microbiology, v. 66, no. 6, p. 2430-2437.

Le Faucheur, S., Campbell, P. G., Fortin, C., and Slaveykova, V. I., 2014, Interactions between mercury and phytoplankton: speciation, bioavailability, and internal handling: Environ Toxicol Chem, v. 33, no. 6, p. 1211-1224.

Lehnherr, I., St. Louis, V. L., Hintelmann, H., and Kirk, J. L., 2011, Methylation of inorganic mercury in polar marine waters: Nature Geoscience, v. 4, no. 5, p. 298-302.

Liu, B., Yan, H., Wang, C., Li, Q., Guédron, S., Spangenberg, J. E., Feng, X., and Dominik, J., 2012, Insights into low fish mercury bioaccumulation in a mercury-contaminated reservoir, Guizhou, China: Environmental Pollution, v. 160, p. 109-117.

Mason, R. P., and Fitzgerald, W. F., 1990, Alkylmercury species in the equatorial Pacific: Nature, v. 347, no. 6292, p. 457-459.

Mason, R. P., and Sullivan, K. A., 1997, Mercury in Lake Michigan: Environmental Science & Technology, v. 31, p. 942-947.

Miracle, M. R., 1974, Niche structure in freshwater zooplankton: A principal components approach: Ecology, v. 55, p. 1306-1316.

Monperrus, M., Tessier, E., Amouroux, D., Leynaert, A., Huonnic, P., and Donard, O. F. X., 2007a, Mercury methylation, demethylation and reduction rates in coastal and marine surface waters of the Mediterranean Sea: Marine Chemistry, v. 107, no. 1, p. 49-63.

Monperrus, M., Tessier, E., Point, D., Vidimova, K., Amouroux, D., Guyoneaud, R., Leynaert, A., Grall, J., Chauvaud, L., Thouzeau, G., and Donard, O. F. X., 2007b, The biogeochemistry of mercury at the sediment–water interface in the Thau Lagoon. 2. Evaluation of mercury methylation potential in both surface sediment and the water column: Estuarine, Coastal and Shelf Science, v. 72, no. 3, p. 485-496.

Ortiz, V. L., Mason, R. P., and Evan Ward, J., 2015, An examination of the factors influencing mercury and methylmercury particulate distributions, methylation and demethylation rates in laboratory-generated marine snow: Marine Chemistry, v. 177, p. 753-762.

Paranjape, A. R., and Hall, B. D., 2017, Recent advances in the study of mercury methylation in aquatic systems: FACETS, v. 2, no. 1, p. 85-119.

Parks, J. M., Johs, A., Podar, M., Bridou, R., Hurt, R. A., Smith, S. D., Tomanicek, S. J., Qian, Y., Brown, S. D., Brandt, C. C., Palumbo, A. V., Smith, J. C., Wall, J. D., Elias, D. A., and Liang, L., 2013, The Genetic Basis for Bacterial Mercury Methylation: Science, v. 339, no. 6125, p. 1332-1335.

Peterson, B. D., McDaniel, E. A., Schmidt, A. G., Lepak, R. F., Janssen, S. E., Tran, P. Q., Marick, R. A., Ogorek, J. M., DeWild, J. F., Krabbenhoft, D. P., and McMahon, K. D., 2020, Mercury Methylation Genes Identified across Diverse Anaerobic Microbial Guilds in a Eutrophic Sulfate-Enriched Lake: Environmental Science & Technology, v. 54, no. 24, p. 15840-15851.

Pickhardt, P. C., and Fisher, N. S., 2007, Accumulation of Inorganic and Methylmercury by Freshwater Phytoplankton in Two Contrasting Water Bodies: Environmental Science & Technology, v. 41, no. 1, p. 125-131.

Podar, M., Gilmour, C. C., Brandt, C. C., Soren, A., Brown, S. D., Crable, B. R., Palumbo, A. V., Somenahally, A. C., and Elias, D. A., 2015, Global prevalence and distribution of genes and microorganisms involved in mercury methylation: Science Advances, v. 1, no. 9, p. e1500675-e1500675.

Porvari, P., and Verta, M., 1995, Methylmercury production in flooded soils:

A laboratory study: Water Air & Soil Pollution, v. 80, p. 765-773.

Quiroga-Flores, R., Guedron, S., and Acha, D., 2021, High methylmercury uptake by green algae in Lake Titicaca: Potential implications for remediation: Ecotoxicol Environ Saf, v. 207, p. 111256.

Rasconi, S., Anneville, O., Rimet, F., and Perney, P., 2019, Chlorophyll A Biomass and Primary production in Lake Geneva: CIPEL.

Rienmann, B., 1985, Potential Importance of Fish Predation and Zooplankton Grazing on Natural Populations of Freshwater Bacteria: Applied and Environmental Microbiology, v. 50, p. 187-193.

Rosa, F., 1985, Sedimentation and sediment resuspension in lake ontario: Journal of Great Lakes Research, v. 11, p. 13-25.

Roulet, M., Guimaraes, J. R. D., and Lucotte, M., 2001, Methylmercury production and accumulation in sediments and soils of an amazonian floodplain - effect of seasonal inundation: Water Air & Soil Pollution, v. 128, p. 41-60.

Si, Y., Zou, Y., Liu, X., Si, X., and Mao, J., 2015, Mercury methylation coupled to iron reduction by dissimilatory iron-reducing bacteria: Chemosphere, v. 122, p. 206-212.

Soerensen, A. L., Schartup, A. T., Skrobonja, A., Bouchet, S., Amouroux, D., Liem-Nguyen, V., and Björn, E., 2018, Deciphering the Role of Water Column Redoxclines on Methylmercury Cycling Using Speciation Modeling and Observations From the Baltic Sea: Global Biogeochemical Cycles, v. 32, no. 10, p. 1498-1513.

Stabel, H.-H., 1987, Settling velocity and residence time of particles in Lake Constance: Schweiz. Z. Hydrol., v. 49, p. 284-293.

Sunderland, E. M., Gobas, F. A. P. C., Branfireun, B. A., and Heyes, A., 2006, Environmental controls on the speciation and distribution of mercury in coastal sediments: Marine Chemistry, v. 102, no. 1-2, p. 111-123.
Tomczak, W., Boyer, P., Krimissa, M., and Radakovitch, O., 2019, Kd distributions in freshwater systems as a function of material type, mass-volume ratio, dissolved organic carbon and pH: Applied Geochemistry, v. 105, p. 68-77.
Topping, G., and Davies, I. M., 1981, Methylmercury production in the marine water column: Nature, v. 290, no. 5803, p. 243-244.
Ullrich, S. M., Tanton, T. W., and Abdrashitova, S. A., 2001, Mercury in the Aquatic Environment: A Review of Factors Affecting Methylation: Critical Reviews in Environmental Science and Technology, v. 31, no. 3, p. 241-293.
US-EPA, 2001a, Appendix to Method 1631 Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation, in Agency, U. S. E. P., ed., Volume EPA-821-R-01-013, p. 13.
-, 2001b, Method 1630, Methyl mercury in water by distillation, Aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry, in Agency, U. S. E. P., ed., Volume EPA-821-R-01-020, p. 55.
-, 2002, Method 1631, Revision E: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry, in Agency, U. S. E. P., ed., Volume. EPA-821-R-02-019, p. 45.
Whalin, L., Kim, E.-H., and Mason, R., 2007, Factors influencing the oxidation, reduction, methylation and demethylation of mercury species in coastal waters: Marine Chemistry, v. 107, no. 3, p. 278-294.
Wu, H., Ding, Z., Liu, Y., Liu, J., Yan, H., Pan, J., Li, L., Lin, H., Lin, G., and Lu, H., 2011, Methylmercury and sulfate-reducing bacteria in mangrove sediments from Jiulong River Estuary, China: Journal of Environmental Sciences, v. 23, no. 1, p. 14-21.
Wu, P., Zakem, E. J., Dutkiewicz, S., and Zhang, Y., 2020, Biomagnification of Methylmercury in a Marine Plankton Ecosystem: Environ Sci Technol, v. 54, no. 9, p. 5446-5455.
Zhang, L., and Planas, D., 1994, Biotic and abiotic mercury methylation and demethylation in sediments: Bulletin of Environmental Contamination and Toxicology, v. 52, no. 5.

Supplementary Material

River	Flow Regime	Discharge (m^3/s)	Particles Load (g/s)	Particles concentrations (g/L)
Rhone	High	218	29057 ± 663.7	7.51 ± 0.24
	High	188	9221.9 ± 333.1	20.41 ± 1.04
	Low	154	5179.5 ± 452.2	29.96 ± 3.7
	Low	126	2277.4 ± 657.9	60.36 ± 24.66
Aubonne	High	15	217.5 ± 0.9	68.97 ± 0.42
	High	5.5	15.4 ± 1.6	360.82 ± 51.55
	Low	18	1396.2 ± 443.8	14.34 ± 6.44
	Low	0.2	0.36 ± 0.09	729.82 ± 266.58
Venoge	High	8.0	97.8 ± 15.6	83.37 ± 18.78
	High	3.5	24.7 ± 10.8	174.55 ± 107.40
	Low	15	889.4 ± 62	16.95 ± 1.67
	Low	2.1	17.3 ± 2.5	123.97 ± 25.44
Dranse	High	26.5	190.8 ± 2.7	138.92 ± 2.73
	Low	12.8	46.56 ± 4.3	277.28 ± 36.21

Table II.S1: Particles load calculated for the different rivers in different flow regimes. The discharge data were collected from the FOEN (website for the Swiss rivers and from the Hydroreel website for the Dranse River).

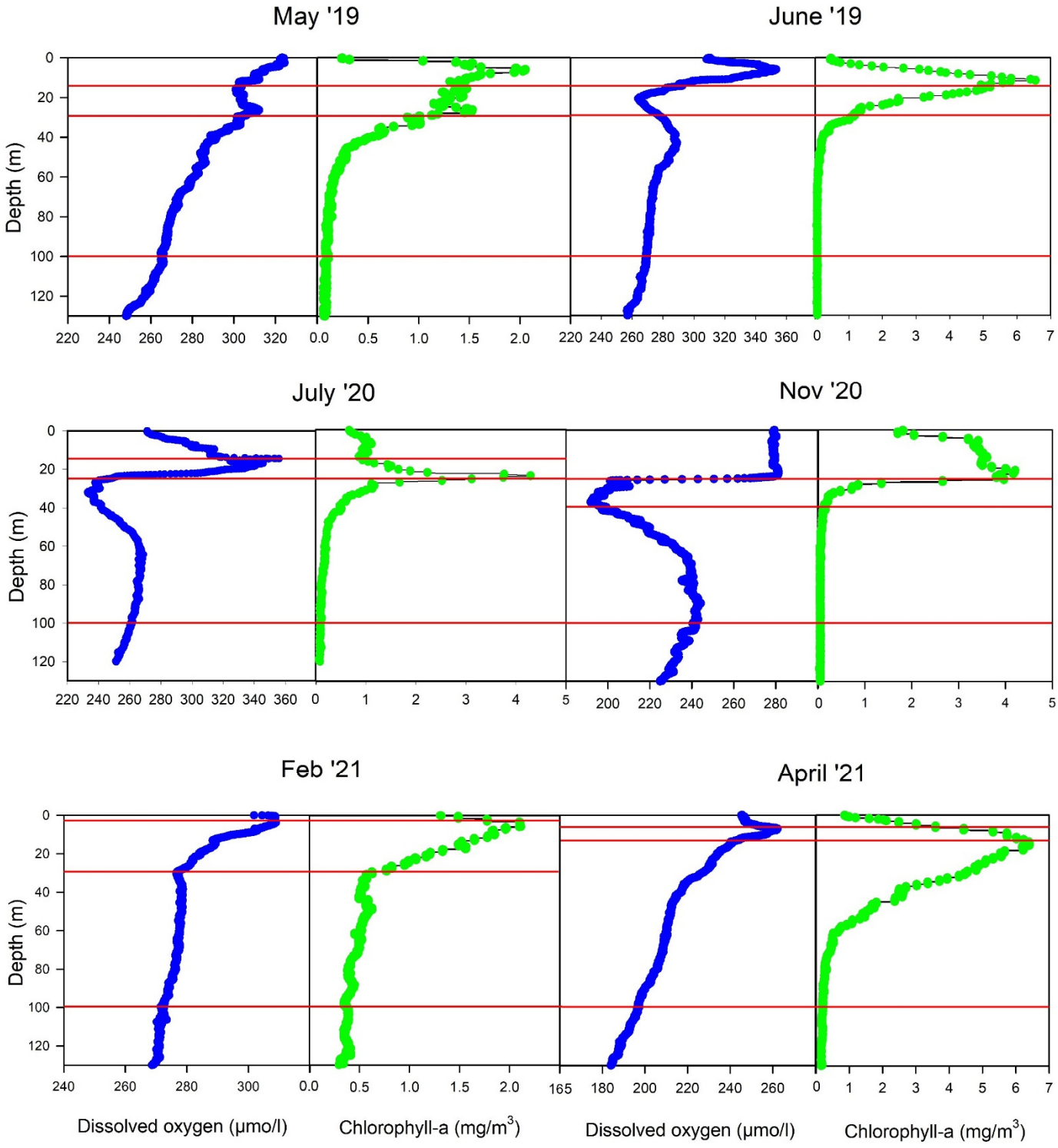


Figure II.S1: Dissolved oxygen and chlorophyll-a vertical profiles collected during the sampling campaigns. In red, the three sampling depths for each month.

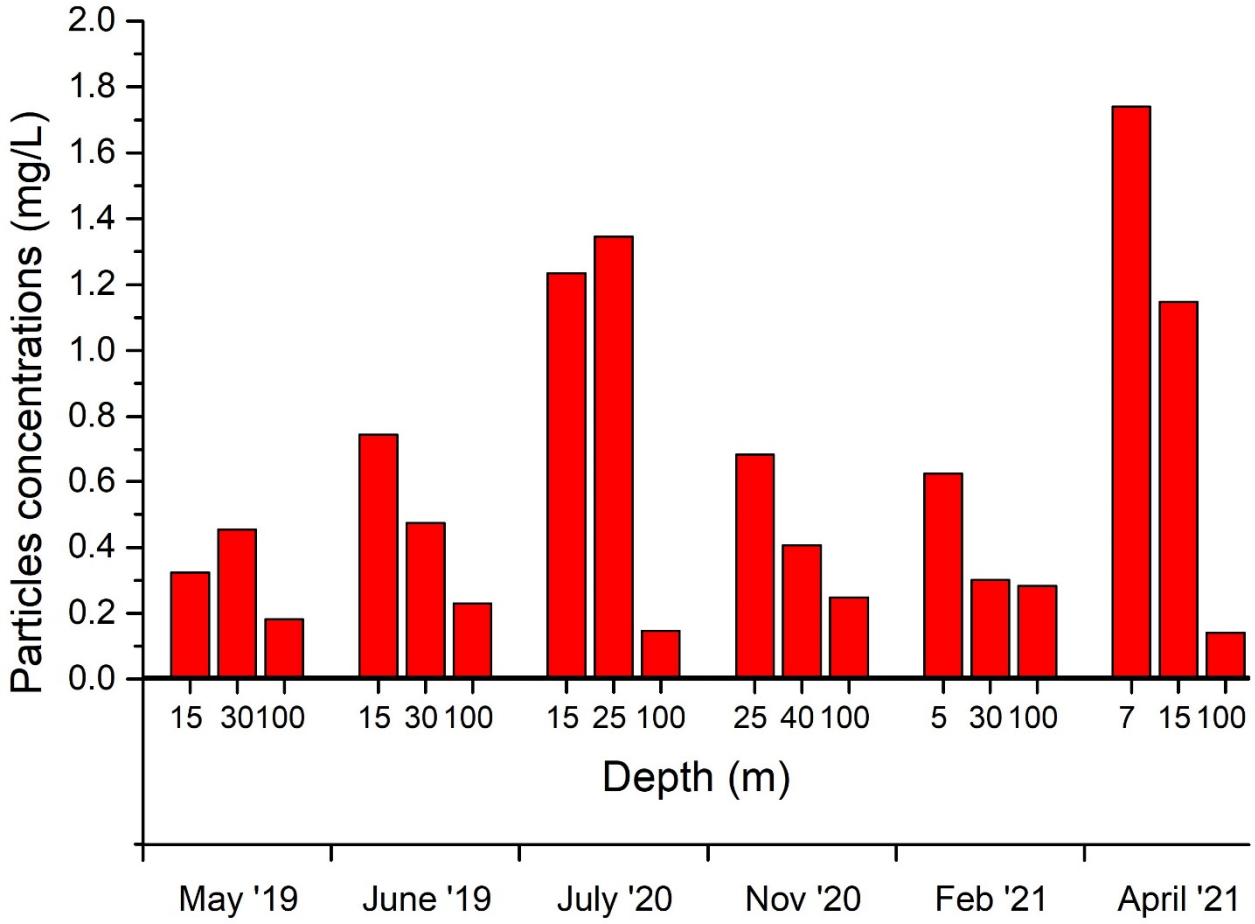
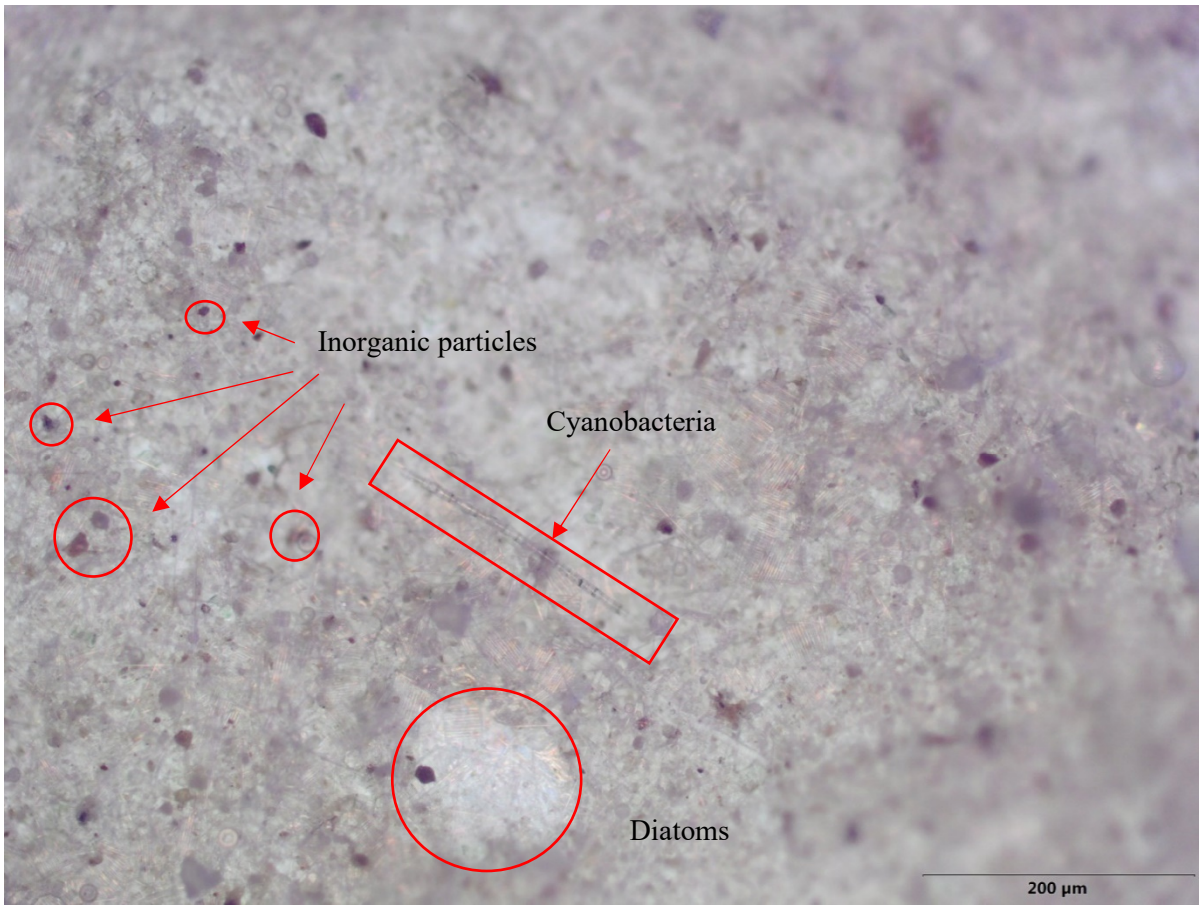
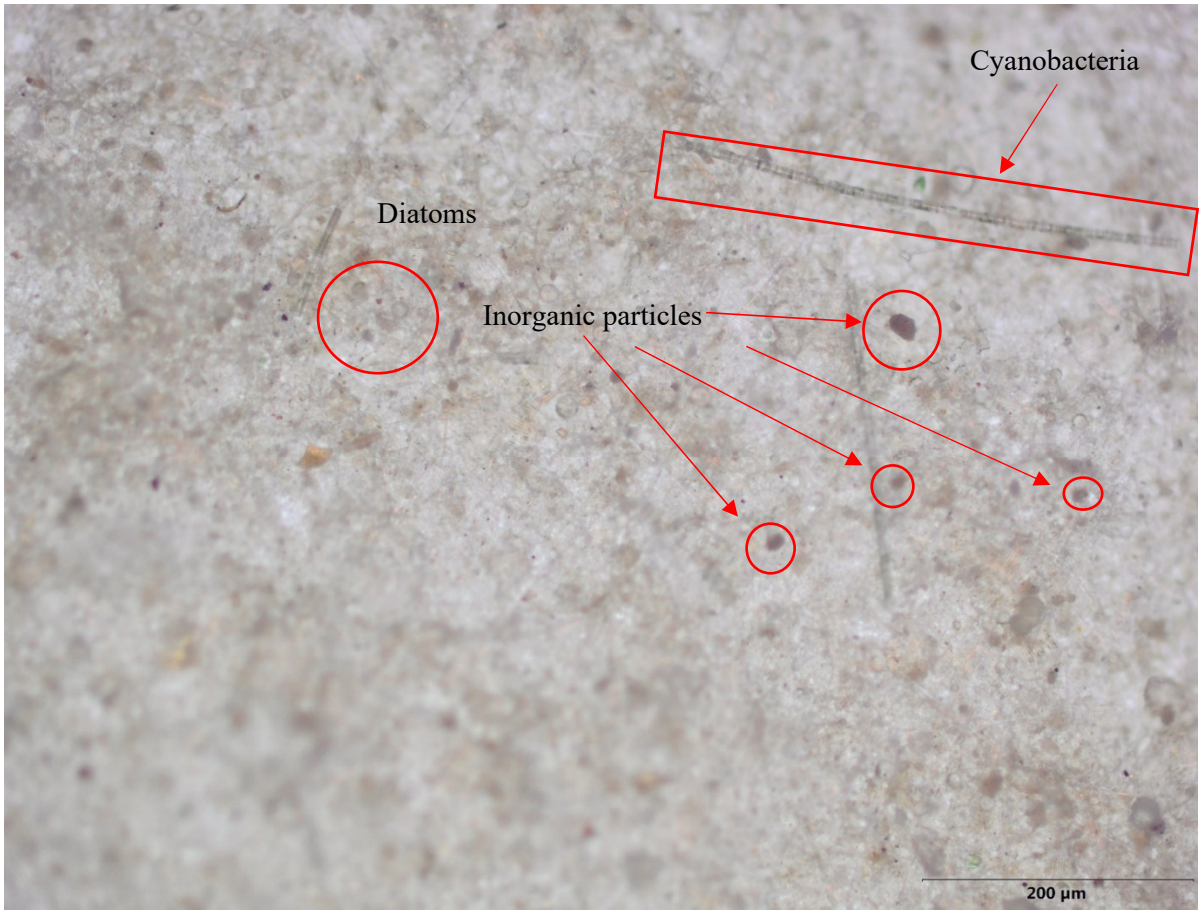


Figure II.S2: Concentrations of suspended particles in the water column of Lake Geneva as a function of depth and sampling periods.



A



B

Figure II.S3: Microscopic images of sampled filters collected at 15m (A) and at 100m (B) during the sampling campaign in May 2019. There is no macroscopic difference in the composition of the two filters as far as it is possible to discern by a microscopic imaging.

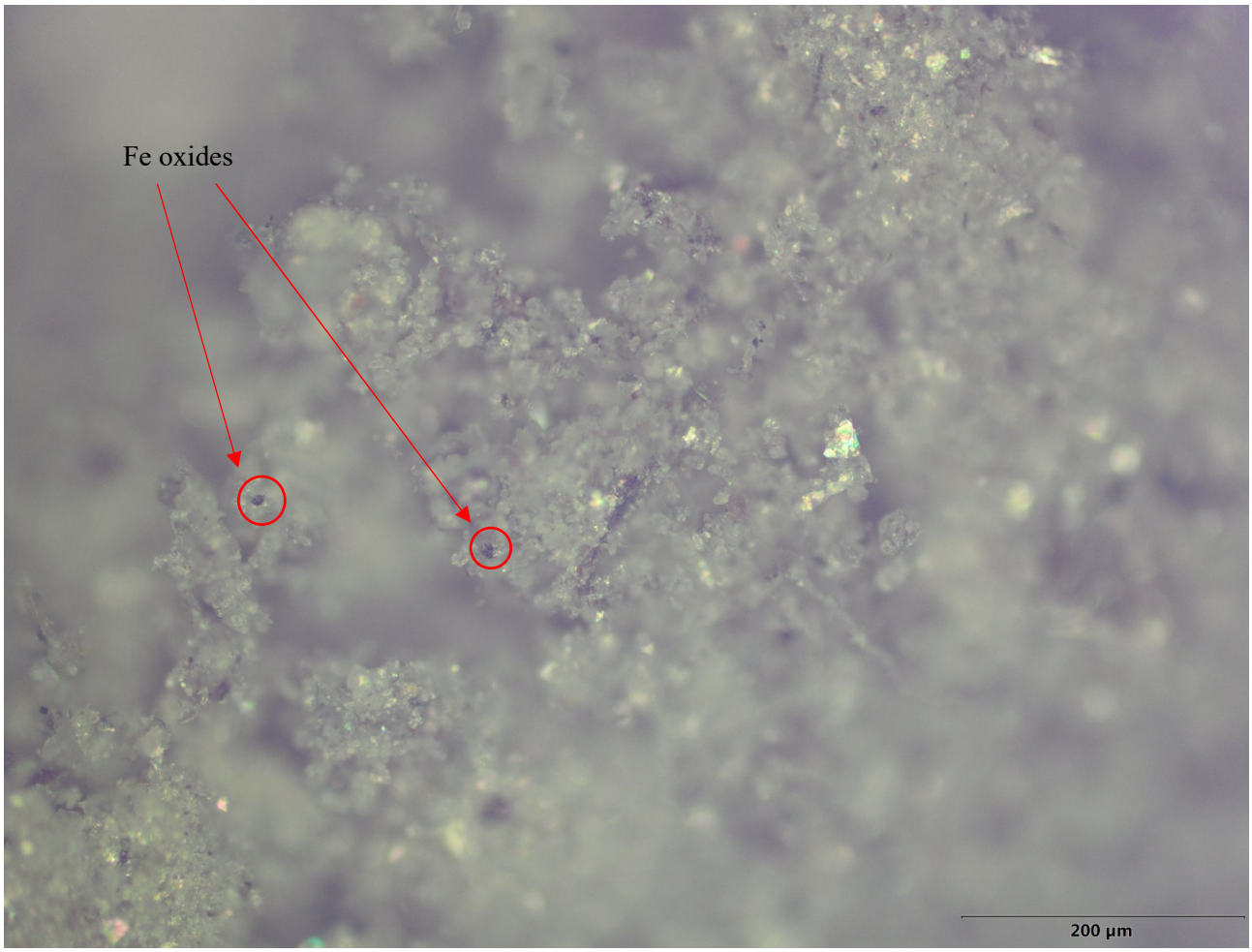


Figure II.S4: Microscope imaging of particles sampled from the Rhone River. The main component that are discernible from an optical imaging are, calcite crystals, which account for the majority of the particles and Fe oxides.

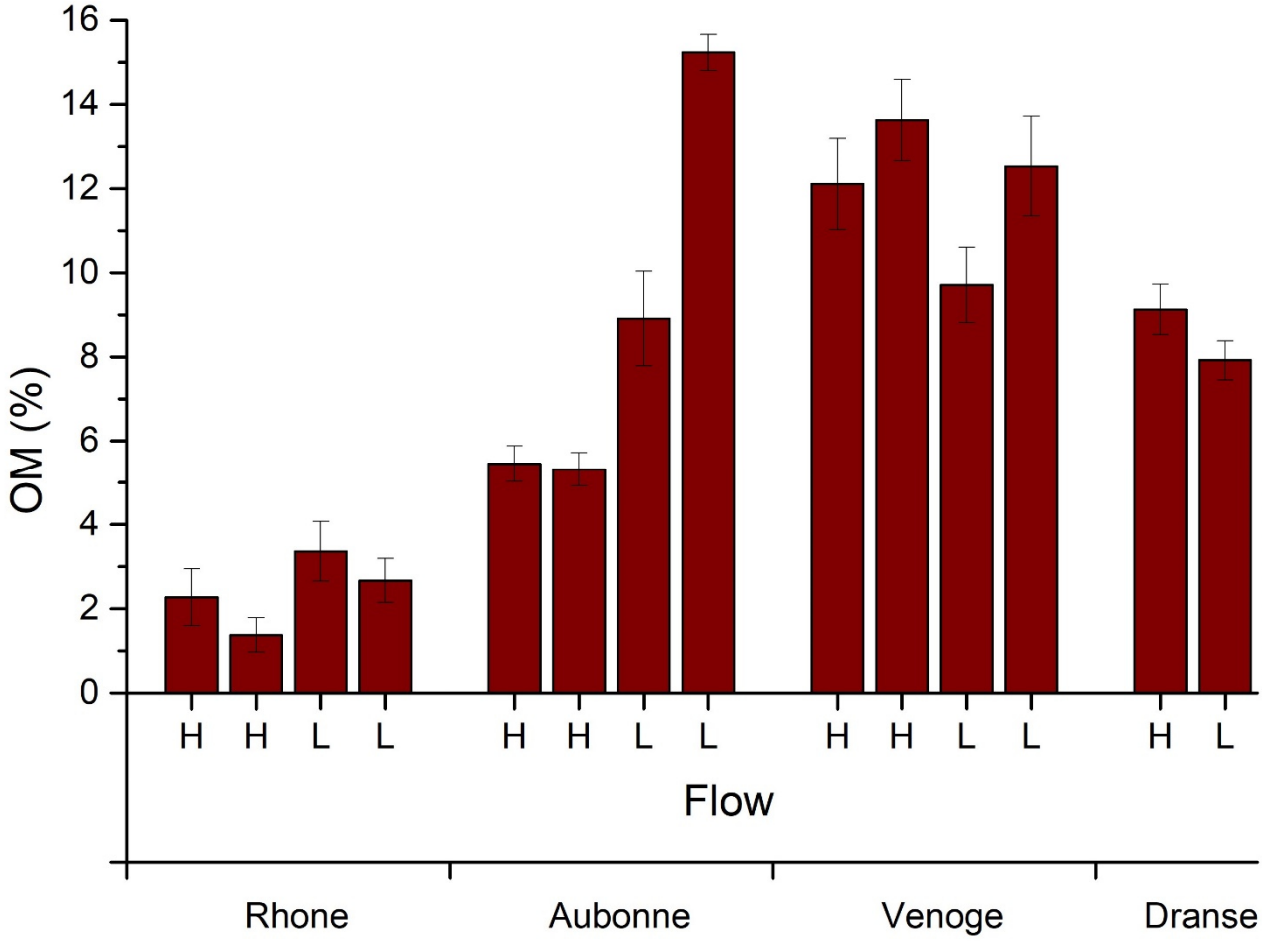


Figure II.S5: Organic matter content (%) in the rivers particles determined via L.O.I.

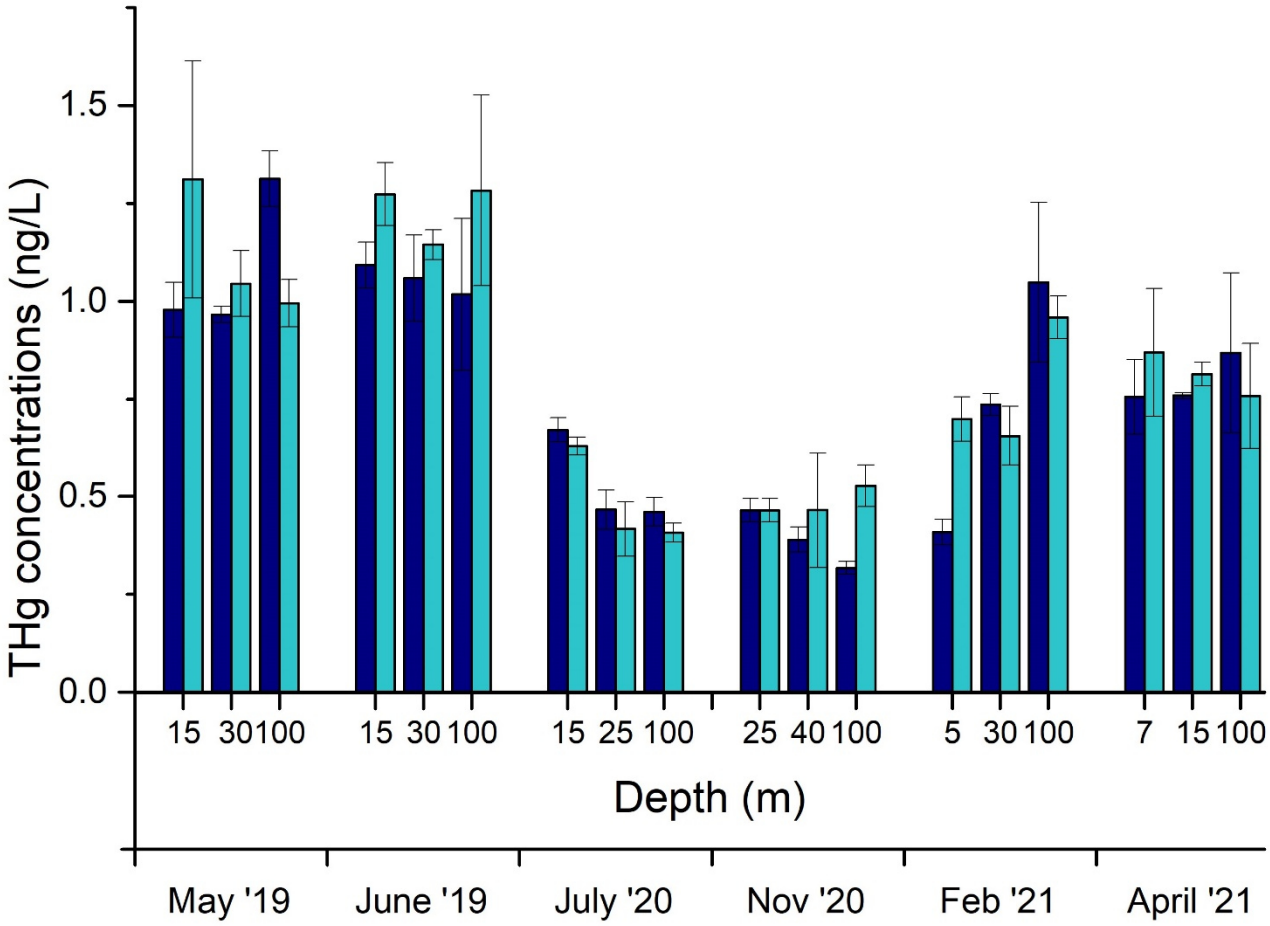


Figure II.S6: THg concentrations in the raw (blue) and filtered (cyan) water samples collected in combination with the suspended particles sampling campaign.

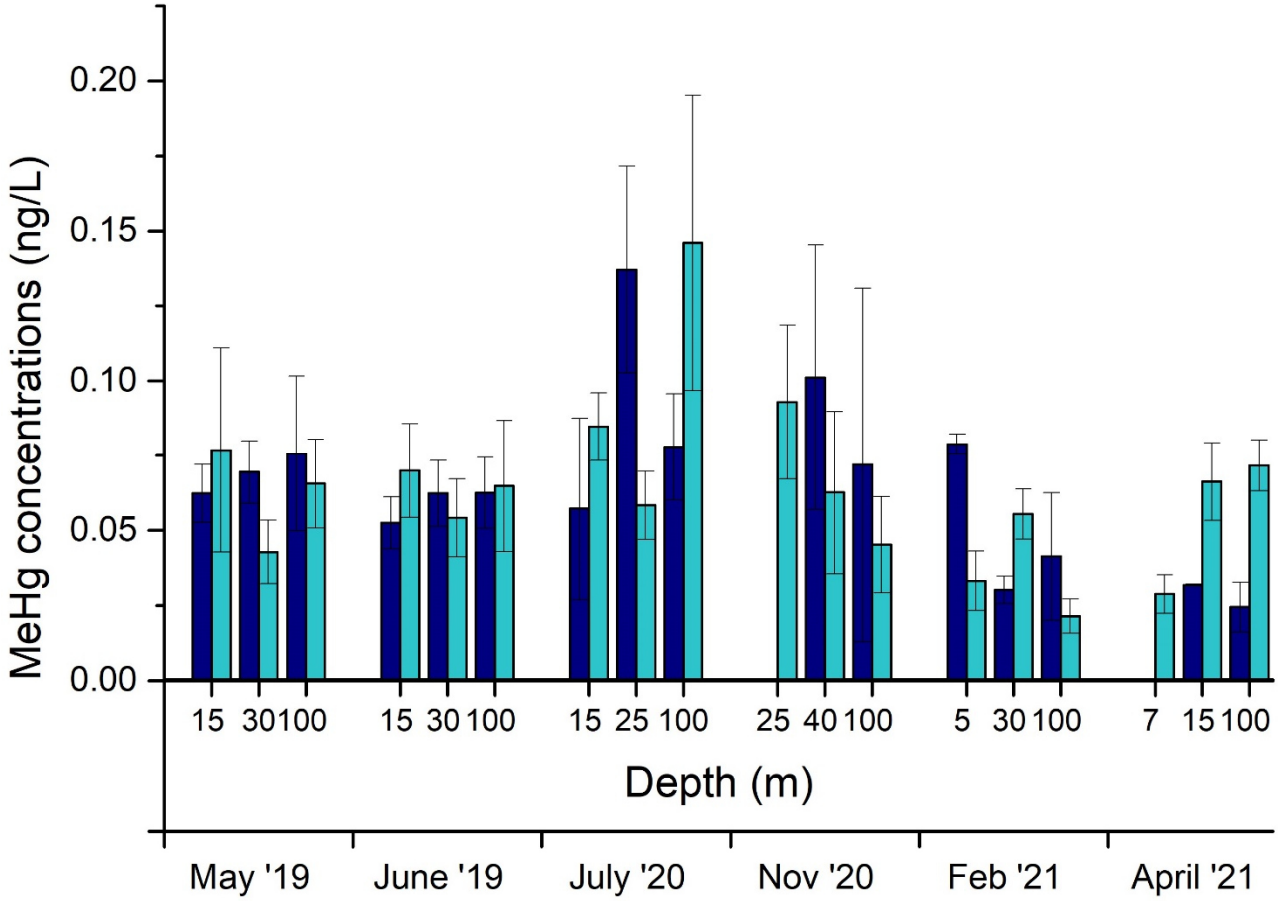


Figure II.S7: MeHg concentrations in the raw (blue) and filtered (cyan) water samples collected in parallel with the suspended particles sampling campaign. The first sampling depth of November and April were below the detection limit (B.D.L.).

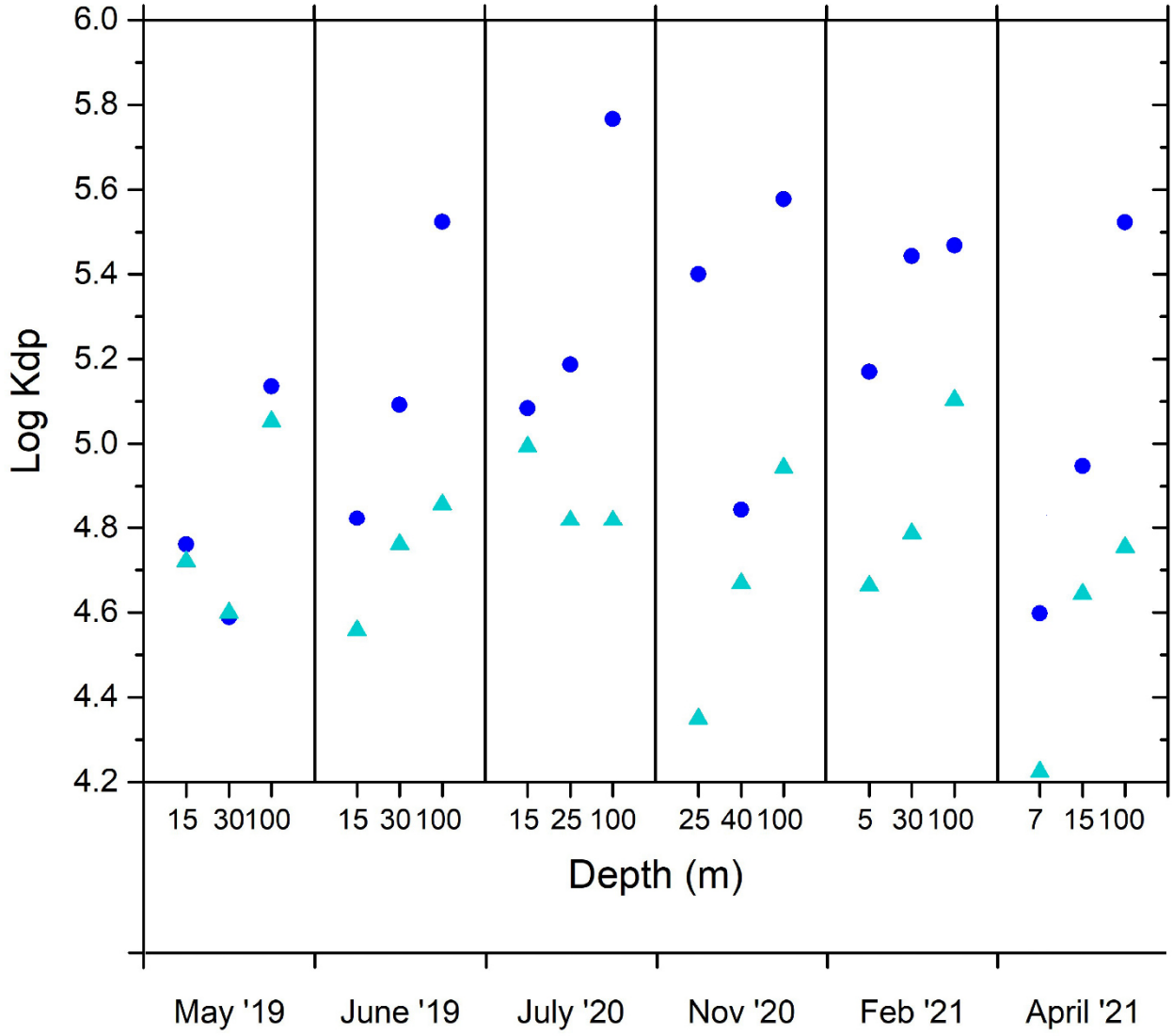


Figure II.S8: Log Kpd for THg (blue circles) and MeHg (light blue triangles).

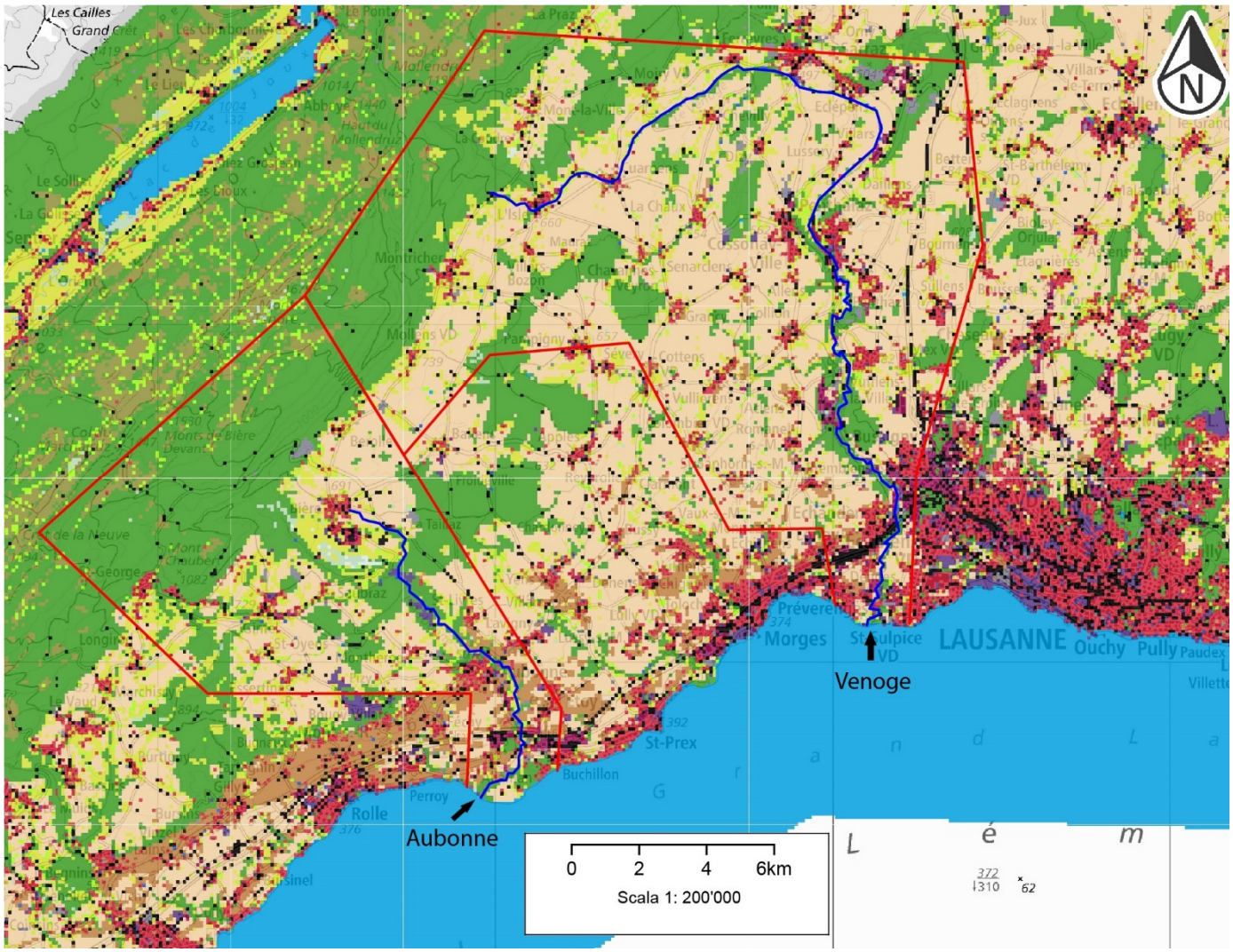


Figure II.S9: Land use map of the watersheds (highlighted by the red lines) of Aubonne and Venoge rivers (blue lines). Shades of green represent woodlands and forests; shades of red and purple represent urban and industrial areas, shades of yellow represent agricultural areas.

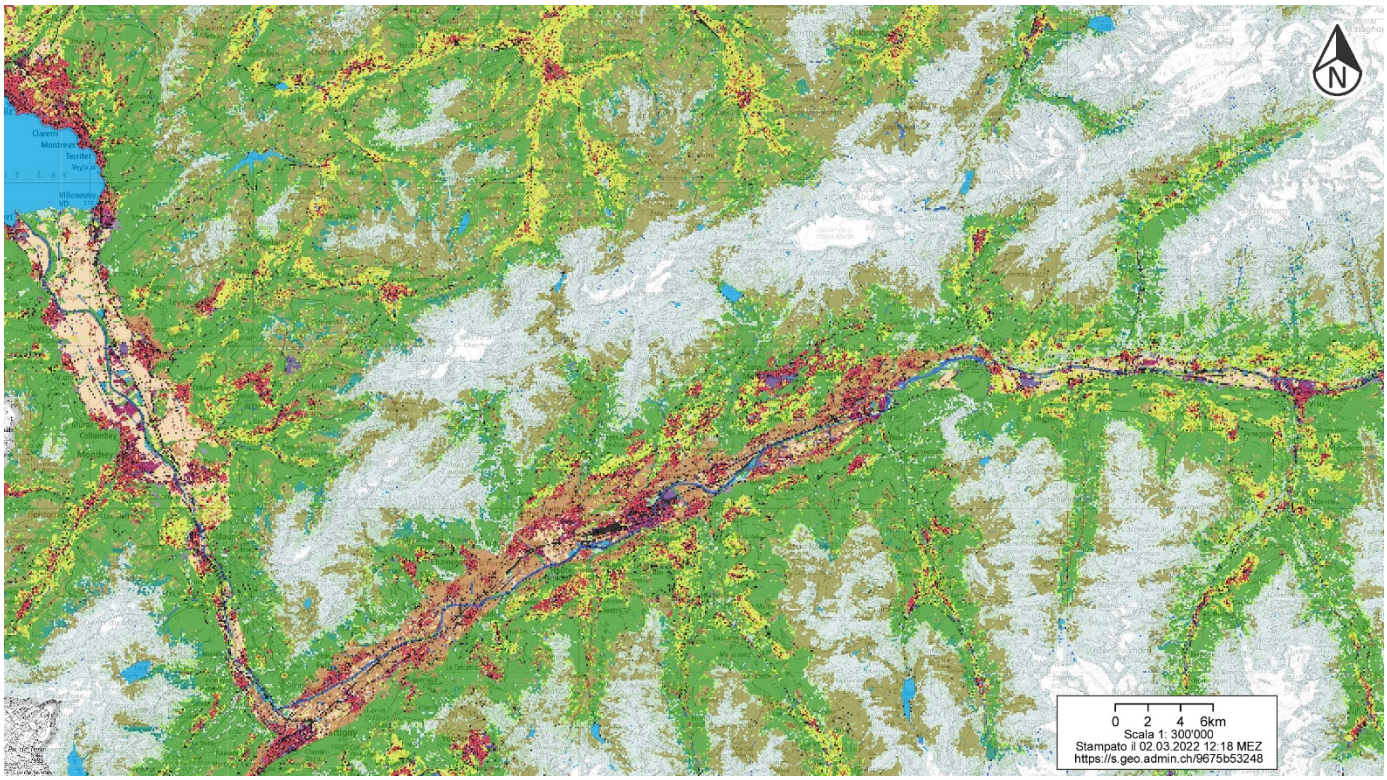


Figure II.S10: Land use map of the watershed of Rhone River. Shades of green represent woodlands and forests; shades of red and purple represent urban and industrial areas, shades of yellow represent agricultural areas.

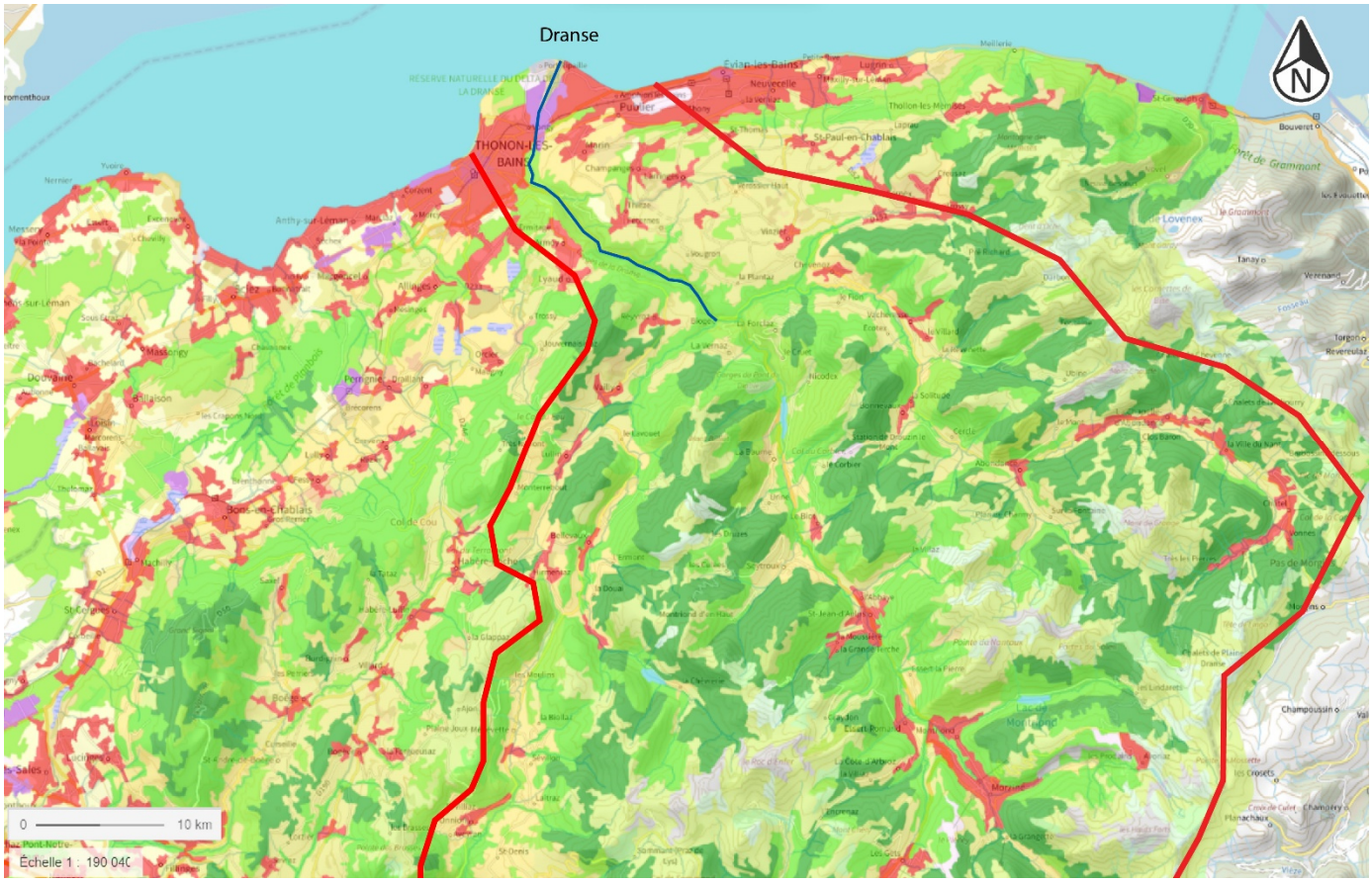


Figure II.S11: Land use map of the watershed (highlighted by the red lines) of Dranse River (blue line). Shades of green represent woodlands and forests; shades of red and purple represent urban and industrial areas, shades of yellow represent agricultural areas.

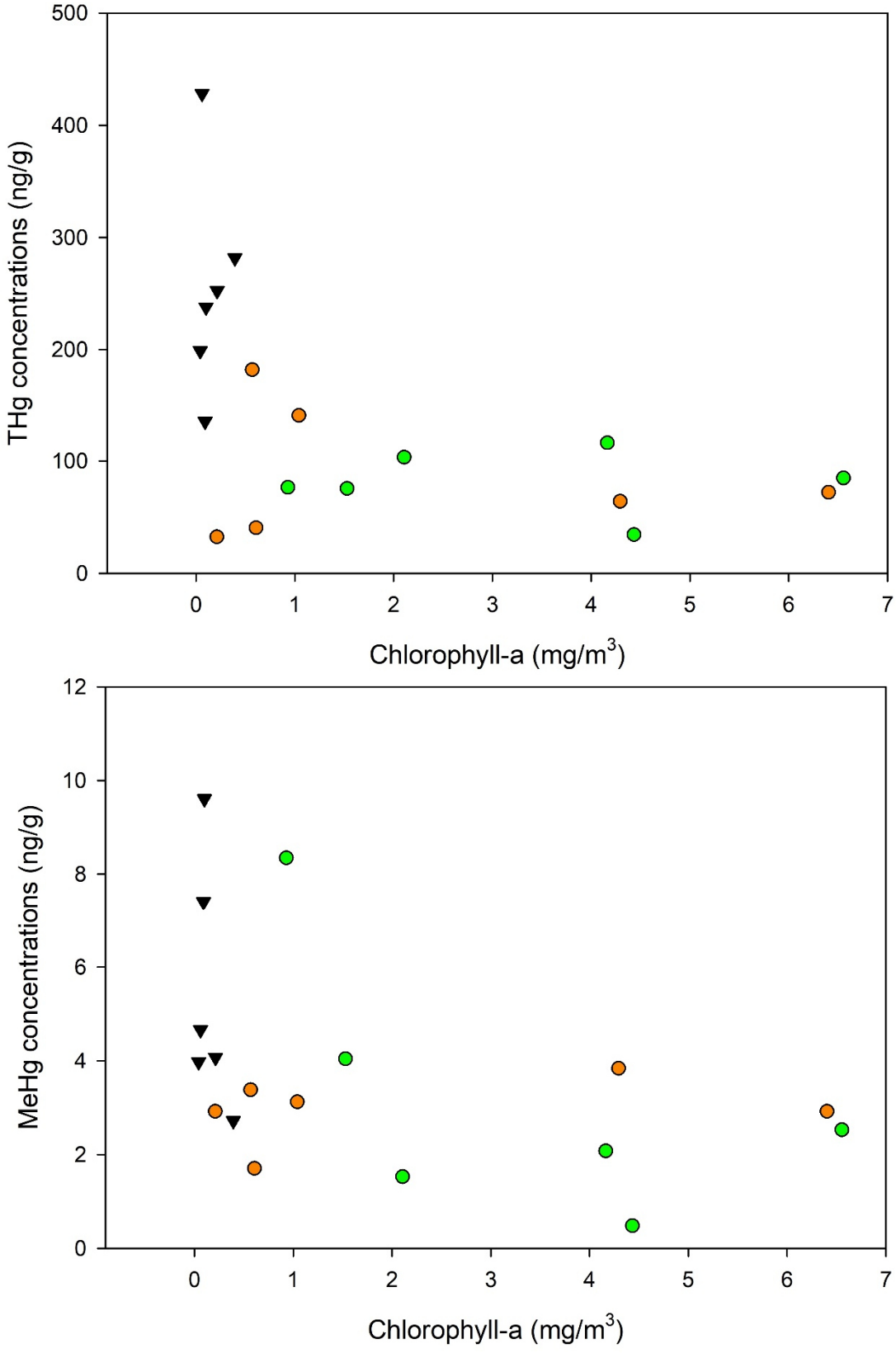


Figure II.S12: THg and MeHg concentrations plotted against fluorescence, which is the measure of the chlorophyll-a. Green circles represent the production zone, orange circles the mineralization zone and the black triangles the hypolimnion.

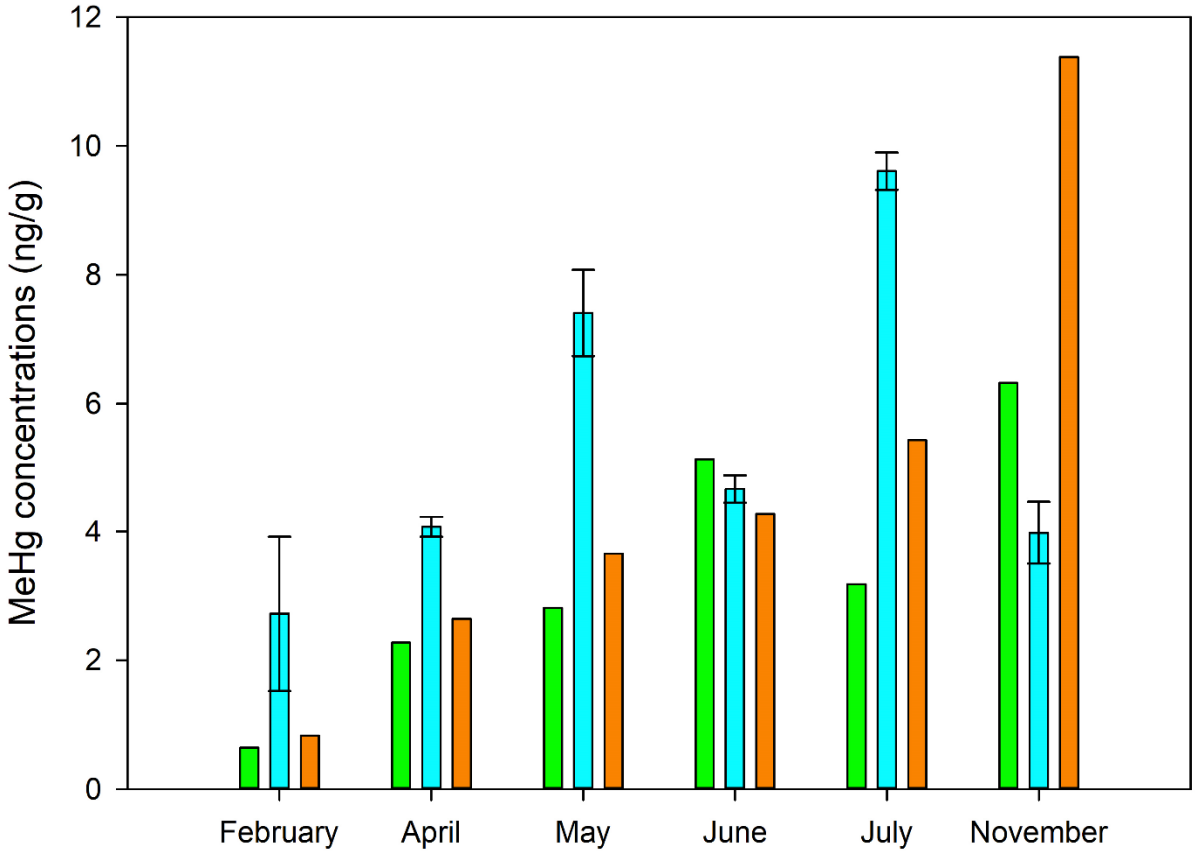


Figure II.S13: MeHg concentrations comparison in particles between this work and the work of Díez et al. (2018). In green and orange data from Díez et al. (2018) (75m and 132m respectively), in cyan data from this work (100m).

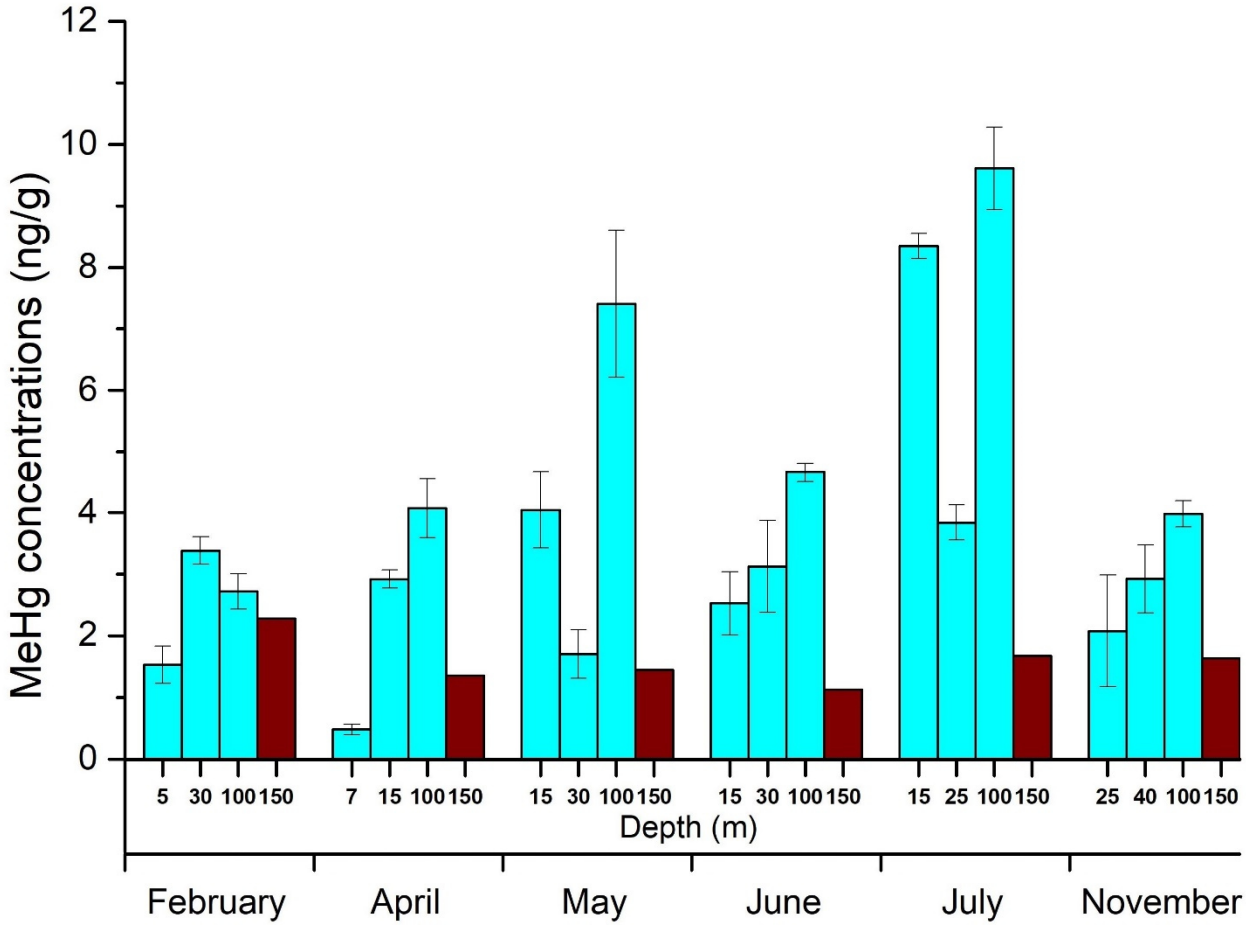


Figure II.S14: MeHg concentrations comparison between suspended particles (cyan) and bottom sediments (brown) (Díez et al., 2016).

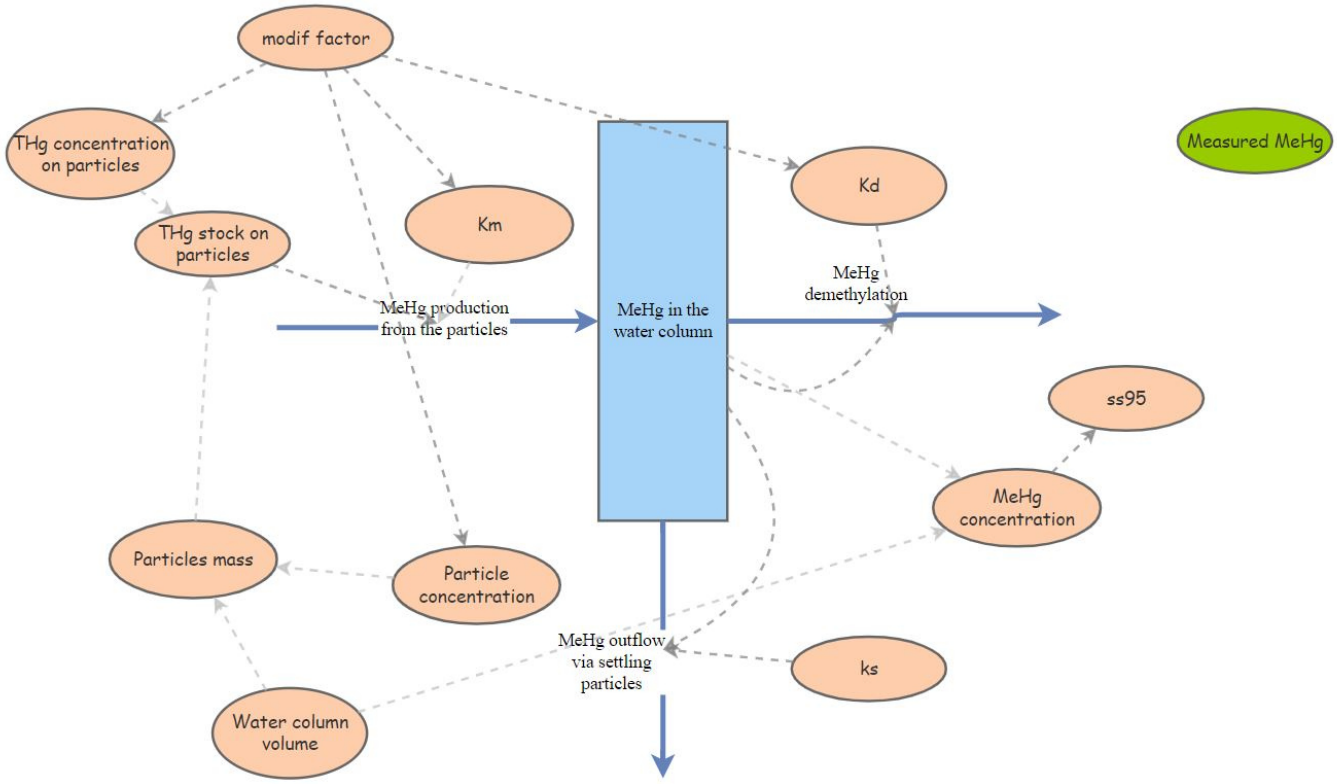


Figure II.S15: Model produced using Insight Maker (link to the model MeHg Model), used to determine the necessary days to obtain the steady-state between MeHg production of the particles and MeHg in the dissolved phase in the water column. In the model, Km (Hg methylation rate), Kd (Hg demethylation rate) and ks (removal rate of MeHg via settling particles).

	Weighted average MeHg concentrations in the water	Weighted average Particles concentration	Weighted average THg concentrations in the particles	Water column volume	Km	Kd
	ng/m^3	g/m^3	(ng/g)	m^3	1/d	1/d
May '19	63.28	0.26	104.89	100.00	0.02	0.1
June '19	63.73	0.38	302.20	100.00	0.045	0.1
July '20	116.17	0.60	170.88	100.00	0.06	0.1

Table II.S2: Data from this work used into the conceptual model of figure S10.

Díez, Elena Gascón, Neil D. Graham, and Jean-Luc Loizeau. 2018. 'Total and methyl-mercury seasonal particulate fluxes in the water column of a large lake (Lake Geneva, Switzerland)', Environmental Science and Pollution Research, 25: 21086-96.

Díez, Elena Gascón, Jean-Luc Loizeau, Claudia Cosio, Sylvain Bouchet, Thierry Adatte, David Amouroux, and Andrea G. Bravo. 2016. 'Role of Settling Particles on Mercury Methylation in the Oxic Water Column of Freshwater Systems', Environmental Science & Technology, 50: 11672-79.

FOEN. https://www.geo.admin.ch/en/geo-information-switzerland/geodata-index-inspire/surface-representation/land-cover.html.

Hydroreel. https://www.rdbrmc.com/hydroreel2/index.php.

Additional information regarding the sampling procedures

During the sampling via continuous flow filtration, the flow was measured every 15 minutes thanks to a metered container and a chronometer. This procedure was necessary in order to verify the regularity of the flow and to measure the total liters of water filtrated. The output flow from the peristaltic pump was of about 4 liter per minute, which was divided for the two filter holders with an

output flow from each filter holders of about 2 liters per minutes. The total amount of filtered liters of water varies between depths, ranging from 20 to 120 liters in the epilimnion, from 30 to 190 liters in the metalimnion and from 150 to 240 liters in the hypolimnion. With the volume of filtered water and the dry weight of the samples on the filters, we were able to calculate the concentration of particulate matter per liter of water as showed in figure II.S2.

Chapter III

As presented in the previous chapter, MeHg concentrations higher than the bottom sediments were found in the suspended particles collected in Lake Geneva water column. Moreover, no other source were found to be able to produce the MeHg concentrations on the suspended particles, other than the in-situ production in the *lake snow*. In chapter III the second module of the project will be developed following the results and conclusions of the second chapter, by designing a new experiment to determine the presence of anoxic micro-environments inside the *lake snow* in order to further confirm the hypothesis that in-situ production was the process responsible for the MeHg found on the suspended particles along the water column of Lake Geneva.

This chapter was proposed as an article for publication in the Aquatic Science journal and it is now under revision.

Hypoxic and anoxic micro-environments in the water column of a peri-alpine lake: the potential role of *lake snow* in Hg methylation

Abstract

Hg methylation has been classically placed in the bottom sediments of aquatic environments due to the presence of anoxic layers at the sediment water interface. In fact, Hg methylation is commonly referred to the activity of microorganisms that are obligate anaerobes and need anoxic niches to be able to live and methylate. However, several researchers highlighted the importance of the water column in the production of MeHg in aquatic systems and especially the role of *marine* and *lake snow* in this process. In order for marine and *lake snow* to methylate Hg they need to have an anoxic environment capable of hosting the methylators. Following this hypothesis some researchers found oxygen gradients in *marine snow* and full anoxic micro-environments inside large aggregates floating along the marine water column, showing evidence that marine snow can actively sustain the activity of methylators. On the other hand, *lake snow* has been severely understudied in this regard and even if several authors proved the similarities that exists between *lake snow* and its marine counterpart, there are still only few works that aim to study *lake snow* as an actively micro-niche in the MeHg production in limnic environments. In this paper we present novel data showing that hypoxic and anoxic micro-environments exist in *lake snow* of Lake Geneva (Switzerland-France). Using sediment

traps coated with polyacrylamide gel, we sampled undisturbed settling particles, which were analyzed with an oxygen micro-probe. Our results showed hypoxic conditions in all the analyzed aggregates and a full anoxic micro-environment (0.22 mg/L of dissolved oxygen) in one sampled aggregate. These results are a first step in the identification of anoxic micro-environments in *lake snow* and they contribute in the hypothesis that *lake snow* represent an important step in the Hg methylation in limnic systems.

III.1. Introduction

Mercury (Hg) is a trace metal and a global pollutant of great concern, especially in its methylated forms, monomethylmercury and dimethylmercury, collectively referred to as methylmercury (MeHg). MeHg is a potent liposoluble neurotoxin with the ability to biomagnify across the food chain, posing a direct threat to human health and wildlife.

MeHg has been shown to be produced by various strains of microorganisms, including sulfate-reducing bacteria (SRB) (Compeau and Bartha, 1985; King et al., 2000), methanogens (Parks et al., 2013; Podar et al., 2015), iron-reducing bacteria (Bravo et al., 2018; Correia and Guimarães, 2017; Fleming et al., 2006; Si et al., 2015), Firmicutes (Gilmour et al., 2013) and fermenters (Peterson et al., 2020). In both freshwater and marine environments, bottom sediments are the most studied MeHg production compartments, but in recent years increasing emphasis has been placed on the water column as a new zone of MeHg production (Díez et al., 2016; Gallorini and Loizeau, 2021, 2022; Lehnherr et al., 2011). Several studies have focused on the possibility that MeHg production in the water column of oceans and lakes may take place inside micro- and macro-aggregates known as marine and *lake snow*, respectively (Díez et al., 2016; Grossart and Simon, 1993; Ortiz et al., 2015), where suitable conditions for Hg methylation could exist. Since all known methylating microorganisms are obligate anaerobes (Gilmour et al., 2013), anoxic conditions are required to support Hg methylation. Persistent oxygen and pH gradients around macro-aggregates of 1-4 mm in size (Alldredge and Cohen, 1987), and evidence of anoxic microenvironments with high microbial activity inside copepod carcasses floating in oxic water layers, was found in marine snow (Glud et al., 2015). Moreover, Ortiz et al. (2015) designed a microcosm in which marine settling particles were produced and, using isotopically enriched Hg spikes, they found Hg methylation rates comparable to those determined in bottom sediments. These findings suggest that anoxic microenvironments suitable for the MeHg-producing microbial population may exist inside marine snow. *Lake snow* has several similarities with marine snow in terms of abundance, chemical composition, settling velocity, microbial colonization, and bacterial production (Grossart and Simon, 1993); thus *lake snow* may have a similar potential to host a microbial population capable of Hg methylation. As mentioned,

anoxic conditions are necessary to sustain the activity of methylators, therefore, as our primary aim, we tested the presence of anoxic micro-environments in the *lake snow* of Lake Geneva (Switzerland – France), and showed that hypoxic and anoxic micro-environments are present in the aggregates across the water column. To fulfill this objective, we used sediment traps with polyacrylamide gel on the bottom, to collected undisturbed settling particles and aggregates to measure their internal dissolved oxygen (DO) concentrations using an oxygen microprobe. In addition, by means of scanning electron microscopy (SEM) and microscopic analysis, we characterized the main components of the *lake snow*, focusing particularly on the OM content, which is a key driver in the microbial activity that can promote the DO depletion and the formation of an anoxic micro-environment where Hg methylation can occur.

III.2. Study area

Lake Geneva is a warm monomictic lake of glacial origin situated between Switzerland and France (figure III.1). With a surface area of 580 km2, a volume of 89 km3 and a maximum depth of 309 m, Lake Geneva is one of the largest lakes in Western Europe. Its main tributary is the Rhone River, which enters at the eastern end of the lake and contributes about 73% of the water and 80% of detrital particles to the lake. The Rhone River originates in the Rhone Glacier, at an altitude of about 2200 m. The Upper Rhone watershed has a drainage area of 5238 km2, which represents about 70% of the total drainage of Lake Geneva (Burrus et al., 1990).

Lake Geneva was chosen as a study area for the following reasons: i) the presence of the LéXPLORE platform (Wüest et al., 2021), which greatly facilitated the sampling campaigns by giving an appropriate location to set up sampling devices; ii) the accessibility to the ancillary data of the water column from LéXPLORE database; iii) the proximity to our laboratories, which facilitates sample collection and reduces delivery time from the sampling site to the laboratory.

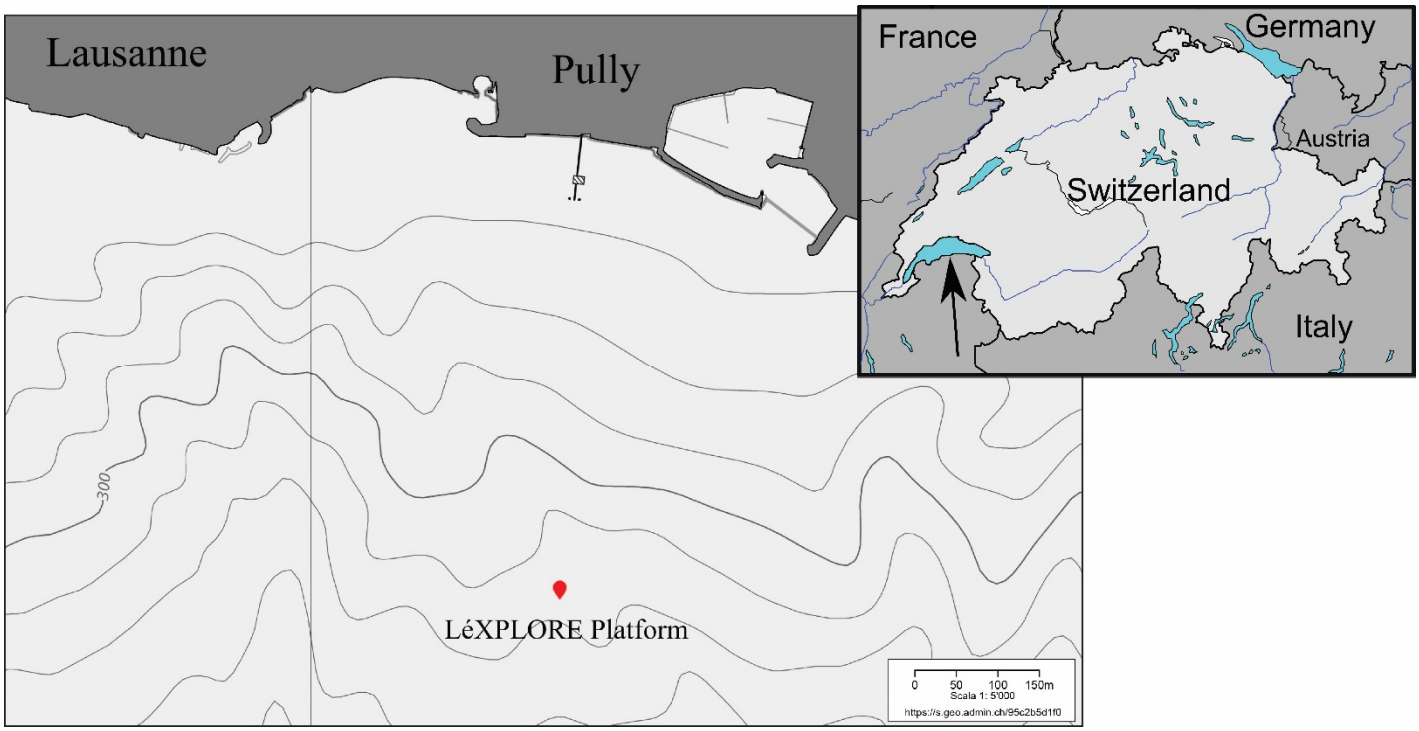


Figure III.1: Map of the study area. The platform is located in the eastern part of Lausanne in Lake Geneva, Switzerland. Contour lines indicate elevation (m asl), contour interval is 20 m; lake surface elevation is 372 m asl. Inset map. Location of Lake Geneva (black arrow) at the border between Switzerland and France.

The aforementioned platform is located east of the City of Lausanne (46°30'0.8″ N and 6°39'39.0″ E), 570 m from shore, anchored at a depth of 110 m (Figure III.1). The platform is located approximately 20 km from the mouth of the Rhone River.

III.3. Material and methods

III.3.1. Sampling setup

The sampling setup developed for this research was designed to meet the following requirements: i) to sample settling particles from different water depths simultaneously; ii) to collect settling particles with different sampling techniques required to perform different analysis; iii) to reduce as much as possible the biotic processes and avoid unwanted effects (artificial redox conditions). To respond to these requirements, 6 Plexiglas tubes of 80 cm length and 11 cm diameter were exposed at three different water depths (6 tubes per depth) using a nylon rope attached to a clamp weight and kept in tension by buoys at the surface. Depths were chosen to sample the epilimnion (13 m), the metalimnion (28 m) and the hypolimnion (100 m), and to be similar with the ones sampled in a previous work on the MeHg in the *lake snow* of Lake Geneva (Gallorini and Loizeau, 2022). At each sampling depth, 2 tubes were deployed with a 0.5 cm thick layer of polyacrylamide gel at the bottom of the tube, and 4 tubes without the gel. With this configuration, it was possible to sample undisturbed particles embedded in the gel for imaging and DO determination, and to collect particles for bulk OM analysis. The polyacrylamide gel was chosen as a viscous medium to collect the settling particles for its

following characteristics: ideal for imaging under a microscope because of its transparency; stable in water; very dense viscous medium able to keep particles unchanged due to its biological retarding proprieties; ideal for long term storage due to its capability to be frozen and thawed without any loss of structural characteristics (Jannasch et al., 1980; McDonnell et al., 2015). The gel was prepared following the method of Lundsgaard (1995) with slight modifications. To account for the sampling environment (freshwater), no salt was added and the gel was prepared using Milli-Q water to avoid any unwanted particulate. Two different brands of acrylamide solution at 40% were used in the preparation of the gel due to the shortage of the first one (VWR Acryl-40 solution), but the second brand (Applichem Acrylamide solution 40%) proved to be difficult to polymerize and didn't reach the viscosity of the first brand. Moreover, the gel produced with the second brand showed increased dissolved oxygen concentrations in the blank compared to the other brand. The reason for this effect is unknown.

The sediment traps were deployed in 2020 and 2021, during two periods of high MeHg concentrations in the settling particles Díez et al. (2018). For both years, the first deployment took place in the second half of July and the second one in early September. The exposure time for all sampling campaigns was one week in order to collect an ideal concentration of particles for subsequent analyses and imaging. During the July 2020 deployment, the sample at 13m depth was unfortunately lost due to a strong current that knocked over the sediment traps.

III.3.2. Laboratory analysis

After every retrieval, the sediment traps were frozen and completely freeze-dried in the case of the traps for OM analysis, and partially for the traps with polyacrylamide gel, in order to leave a small aliquot of water to avoid the complete dehydration of the gel. Specific fluxes of settling particles (g cm-2 d-1) were determined by dividing particle weights by exposition time (7 days) and total tube surface area (4 tubes per depth × 95 cm^2). Settling particles embedded in the polyacrylamide gel were used to obtain microscopic images of the particles and the macro-aggregates present in the collected samples, and to measure the concentrations of dissolved oxygen (DO) present inside these macro-aggregates and in the surrounding gel.

The DO measurements were carried out using an oxygen meter (Presens, Oxygen Meter OXY-1 SMA-BT) connected to an oxygen microprobe (Presens, Oxygen Microsensor PM-PSt7) with a tip diameter of ≈50 µm mounted on a manual micromanipulator (Marzhauser Wetzlar, MM 33 right). DO was measured in the macro-aggregates larger than 50 µm and in the particle-rich regions of the gel, when macro-aggregates were absent from the sample. Under a microscope (Olympus BX40, 4x) and using the micromanipulator, the microprobe was inserted into the selected aggregate and left for several minutes to stabilize (figure III.S1). All sampled gels were investigated using microscopic

imaging to identify suitable aggregates for the analysis. All suitable aggregates were then analyzed for DO concentrations. A very low number of aggregates suitable for the analysis (large enough for the microprobe to enter) were available, ranging from 3 to 6 per depth, and whenever large aggregates were not present (e.g. July samplings), a similar number of measurements were taken in particle-rich regions. Moreover, for every aggregate or particle-rich region analyzed, a blank value measured in a particle-free region of the gel was recorded to determine the DO concentration of the gel itself. The Presens probes were factory calibrated and a 2.0 M sodium sulfite (Na_2SO_3) solution was used to test the probes and to have an anoxic solution references (figure III.S2).

The settling particles collected in absence of polyacrylamide gel (particles and macro-aggregates) were characterized under optical microscope (Olympus BX40, 4x, 10x), and SEM. The SEM images were taken with secondary electrons using a Jeol JSM 7001F Scanning Electron Microscope system (Department of Earth Sciences, University of Geneva, Switzerland) on gold covered samples. In addition, a semi-quantitative analysis of the major component of the particles were carried out using an Energy-Dispersive X-Ray Spectroscopy (EDS) detector (model JEOL EX-94300S4L1Q) (Martignier et al., 2018). After the SEM analysis, the settling particles collected in the sediment traps without the gel were grinded. OM content was determined via loss on ignition (LOI), heating 1 g of sample at 550 °C for one hour (Heiri et al., 2001).

III.4. Results

III.4.1. Settling particles fluxes and organic matter contents

Sediment fluxes showed an increase with depth for all sampling periods, except for July 2020, and an overall higher flux in July (figure III.2). Comparing the same months in the different years, 2021 showed a higher sediment flux compared to 2020, marked in July and relatively small in September. Organic matter content in the settling particles varied from 1.60 % ± 0.54 % to 9.73 % ± 0.45 %. OM contents were lower in 2020 than in 2021 (figure III.S3). In 2020, OM was higher in July than in September, whereas in 2021, similar values were recorded during the two sampling periods. This similarity was probably linked to the strong rainfalls that occurred during the summer 2021, especially during May, June and July (FOMC, 2021), which tend to increase the particle inputs into the lake from the watershed. Moreover, no difference in the OM percentage was found with depth apart for July 2021 that showed a decrease in the OM content with depth.

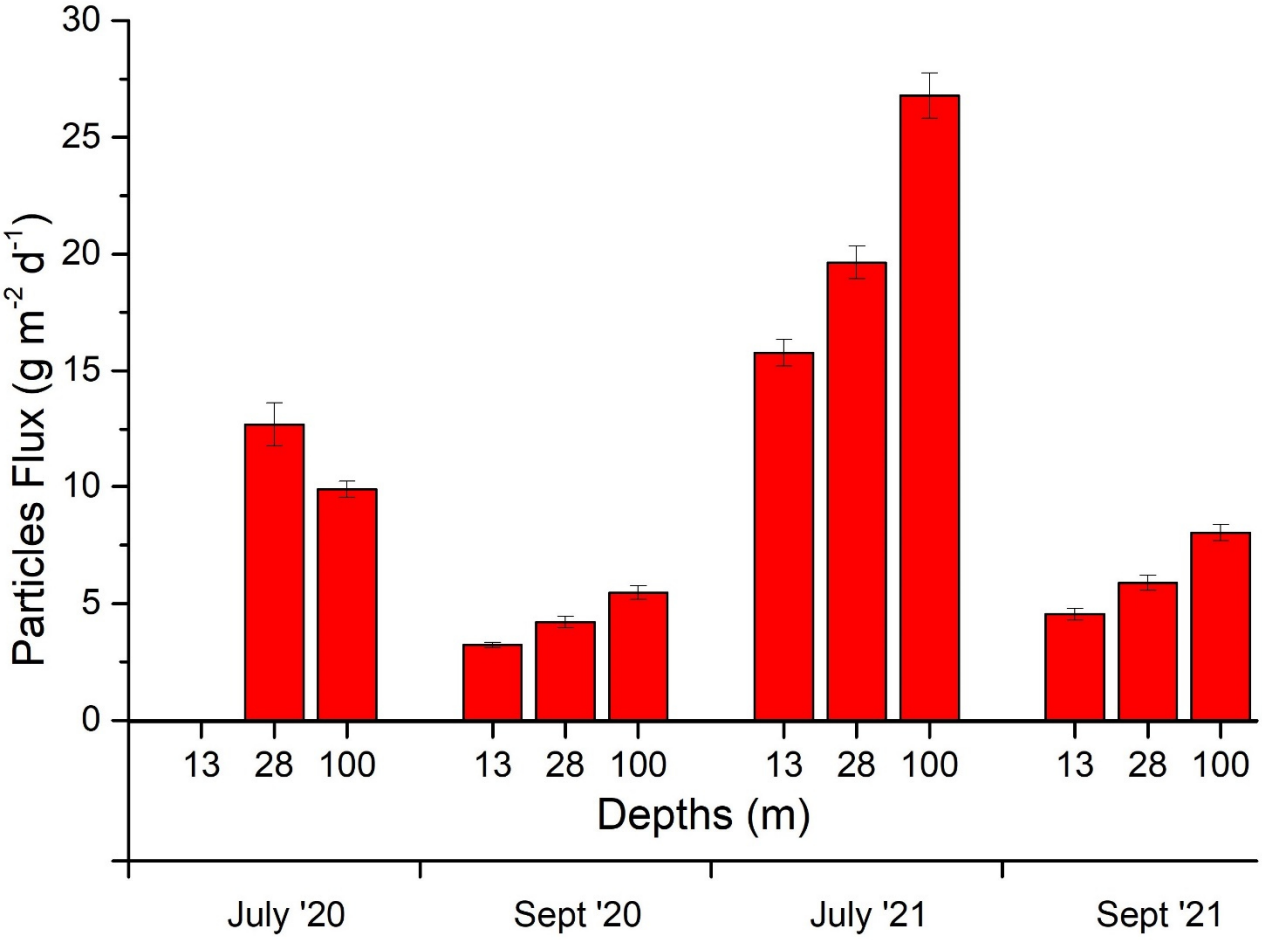


Figure III.2: Sediment flux into the sediment traps at all depths and sampling periods.

III.4.2. Microscopic imaging and SEM analysis

Microscopic imaging was carried out on the settling particles collected in the sediment traps without gel, to identify the main components of the settling particles. Differences in the macro-components were observed between the July and September sampling campaigns, while no differences were found between the same sampling period over the two years (July 2020 ≈ July 2021; September 2020 ≈ September 2021).

The settling particles sampled in July were mainly composed of an inorganic mineral fraction and by various organic-derived components like cyanobacteria and diatoms (figure III.S4), with no noticeable differences between depths. A similar composition was observed in September but, unlike the July particles, additional organic components like red algae (figure III.S5) and other organic components (figure III.S6) were present. Moreover, a fragment of a small crustacean was found among the particles from 100 m depth (figure III.S7).

The SEM analyses had the objective to determine the nature of the micro components of the settling particles and to have an estimation of the main chemical composition. Regarding the mineralogy, the settling particles are mainly composed of calcite (figure III.S8-S9 and III.S10-S12) and

phyllosilicates (clay) (figure III.S10 and III.S11). In September only, the presence of gypsum ($CaSO_4$) was noted (figure III.S13 and III.S14), which may be related to a more intense mineralization of the organic matter bound to the settling particles, releasing sulphur into the surrounding environment (Coleman and Raiswell, 1993). Concerning the biological components, SEM observations revealed the presence of diatoms (figure III.S15), green algae (figure III.S16) identified as the genus *Phacotus* (figure III.S17), and probably *Ceratium hirundinella* (figure III.S18). Moreover, in almost all the investigated settling particles, we observed small regions covered by films of organic matter. In some of these regions, we have identified the presence of bacteria (figure III.S19).

III.4.3. Differences in aggregate structure between sampling periods

During this work, different particle aggregation states were analyzed and called by different names to easily identify them. This classification was built around our sampling resolution given by the microprobe tip (≈ 50 µm), and it consists of: i) macro-aggregate: coherent aggregate of particles with a size equal or bigger than 100 µm (during the analysis we defined this size as a minimum for the tip to easily enter and to not be influenced by the surrounding gel); ii) micro-aggregate: coherent aggregate of particles with a size lower than 100 µm; iii) particle: the smaller fraction of the settling particles with a size inferior to 10 µm; iv) particles-rich region: a region in the gel with high concentration of particles and micro-aggregates which create a non-coherent aggregate often called loose aggregate.

The aggregate types between the two sampling months in both years (2020-2021) are quite different in structure. In July, we do not record the presence of macro-aggregates but only particles and micro-aggregates forming loose aggregates (figure III.3). This type of aggregates turns out to breaks up easily upon the insertion of the microprobe, allowing oxygen from the outer layers of the gel to diffuse into the aggregate, as evidenced by the strong DO increase within the first 2 minutes after the beginning of the analysis (figure III.3).

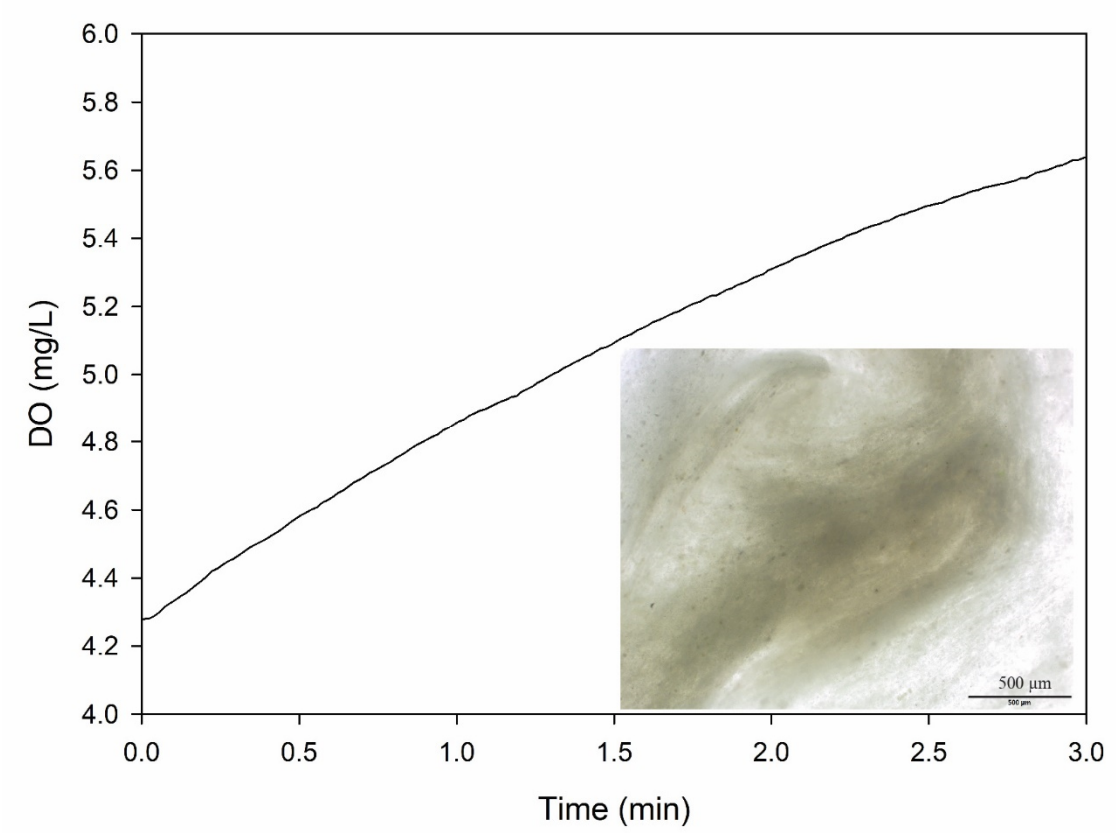


Figure III.3: DO concentrations found in a particle aggregate from the July campaign 2020. The sample was collected at a depth of 28m. Inset: Microphotography of the aggregate analyzed.

On the other hand, macro-aggregates sampled during the September campaigns were more compact (figure III.4) than the July aggregates, and at the insertion of the microprobe, no DO increase was recorded within two minutes of the insertion of the microprobe (figure III.4). The stronger increase in oxygen observed in the September sample after the first 2 minutes (figure III.4), which was more gradual in the July sample (figure III.3), is probably due to the actual size of the two aggregates.

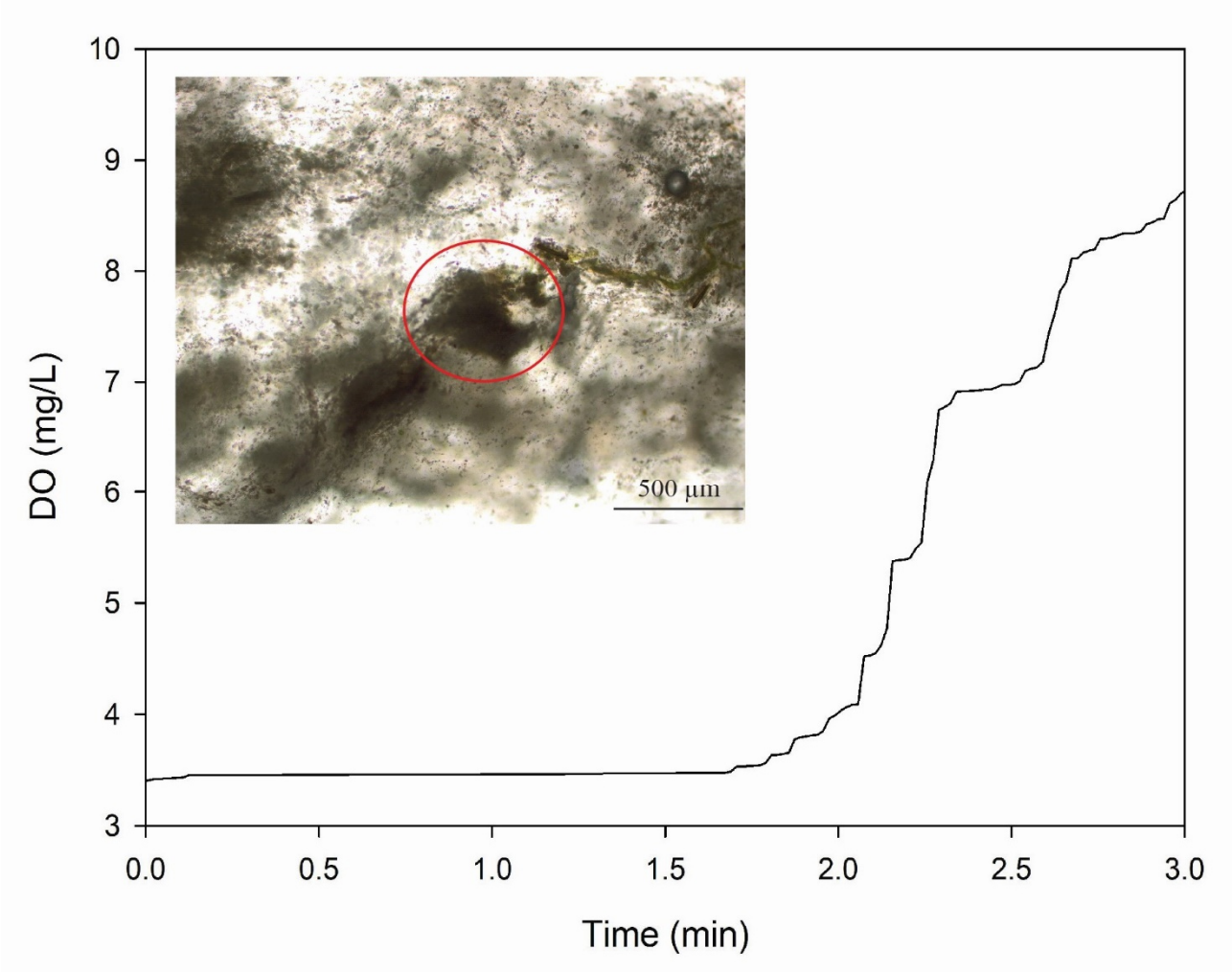


Figure III.4: DO concentrations found in a particle aggregate from the September campaign 2020. The sample was collected at a depth of 28m. Included in the graph the image of the aggregate analyzed (inside the red circle).

Among the several macro-aggregates investigated in the September samples, one recorded much lower DO concentration (0.22 mg/L) that the others (figure III.5), indicating the presence of a fully anoxic environment inside the aggregate. It was the darkest and the biggest among all the analyzed samples.

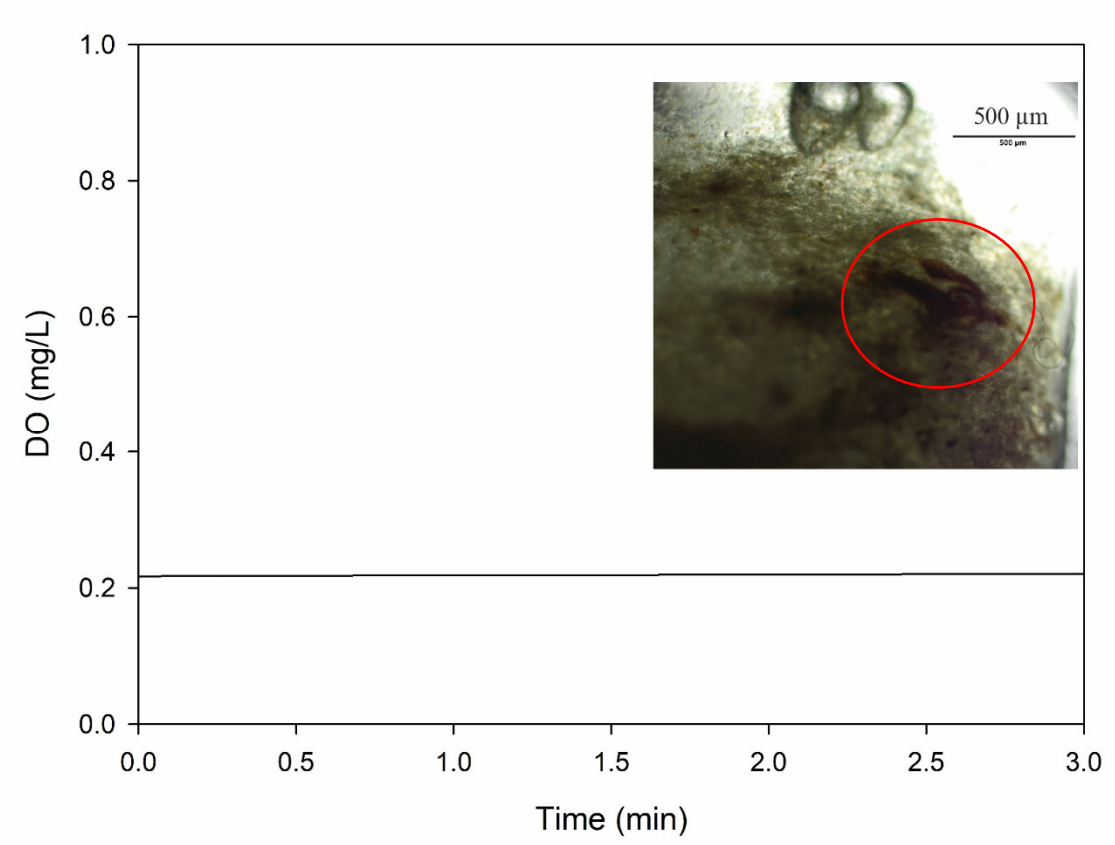


Figure III.5: DO concentrations found in a particle aggregate from the September campaign 2020 at 13m depth. Included in the graph the images of the aggregate analyzed (inside the red circle).

III.4.4. Dissolved oxygen in the settling particles

Dissolved oxygen measurements in the macro-aggregates and loose aggregates showed oxygen concentrations ranging from 0.22 mg/L to 8.61 mg/L, while gel blanks (free of particles) showed oxygen concentrations ranging from 8.76 mg/L to 11.55 mg/L (figure III.6). The DO concentrations in the water column ranged from 8.24 mg/L to 9.99 mg/L (Datalakes, 2021), showing values comparable to most measured gel blanks. However, some gel blanks showed values significantly higher than DO concentrations of the water column. As mentioned before, the reason of this effect remains unclear; it may be due to differences between the two brands of acrylamide solution as polymerization was difficult to reach for the gels in which high levels of oxygen are observed. As described by Smith (1994) presence of oxygen in the stock solution of acrylamide can result in problem reaching the polymerization, so it can be possible that the second brand of acrylamide contains more DO than the first one.

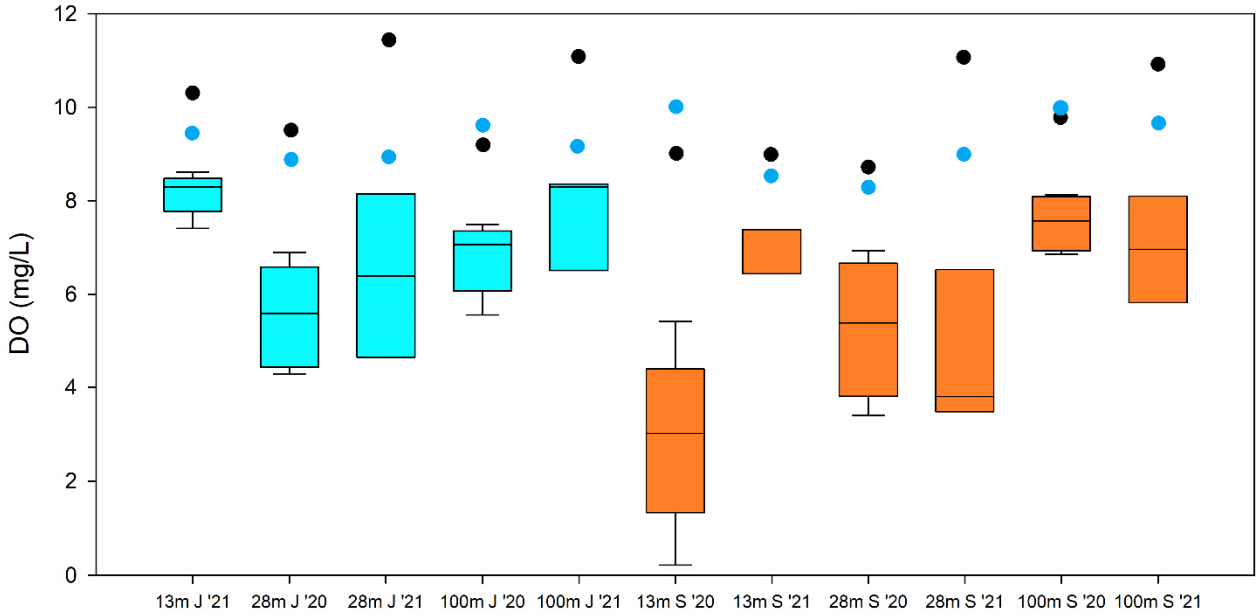


Figure III.6: Box plot of DO concentrations found in the settling particles from July (J) and September (S) for both sampled years. The concentrations are plotted with the average DO concentrations (black circles) of the blank (gel without particles) measured on that particular gel and the average DO concentrations found in the water column at the same sampling depths (blue circles) (Datalakes, 2022). The boxes represent the range of values determined in the loose aggregate (cyan, July samples) and in the macro-aggregates (orange, September samples). The bars represent the minimum and maximum recorded values, if they are outside the first and third quartile (limits of the boxes), and the median (bar inside the boxes).

DO analysis on lake macro-aggregates and loose aggregates showed oxygen concentrations from 16% to 98% lower than the measured gel blanks and from 5% to 98% than DO concentrations in the water column, indicative of an oxygen gradient in the aggregates. Only one macro-aggregate collected in September 2020 from 13m harbored a fully anoxic micro-environment, while the other aggregates from this depth showed higher levels of oxygenation. Furthermore, the aforementioned differences in the oxygen saturation of the gels could have impacted some of the measurements taken inside the loose aggregates (cyan boxes in figure III.6), where some gel exists between the different particles and micro-aggregates, determining an increase in the DO concentrations. This effect probably did not occur during the measurements of the macro-aggregates (orange boxes in figure III.6), due to their more compact nature, which kept the interior of the aggregate free from the gel interference. However, we do not see much difference between July and September samples, apart for the 13m 2020 sample, thus this effect was probably not very important.

III.5. Discussion

III.5.1. Aggregates characterization

OM content of the settling particles (figure III.S3) showed higher values during 2021 in both months (July and September) compared to 2020. This trend can be linked to the aforementioned difference between the July and September aggregates. In the 2020 sampling campaigns, July samples had twice

the OM percentages of September samples. Following our hypothesis, this differences in the OM contents between July and September could be linked to the different settling velocities of the particles and aggregates. Dyer and Manning (1999) found in estuarine systems that flocculation is inversely related to the concentration of suspended particles and the shear stress (wind induced in lake systems), with the concentration of the particles having a greater effect than the shear stress. During July, the higher concentrations of particles suggested by the higher sediment flux (figure III.2) and the higher shear velocities induced by the wind during the exposure time (from $7.293*10^{-5}$ m/s to 0.025 m/s and from $7.592*10^{-5}$ m/s to 0.021 m/s 2020 and 2021 respectively (Datalakes, 2021)), induced less flocculation, therefore keeping the particles separated, as highlighted by the loose aggregates in figure III.3. In contrast, in September, lower concentrations of particles (lower sediment flux in figure 2), and lower shear velocities during the exposure time (from $7.302*10^{-5}$ to 0.014 m/s and from $7.778*10^{-5}$ to 0.014 m/s in 2020 and 2021, respectively (Datalakes, 2021)) increased flocculation that produced the macro-aggregates as depicted in figures III.4 and III.5. Moreover, Dyer and Manning (1999) observed that macro-aggregates tend to break down due to the different densities of their components (inorganic and organic), creating organic and inorganic micro-aggregates that, in turn, have different settling velocities. This process keeps the particulate OM in the water column longer, thus decreasing the amount of OM collected by the sediment traps. Interestingly, this behavior is not observed in 2021, with values from the two sampling periods showing similar ranges of OM content (figure III.S3). This behavior is probably linked to the heavy rainfall that occurred in the summer period of 2021. In May, June and July 2021, monthly rainfall rates were 201 mm, 174 mm and 291 mm, respectively (FOMC, 2021), values significantly higher than those of 2020, which were 72 mm, 146 mm and 31 mm, respectively (FOMC, 2021). This exceptional rainfall could have mobilized terrigenous OM particles from the lake watershed, which is composed in majority by urban, forest and cultivated areas (FOEN) in the portion of the watershed that feeds the small rivers that enter into the lake near the platform. For September, the rainfall values in 2020 and 2021 were more similar (83 mm and 74 mm, respectively), so the high OM content is probably related to the primary production of the lake. Comparison of chlorophyll-a content between the sampling periods showed that July and September 2020 had similar values (from 2.8 to 13 g/L and from 3.5 to 9.9 g/L respectively), while in 2021, July and September showed very different chlorophyll-a concentrations (from 2.7 to 4.2 g/L and from 15 to 24 g/L, respectively) (Datalakes, 2021). As chlorophyll-a is mainly a product of lake primary production, it can be used to hint the differences in lake production for the aforementioned periods. September 2021 showed an increase in chlorophyll-a at least 4 times higher than the other periods, probably linked to the terrestrial OM came from the near watershed during the extreme rain events of July, which cause an increase in nutrients that lead to an increase primary production during September.

Hoover et al. (2010) showed that terrestrial OM tend to sink in aquatic environments with average settling velocities ranging between 0.69 cm/s to 6.03 cm/s, depending on the kind of OM, after complete water imbibition. These allochthonous OM particles settle faster than the fresh OM produced in-situ, thus they reach the bottom, and the sediment traps, at a much higher velocity.

Through SEM and EDS analyses, we identify small regions in many aggregates, found at all depths and sampling periods, covered with OM coatings (figure III.S19). These OM coatings were identified by a high concentration of carbon. In many of these zones, bacteria were found (figure III.S19), whose metabolic activity could represent the first step in creating anoxic conditions.

SEM imaging analysis allow us to recognize, among the macro components of the OM portion of the settling particles, the presence of phytoplankton (figure III.S17 and III.S18) and small crustaceans (figure III.S7), which represent the base of the food chain. Other OM components were impossible to recognize due to degradation, or present as just films of gooey matter (figure III.S19).

III.5.2. Particle aggregates: hypoxic niche in the oxic water column

As shown in figures III.3 and III.4, the density of the aggregates evolves between July and September, with the latter showing macro-aggregates while July samples were composed of loose aggregates only. The difference in the type of aggregates between July and September is probably linked to the effect of the Rhone River during July. In summer, the particle load from the Rhone River increases the overall flux of particles in the water column (figure III.2) (Halder et al., 2013) and the particles concentrations, which in turn lessens the effect of flocculation (Dyer and Manning, 1999). In September, the particle input from the Rhone River is less than in July and the large organic aggregates from primary in situ primary production, which form the *lake snow*, are more prevalent in the settling particles.

The difference between the two types of aggregates present in July and in September created a methodological bias for the measured DO concentrations between the two months. In July, the high density regions analyzed are composed of micro-aggregates clumped together to form a region of high particle density. It is likely that anoxic micro-environments lie inside these micro-aggregates (<100 µm), impossible to investigate with the resolution of the microprobe used (≈50 µm). Thus, the probe probably measured the DO gradient that these anoxic micro-aggregates generate in their close proximity. On the other hand, the larger size of the aggregates collected in September (≥100 µm) allowed the microprobe to be entirely inside the aggregates and to make an unbiased measurement of the DO levels.

Only one macro-aggregates showed a fully anoxic micro-environment, and despite an extensive search for similar aggregates, it was impossible to find other macro-aggregates with the same characteristics. This paucity of fully anoxic aggregates is probably linked to the sampling method

used. Our hypothesis is that these large aggregates are few in number relative to other particles and aggregate size fractions; and they have a high organic content and thus low settling velocities, which reduces their downward flux in the water column and thus decreasing their presence in the sediment traps.

Ortiz et al. (2015) conducted research on marine snow, the sea counterpart of *lake snow*, and its suitability to provide favorable conditions for Hg methylation. These authors produced marine snow in a microcosm using estuarine particles and enriched them with Hg isotopes to determine the methylation rate. Their results showed that the dominant particle-size mass fraction was the 0.2 - 8 µm fraction, followed by the 8 - 300 µm fraction (both collectively called marine aggregates) and finally the >300 µm fraction (actual marine snow). This is in line with our observations on the polyacrylamide gels, where a total of 22 macro-aggregates (>100 µm) were observed and analyzed, while micro-aggregates and particles (<100 µm and <10 µ respectively) were several times more numerous (hundreds of them), even if only 22 were analyzed to match the number of the macro-aggregates. Moreover, Ortiz et al. (2015) showed that the large particle fraction (>300 µm) had the highest methylation rate, followed by the 8 - 300 µm fraction. This highlights the possibility that even in small aggregates, there may be an anoxic micro-environment where Hg methylation can take place. This is consistent with the results on the DO analysis performed on the loose aggregates from the July campaigns (figure III.3), which showed hypoxic conditions around these micro-aggregates.

Furthermore, Stabel (1987) showed in Lake Constance that settling velocities of particles with high organic content can reach a minimum of 0.4 m/d, which is in agreement with our hypothesis that aggregates settle at low velocities and are under-collected by sediment traps, unless they reach a size that allows them to reach higher settling velocities. Moreover, Grossart and Simon (1998) found that in Lake Constance, *lake snow* settling velocities depended on size, form and composition of the different aggregates. They found that highly organic (cyanobacteria), “small” aggregates (3 mm diameter) settle at 5.2 ± 1.3 m/d, while bigger diameters (22 mm), less organic (large diatoms) sink at 25.3 ± 7.4 m/d. In our analyses, we consider highly organic aggregates with sizes at least 10 time smaller than the smallest sample from Grossart and Simon (1998), thus they probably have also much lower settling velocities, due to the velocity being related to the square of the diameter. The smallest aggregates (around or lower than 100 µm) could even reach the settling velocities determined by Stabel (1987).

The presence of these hypoxic and anoxic micro-environments highlights the ability of the particulate present along the lake water column to sustain MeHg production, which agrees with (Gallorini and Loizeau, 2022), who reported significant MeHg concentrations in the suspended matter along the water column of Lake Geneva, and which most likely originated from in situ production within the suspended particles in the water column.

The capability of *lake snow* to be a micro-niche for the production of MeHg in an oxic environment has important repercussion on the exposure of biota (especially fish) to MeHg. Comparison between bottom sediments and *lake snow* MeHg concentrations reveals that the *lake snow* yield more MeHg than the sediments (10% to 77% increase of MeHg into the *lake snow*) (Gallorini and Loizeau, 2022). These findings highlight that the water column could be as important, if not more, than the most referred MeHg production zone (i.e. the bottom sediments). This has great importance for understanding the food chain pathways, as MeHg concentrations in seston predicted 63% of MeHg concentrations in fish (Wu et al., 2019). Having greater MeHg concentrations in the water column, where most fish feed, could make *lake snow* the main source of MeHg for the food chain. As fish consumption is the primary pathway of human exposure to MeHg (Fitzgerald and Lamborg, 2014), it is important to quantify the MeHg production driven by *lake snow* in order to assess its impact on lakes biota and the human diets.

Additional analyses are needed to further investigate the *lake snow* as an anoxic micro-niche capable of Hg methylation. OM specific analysis and characterization of the aggregates (e.g. C/N ratio) could be a major help to better characterize their level of OM mineralization. However, a different sampling protocol might be necessary, because with the present setup, it was impossible to remove the aggregates from the gel and therefore to analyze the OM in the aggregates. Collecting enough material could be sufficient to perform OM analysis in the bulk of the settling particles, but characterization of OM on the same aggregates used for DO analysis would be important, especially if it is possible to characterize the elemental composition of the OM in the aggregates in a same way as for DO analysis with some sort of micro-probe. This characterization of OM could be linked to the metabolism of the methylators inside the *lake snow* highlighting the most probable ones.

Moreover, more samplings are needed to collect data on the DO concentrations of *lake snow* in order to build a more solid dataset with a robust statistical relevance.

Furthermore, the sampling and analytical methods used in this work proved to be promising in the detection of suboxic and anoxic conditions in the *lake snow*, but further improvements are needed to increase its efficiency and capabilities. First and foremost, the use of a micro-probe with a smaller tip can increase the size resolution and allow to analyze smaller aggregates. Moreover, the use of a polyacrylamide gel to collect aggregates does not allow other analysis (e.g. OM characterization) to be performed on the same sample. Therefore, a different sampling configuration, which needs to have specific characteristics, should be identified and applied: i) fast recovery of aggregates or inhibition of microorganism processes to avoid unwanted redox transformations; ii) possibility to use the sampled aggregates for different types of analysis; iii) ability to sample aggregates with low settling velocities with a good recovery efficiency.

III.6. Conclusions

Suboxic to anoxic conditions were detected in the lake aggregates. The combined use of polyacrylamide gel and sediment traps shown to be efficient for collecting settling particles without creating unwanted redox change in the traps and avoiding OM mineralization. The biological retarding effect of the gel (Jannasch et al., 1980) helped to prevent the OM to be further mineralized in the traps, avoiding an artificial alteration of the redox conditions inside the aggregates. SEM imaging analysis of the aggregates showed the presence of bacteria in OM-rich zones, highlighting the presence of OM mineralization that can subsequently give rise to the formation of an anoxic micro-environment in the aggregates. The presence of this anoxic environment, populated by methylators, capable of Hg methylation, inside *lake snow* could represent a direct link of MeHg into the lake food chain, especially fish which are the main pathways for MeHg to reach the human diet. Moreover, (Gallorini and Loizeau, 2022) found that the suspended particles of Lake Geneva present significant MeHg concentrations, which, coupled with the findings of this work, highlight the potential for *lake snow*, like marine snow, to be an important site of Hg methylation and to cover an important role into the MeHg cycle into the limnic environment.

Further analysis is needed to better characterize *lake snow* and its potential to host Hg methylation. OM characterization is needed to identify the level of mineralization and the overall maturation of the OM substrate with depth. Moreover, identification of the microorganism communities is needed to determine which strains are present in the *lake snow* and to identify the potential methylators.

III.7. Acknowledgments

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III.8. References

Alldredge, A. L., and Cohen, Y., 1987, Can Microscale Chemical Patches Persist in the Sea? Microelectrode Study of Marine Snow, Fecal Pellets: Science, v. 235, no. 4789, p. 689-691.

Bravo, A. G., Zopfi, J., Buck, M., Xu, J., Bertilsson, S., Schaefer, J. K., Poté, J., and Cosio, C., 2018, Geobacteraceae are important members of mercury-methylating microbial communities of sediments impacted by waste water releases: The ISME Journal, v. 12, no. 3, p. 802-812.

Burrus, D., Thomas, R. L., Dominik, B., Vernet, J. P., and Dominik, J., 1990, Characteristics of suspended sediment in the Upper Rhone River, Switzerland, including the particulate forms of phosphorus: Hydrological Processes, v. 4, p. 85-98.

Coleman, M. L., and Raiswell, R., 1993, Microbial mineralization of organic matter: mechanisms of self-organization and inferred rates of precipitation of diagenetic minerals: Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, v. 344, no. 1670, p. 69-87.

Compeau, G. C., and Bartha, R., 1985, Sulfate-Reducing Bacteria: Principal Methylators of Mercury in Anoxic Estuarine Sedimentt: APPL. ENVIRON. MICROBIOL., v. 50, no. 2, p. 498-501.

Correia, R. R. S., and Guimarães, J. R. D., 2017, Mercury methylation and sulfate reduction rates in mangrove sediments, Rio de Janeiro, Brazil: The role of different microorganism consortia: Chemosphere, v. 167, p. 438-443.

Datalakes, 2021.

Díez, E. G., Graham, N. D., and Loizeau, J.-L., 2018, Total and methyl-mercury seasonal particulate fluxes in the water column of a large lake (Lake Geneva, Switzerland): Environmental Science and Pollution Research, v. 25, no. 21, p. 21086-21096.

Díez, E. G., Loizeau, J.-L., Cosio, C., Bouchet, S., Adatte, T., Amouroux, D., and Bravo, A. G., 2016, Role of Settling Particles on Mercury Methylation in the Oxic Water Column of Freshwater Systems: Environmental Science & Technology, v. 50, no. 21, p. 11672-11679.

Dyer, K. R., and Manning, A. J., 1999, Observation of the size, settling velocity and effective density of flocs, and their fractal dimensions: Journal of Sea Research, v. 41, p. 87-95.

Fitzgerald, W. F., and Lamborg, C. H., 2014, Geochemistry of Mercury in the Environment, In: Treatise on Geochemistry (Second Edition): Oxford, Elsevier,, p. 91-129.

Fleming, E. J., Mack, E. E., Green, P. G., and Nelson, D. C., 2006, Mercury Methylation from Unexpected Sources: Molybdate-Inhibited Freshwater Sediments and an Iron-Reducing Bacterium: Applied and Environmental Microbiology, v. 72, no. 1, p. 457-464.

FOEN, 2022, Land Cover, accessed 25 January 2022, https://www.geo.admin.ch/en/geo-information-switzerland/geodata-index-inspire/surface-representation/land-cover.html

FOMC, 2021, Swiss Federal Office of Meteorology and Climatology.

Gallorini, A., and Loizeau, J.-L., 2021, Mercury methylation in oxic aquatic macro-environments: a review: Journal of Limnology.

-, 2022, Lake snow as a mercury methylation micro-environment in the oxic water column of a deep peri-alpine lake: Chemosphere, p. 134306.

Gilmour, C. C., Podar, M., Bullock, A. L., Graham, A. M., Brown, S. D., Somenahally, A. C., Johs, A., Hurt, R. A., Bailey, K. L., and Elias, D. A., 2013, Mercury Methylation by Novel Microorganisms from New Environments: Environmental Science & Technology, v. 47, no. 20, p. 11810-11820.

Glud, R. N., Grossart, H.-P., Larsen, M., Tang, K. W., Arendt, K. E., Rysgaard, S., Thamdrup, B., and Gissel Nielsen, T., 2015, Copepod carcasses as microbial hot spots for pelagic denitrification: Copepod carcasses and denitrification: Limnology and Oceanography, v. 60, no. 6, p. 2026-2036.

Grossart, H.-P., and Simon, M., 1993, Limnetic macroscopic organic aggregates (lake snow): Occurrence, characteristics, and microbial dynamics in Lake Constance: Limnology and Oceanography, v. 38, no. 3, p. 532-546.

Grossart, H., and Simon, M., 1998, Significance of limnetic organic aggregates (lake snow) for the sinking flux of particulate organic matter in a large lake: Aquatic Microbial Ecology, v. 15, p. 115-125.

Halder, J., Decrouy, L., and Vennemann, T. W., 2013, Mixing of Rhône River water in Lake Geneva (Switzerland–France) inferred from stable hydrogen and oxygen isotope profiles: Journal of Hydrology, v. 477, p. 152-164.

Heiri, O., Lotter, A. F., and Lemcke, G., 2001, Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results: Journal of Paleolimnology, v. 25, p. 101-110.

Hoover, T. M., Marczak, L. B., Richardson, J. S., and Yonemitsu, N., 2010, Transport and settlement of organic matter in small streams: Freshwater Biology, v. 55, no. 2, p. 436-449.

Jannasch, H. W., Zafiriou, O. C., and Farrington, J. W., 1980, A sequencing sediment trap for time-series studies of fragile particles: Limnology and Oceanography, v. 25, p. 939-943.

King, J. K., Kostka, J. E., Frisher, M. E., and Saunders, F. M., 2000, Sulfate-Reducing Bacteria Methylate Mercury at Variable Rates in Pure Culture and in Marine Sediments: Applied and Environmental Microbiology, v. 66, no. 6, p. 2430-2437.

Lehnherr, I., St. Louis, V. L., Hintelmann, H., and Kirk, J. L., 2011, Methylation of inorganic mercury in polar marine waters: Nature Geoscience, v. 4, no. 5, p. 298-302.

Lundsgaard, C., 1995, Use of a high viscosity medium in studies of aggregates: Sediment trap studies in the Nordic Countries, v. 3, p. 141-152.

Martignier, A., Filella, M., Pollok, K., Melkonian, M., Bensimon, M., Barja, F., Langenhorst, F., Jaquet, J.-M., and Ariztegui, D., 2018, Marine and freshwater micropearls: biomineralization producing strontium-rich amorphous calcium carbonate inclusions is widespread in the genus Tetraselmis (Chlorophyta): Biogeosciences, v. 15, no. 21, p. 6591-6605.

McDonnell, A. M. P., Lam, P. J., Lamborg, C. H., Buesseler, K. O., Sanders, R., Riley, J. S., Marsay, C., Smith, H. E. K., Sargent, E. C., Lampitt, R. S., and Bishop, J. K. B., 2015, The oceanographic toolbox for the collection of sinking and suspended marine particles: Progress in Oceanography, v. 133, p. 17-31.

Ortiz, V. L., Mason, R. P., and Evan Ward, J., 2015, An examination of the factors influencing mercury and methylmercury particulate distributions, methylation and demethylation rates in laboratory-generated marine snow: Marine Chemistry, v. 177, p. 753-762.

Parks, J. M., Johs, A., Podar, M., Bridou, R., Hurt, R. A., Smith, S. D., Tomanicek, S. J., Qian, Y., Brown, S. D., Brandt, C. C., Palumbo, A. V., Smith, J. C., Wall, J. D., Elias, D. A., and Liang, L., 2013, The Genetic Basis for Bacterial Mercury Methylation: Science, v. 339, no. 6125, p. 1332-1335.

Peterson, B. D., McDaniel, E. A., Schmidt, A. G., Lepak, R. F., Janssen, S. E., Tran, P. Q., Marick, R. A., Ogorek, J. M., DeWild, J. F., Krabbenhoft, D. P., and McMahon, K. D., 2020, Mercury Methylation Genes Identified across Diverse Anaerobic Microbial Guilds in a Eutrophic Sulfate-Enriched Lake: Environmental Science & Technology, v. 54, no. 24, p. 15840-15851.

Podar, M., Gilmour, C. C., Brandt, C. C., Soren, A., Brown, S. D., Crable, B. R., Palumbo, A. V., Somenahally, A. C., and Elias, D. A., 2015, Global prevalence and distribution of genes and microorganisms involved in mercury methylation: Science Advances, v. 1, no. 9, p. e1500675-e1500675.

Si, Y., Zou, Y., Liu, X., Si, X., and Mao, J., 2015, Mercury methylation coupled to iron reduction by dissimilatory iron-reducing bacteria: Chemosphere, v. 122, p. 206-212.

Smith, B. J., 1994, SDS Polyacrylamide Gel Electrophoresis of Proteins, Humana Press, p. 23-34.

Stabel, H.-H., 1987, Settling velocity and residence time of particles in Lake Constance: Schweiz. Z. Hydrol., v. 49, p. 284-293.

Wu, P., Kainz, M. J., Bravo, A. G., Åkerblom, S., Sonesten, L., and Bishop, K., 2019, The importance of bioconcentration into the pelagic food web base for methylmercury biomagnification: A meta-analysis: Science of The Total Environment, v. 646, p. 357-367.

Wüest, A., Bouffard, D., Guillard, J., Ibelings, B. W., Lavanchy, S., Perga, M. E., and Pasche, N., 2021, LéXPLORE : A floating laboratory on Lake Geneva offering unique lake research opportunities: WIREs Water.

Supplementary Material

Figure III.S1: Example of a DO analysis of the aggregates in the polyacrylamide gel. In the image the tip of the micro-probe inserting into an aggregate. In the red circle the probe tip (≈50 µm).

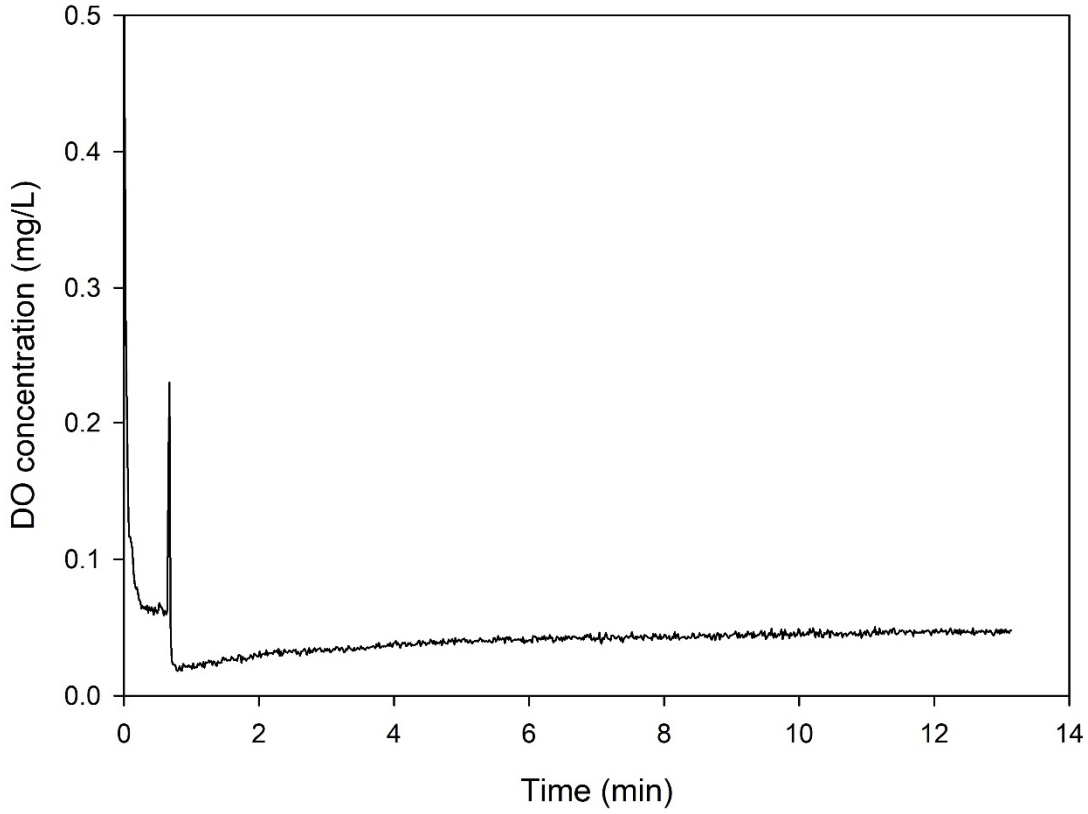

Figure III.S2: DO concentration measured in the sodium sulphite solution.

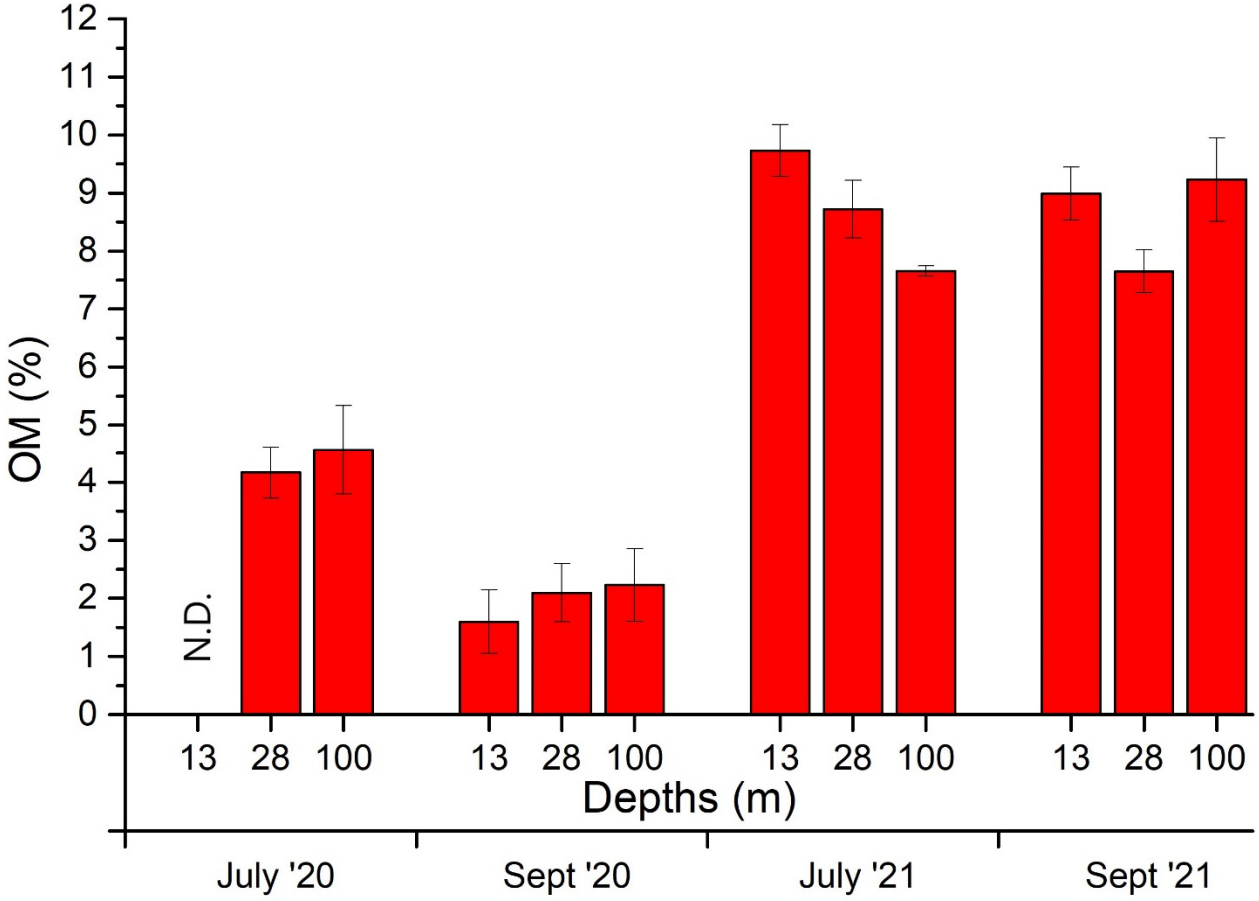

Figure III.S3: OM percentages measured in the settling particles for every depth and sampling periods.

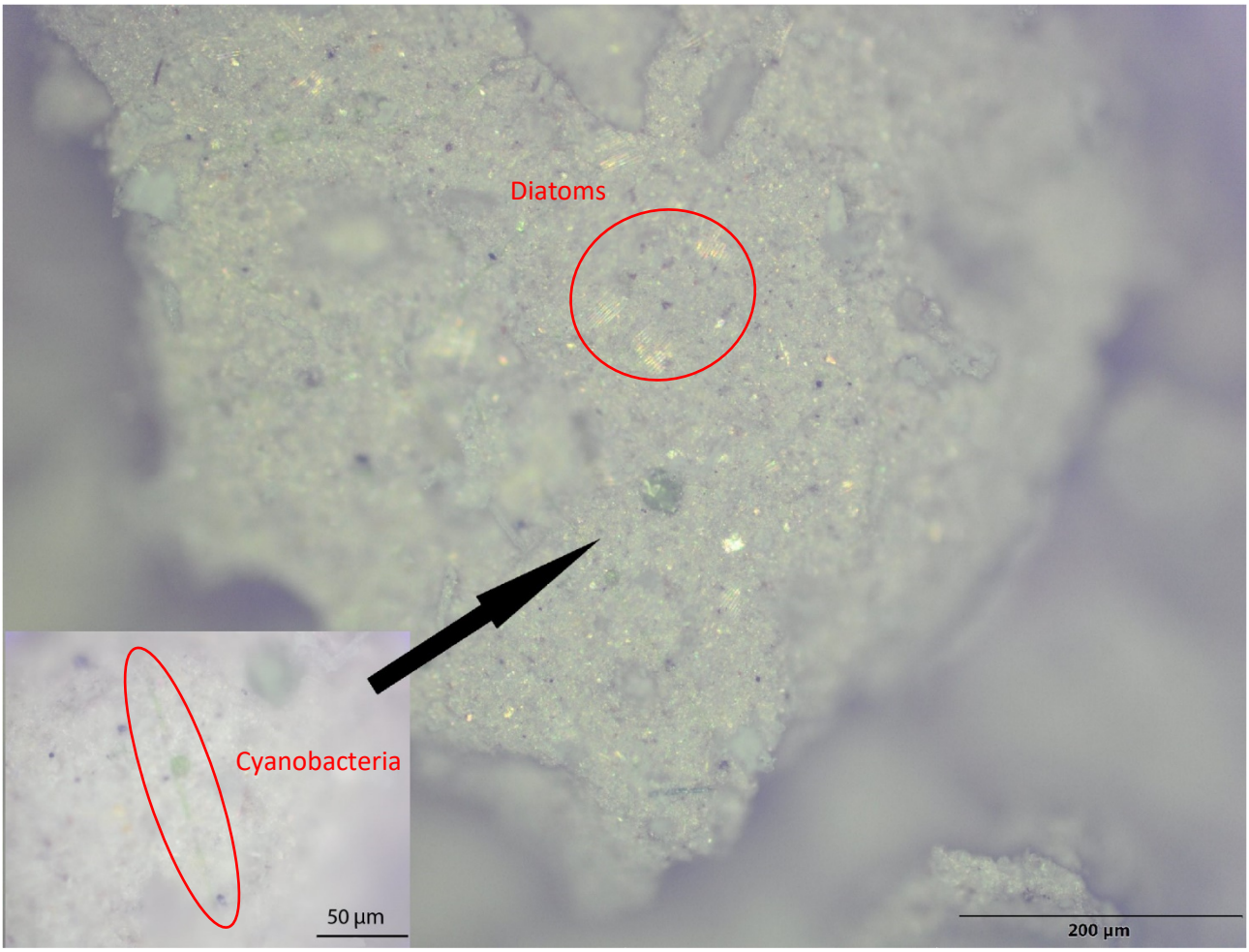

Figure III.S4: Microscopic image of a settling aggregate collected at 13m during the sampling campaign of July (2021).

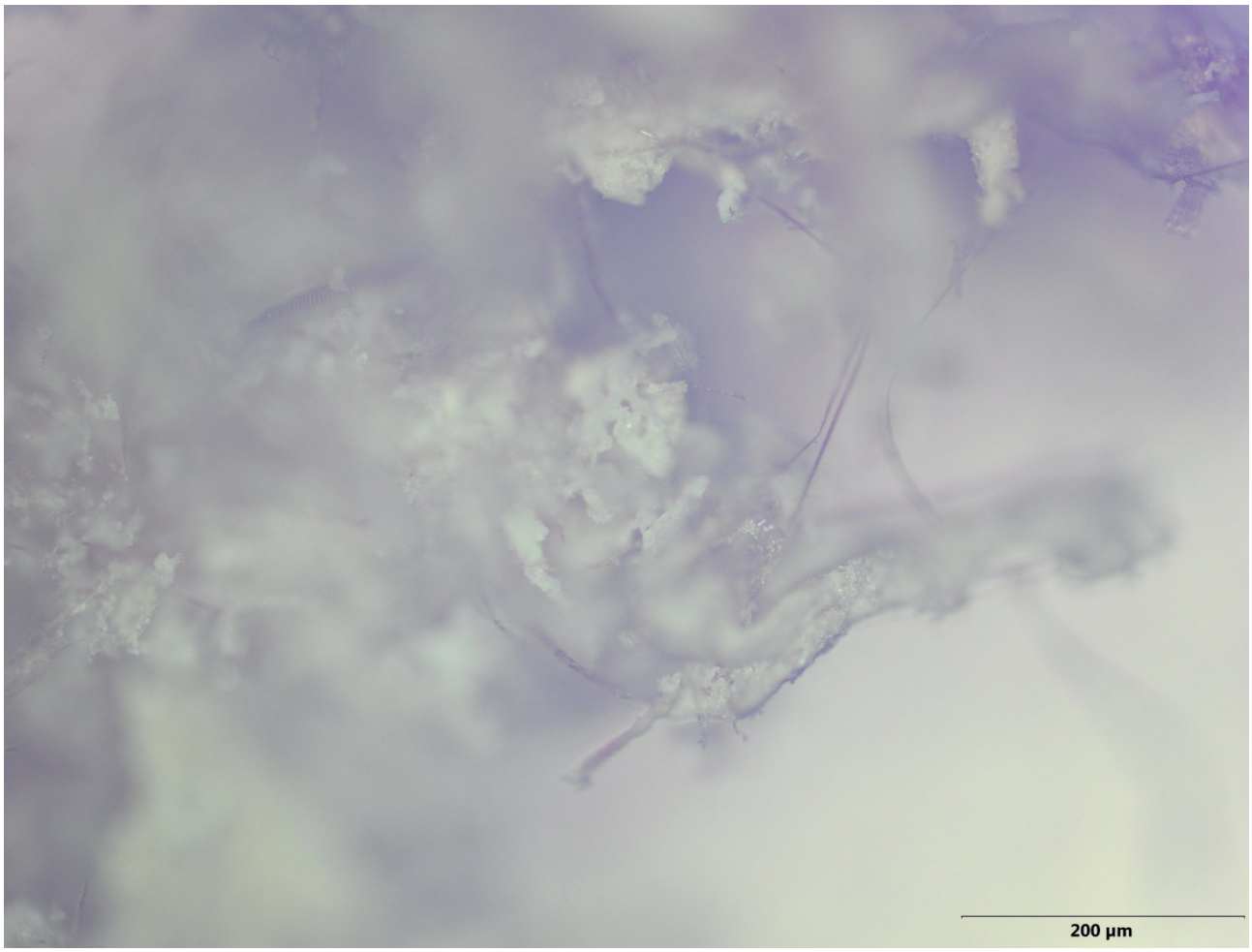

Figure III.S5: Microscopic image of some settling aggregates collected at 13m during the sampling campaign of September (2021).

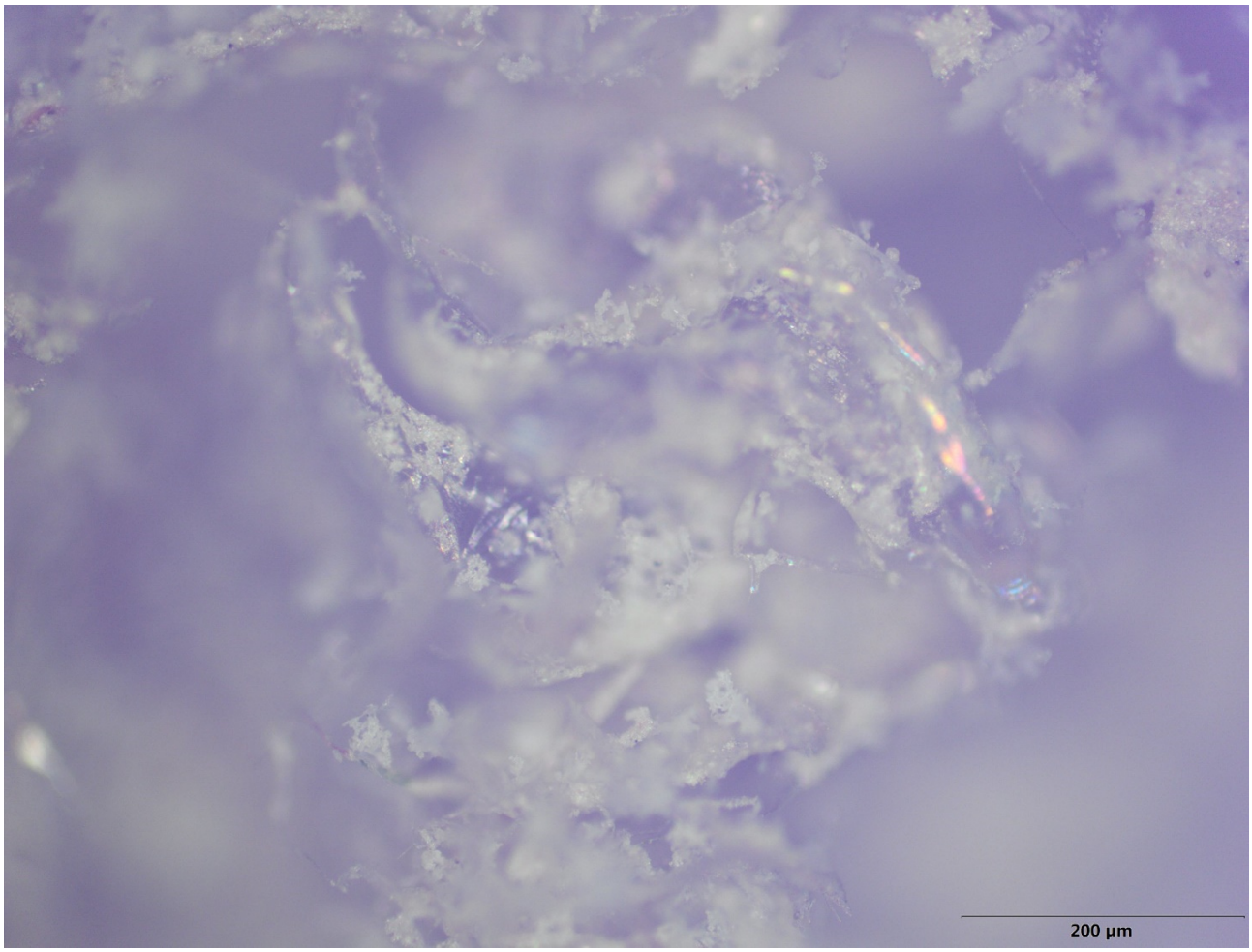

Figure III.S6: Microscopic image of some settling aggregates collected at 28m during the sampling campaign of September (2021).

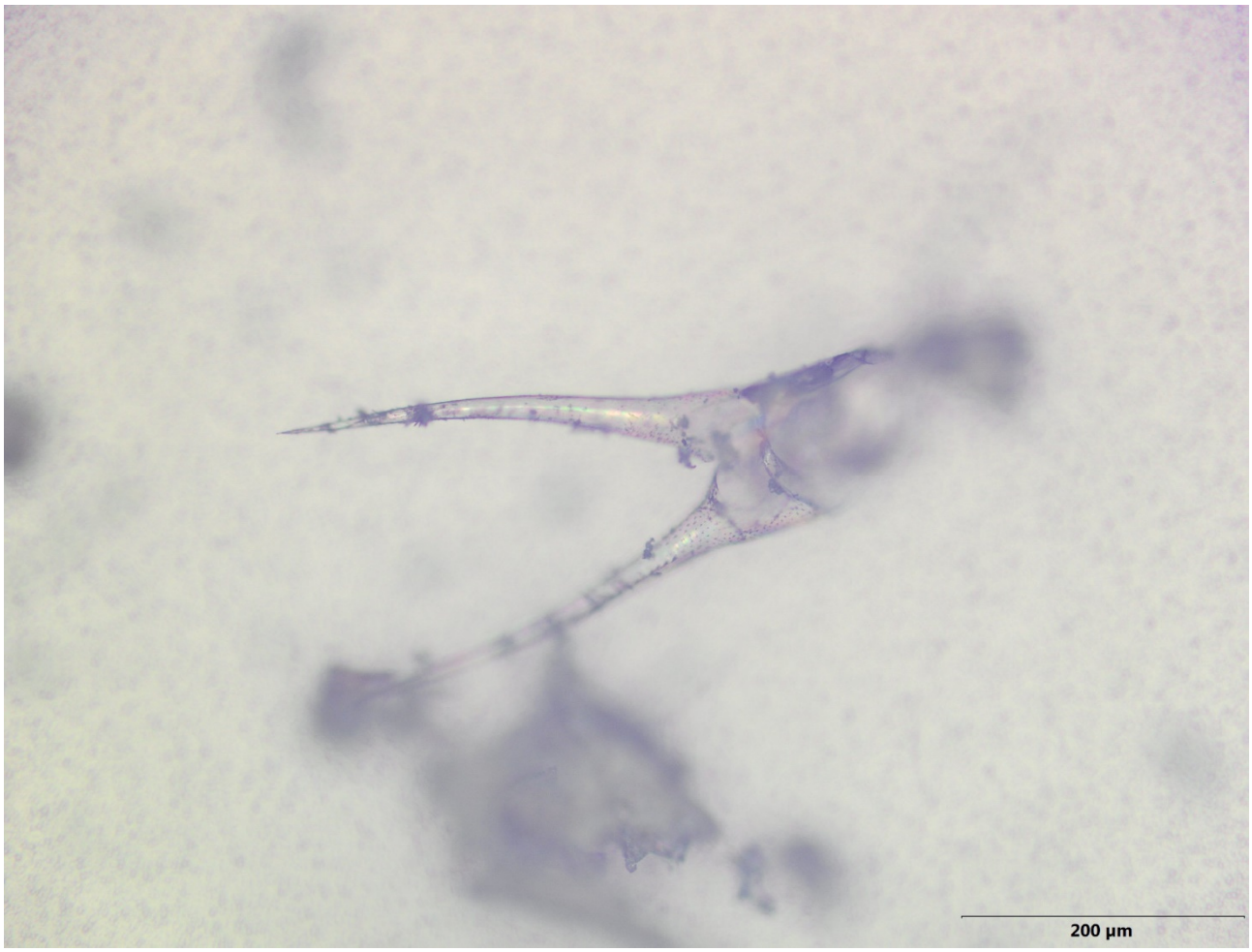

Figure III.S7: A portion of a small crustacean took in the September samples (2021) at 100m dept.

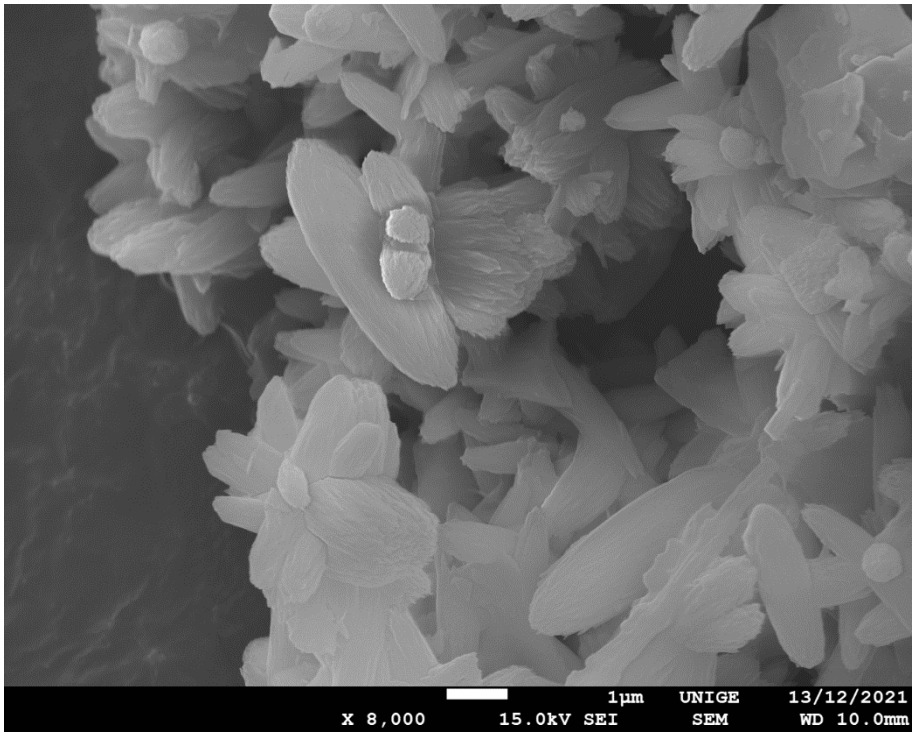

Figure III.S8: Concretions of bio-calcite on a settling particle from the 13m July (2021) sample.

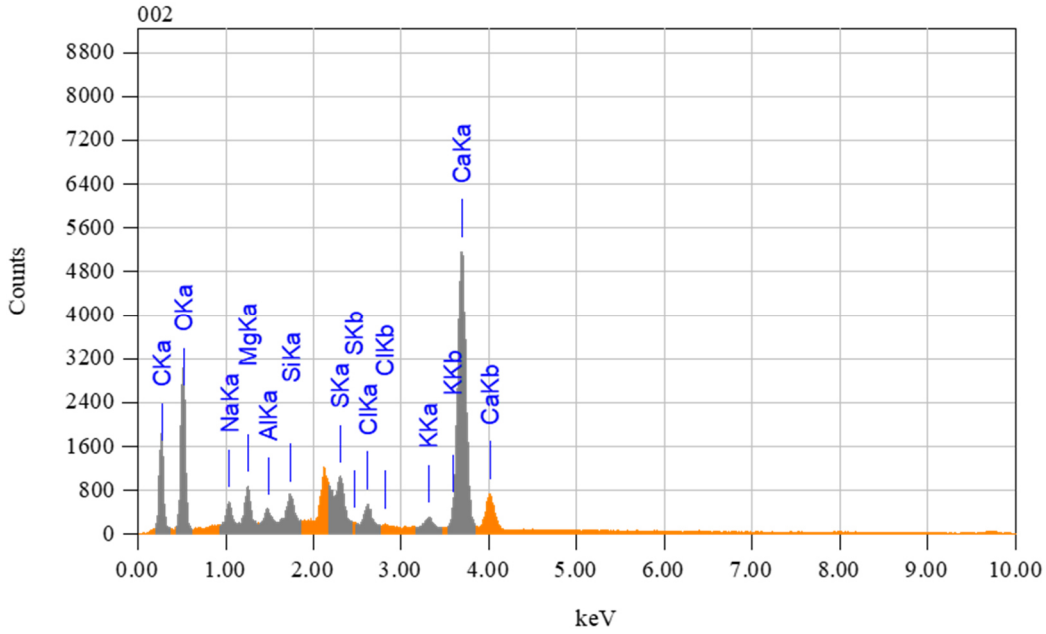

Figure III.S9: Chemical composition for the bio-calcite concretions in figure S6.

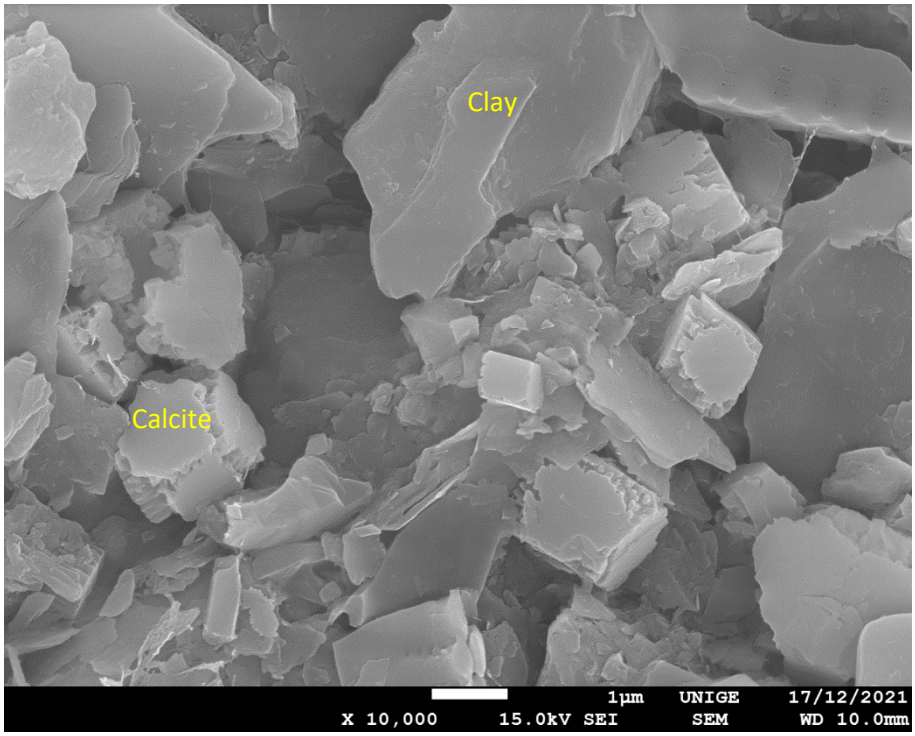

Figure III.S10: Calcite and clay crystals on a settling particle from the 13m September (2021) sample.

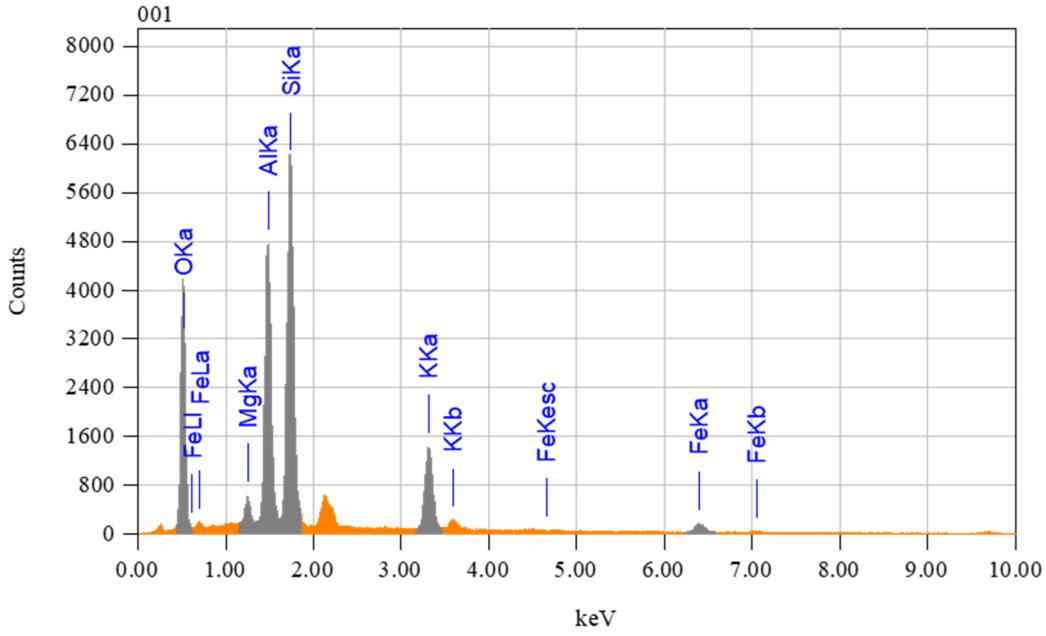

Figure III.S11: Chemical composition for the clay in figure S8

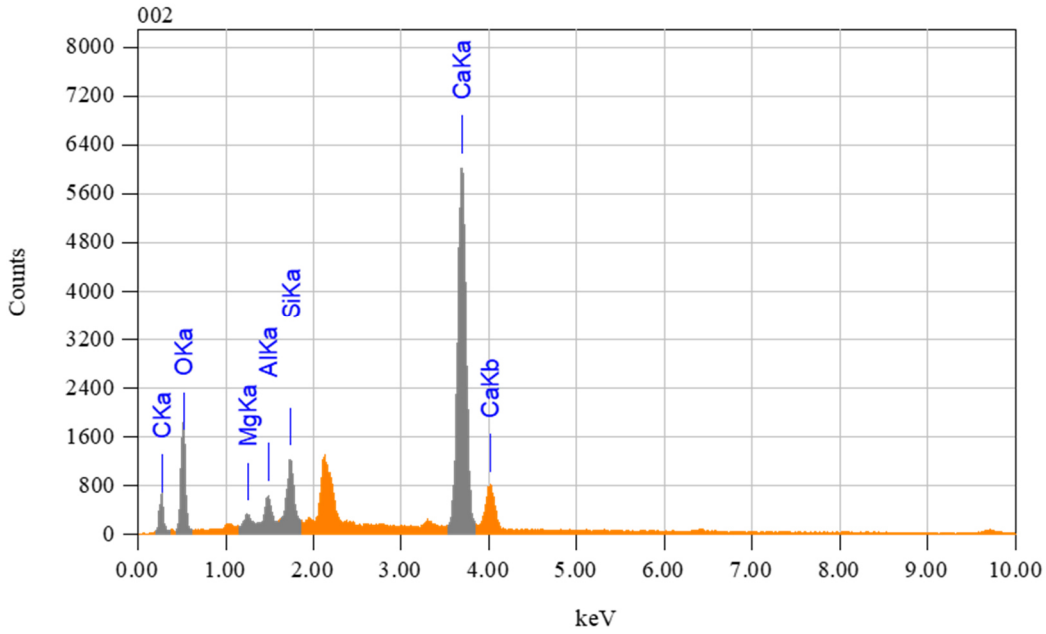

Figure III.S12: Chemical composition for the calcite in figure S8

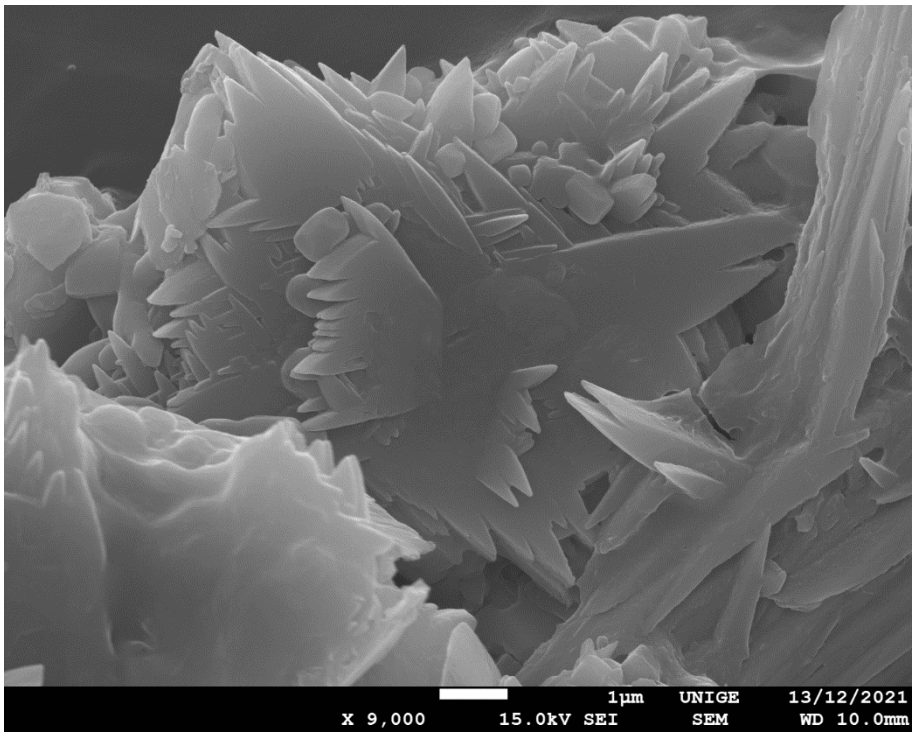

Figure III.S13: Gypsum crystals on a settling particle from the 13m September (2021) sample.

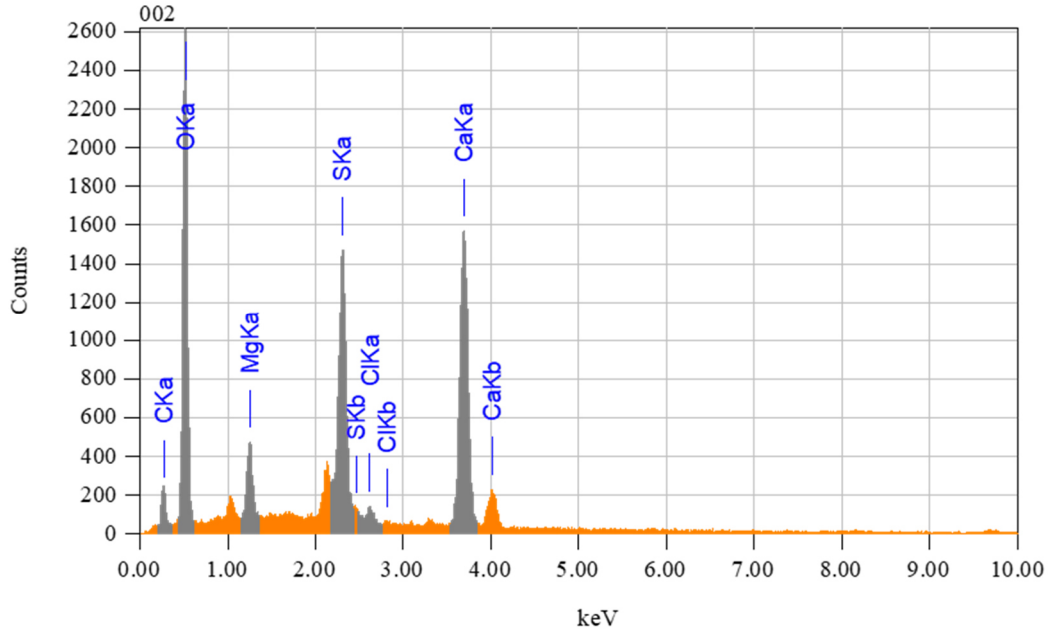

Figure III.S14: Chemical composition for the gypsum in figure S11

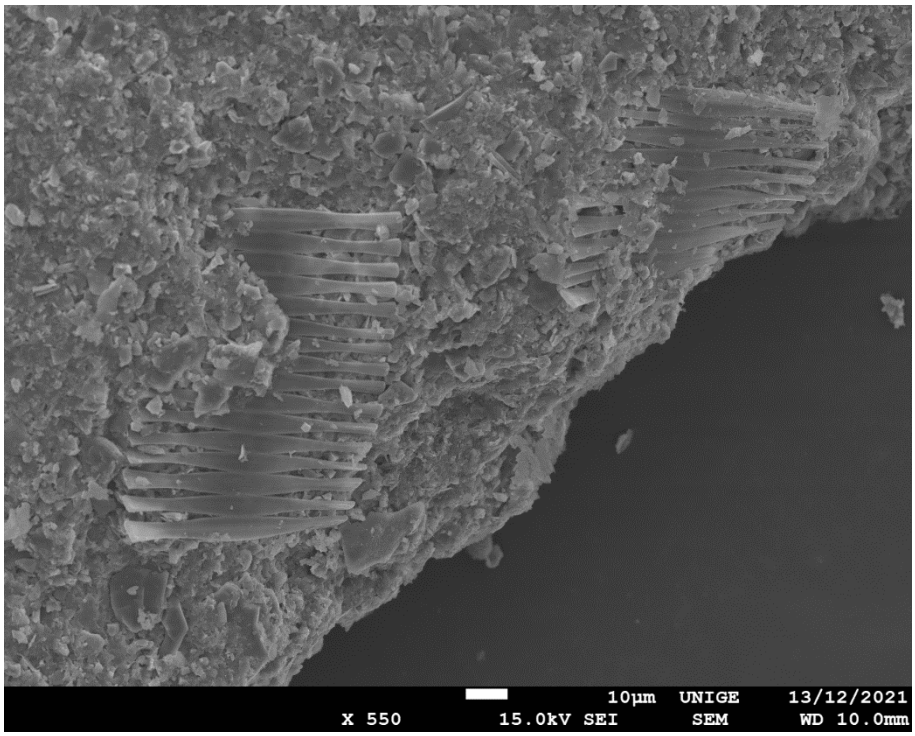

Figure III.S15: Diatoms on a settling particle from the 13m July (2021) sample.

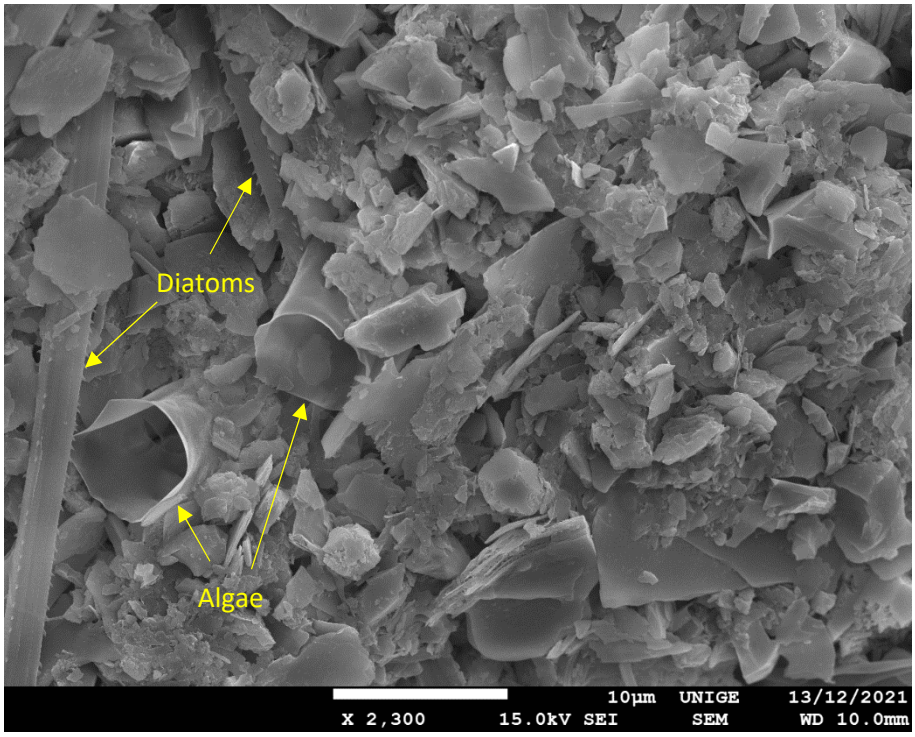

Figure III.S16: On the right side, diatoms and algae on a settling particle from the 28m September (2020) sample.

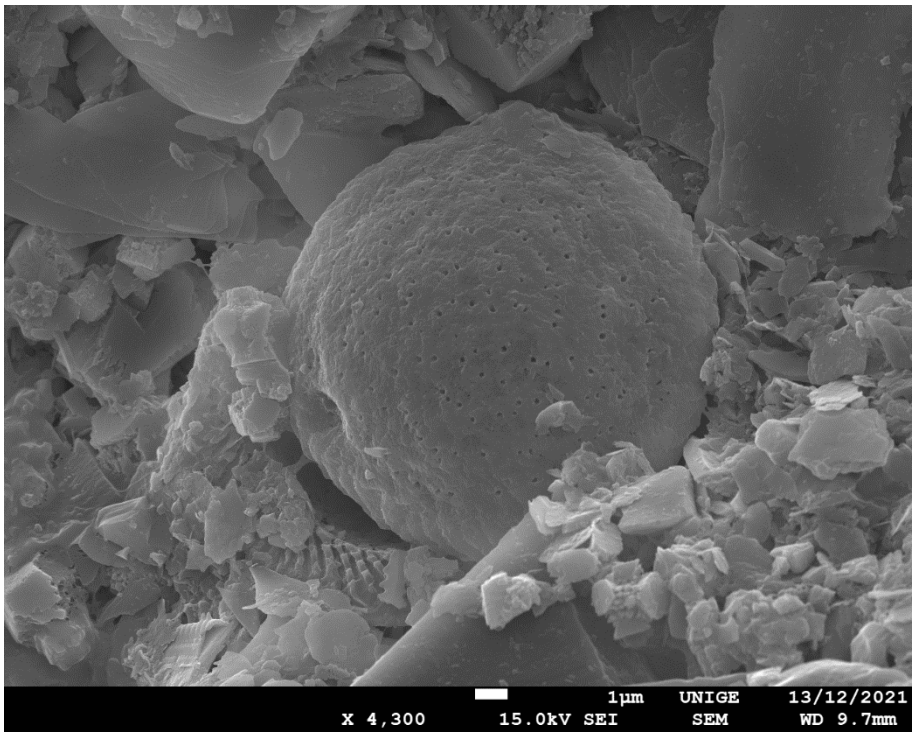

Figure III.S17: A Phacotus on a settling particle from 100m July (2020) sample.

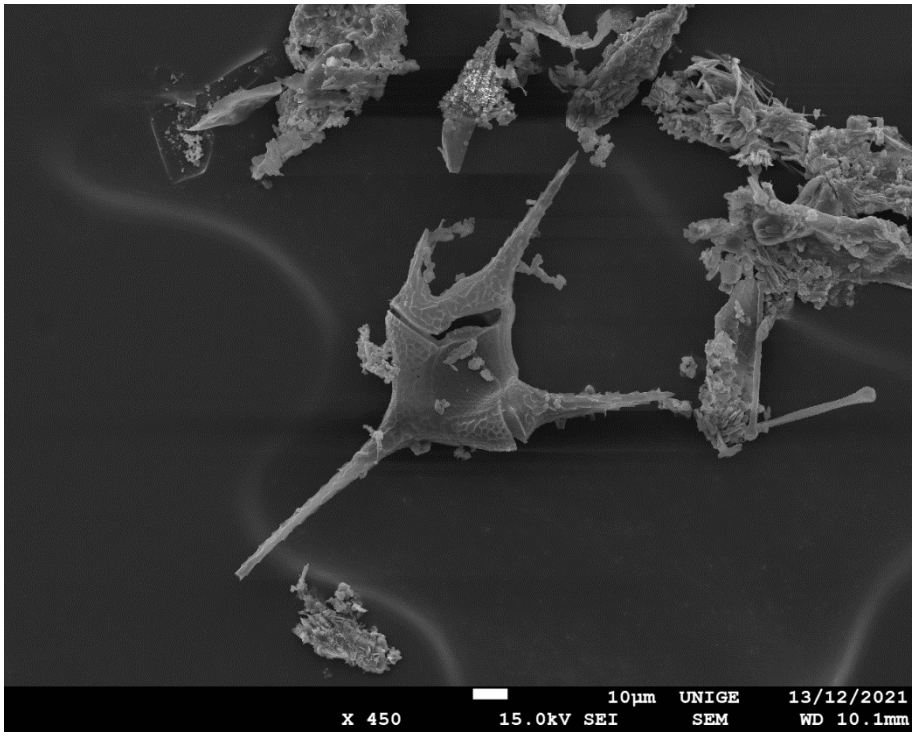

Figure III.S18: A Ceratiom Hirondella among the settling particles from the 13m September (2020) sample.

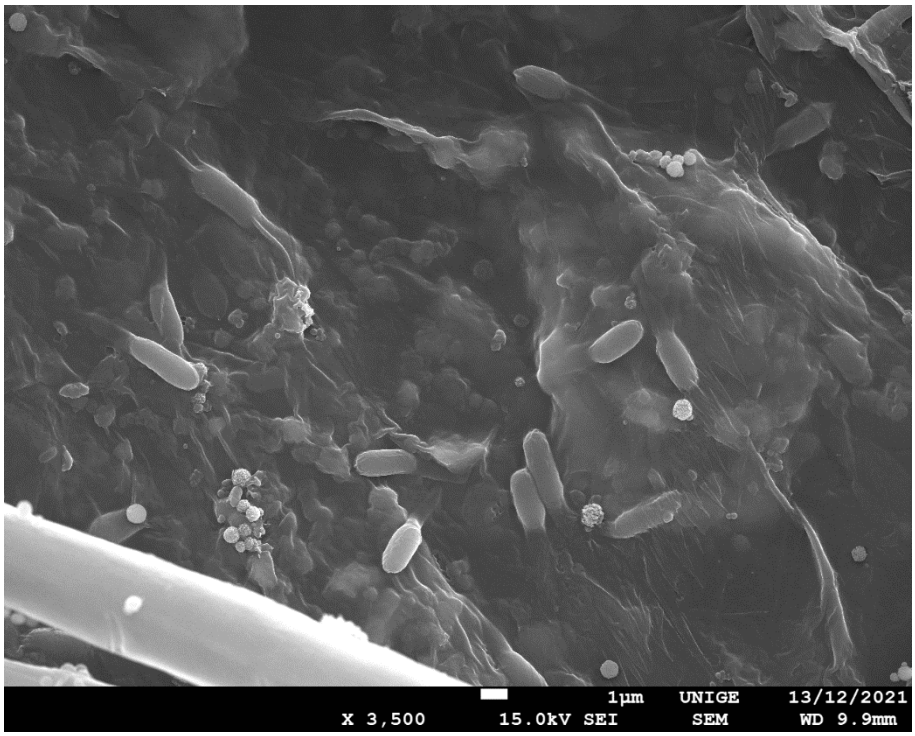

Figure III.S19: Bacteria inside a "pond" of organic matter on a settling particle from the 100m July (2020) sample.

Chapter IV

Discussion and conclusions

IV.1. General discussion

The present thesis aimed to highlight the presence of MeHg in a limnic environment (i.e. Lake Geneva) and to investigate the possibility that MeHg could be produced in association with the particulate matter along the water column, known as *lake snow* (>300 µm) and lake aggregates (<300 µm). To that end, firstly, literature on the subject has been extensively reviewed to assess the state of the art and to highlight knowledge gaps and future research needs. Our assessment highlighted that marine and *lake snow* present the potential to be important compartments for MeHg production in the oxic water column in both marine and lacustrine environments. As discussed, *marine snow* has already been the subject to numerous studies highlighting its capability to play a niche role in MeHg production, whereas *lake snow* has not received the same interest. Few studies observed similarities between marine and lake snow and the possibility for the latter to act similarly to *marine snow*.

IV.1.1. MeHg production in limnic water column: the role of *lake snow*

In order to investigate *lake snow* as a micro-niche for MeHg production, specific sampling and analytical setups were designed and tested to obtain particles and aggregates from the water column of Lake Geneva without creating artefacts (e.g. induced anoxic conditions) that can result in MeHg production not linked to natural *lake snow* conditions in the aquatic environment.

Continuous flow filtration of large volume of water (hundreds of liters) made possible to collect suspended particles along Lake Geneva water column in sufficient quantity to analyze THg and MeHg concentrations, while continuous flow centrifugation allowed the sampling of suspended particles in the four major tributaries of Lake Geneva to determine the overall concentrations of MeHg from the watershed.

The MeHg concentrations found in suspended particles of the water column of Lake Geneva were compared to the concentrations determined on the rivers particles to assess the possibility that the watershed was the actual production environment for the MeHg found on the lake suspended particles. This comparison showed that the ranges of MeHg concentrations in the river particles are too low to be the only source of MeHg for the lake water column. Other possible sources of particulate MeHg in the lake system are represented by the resuspension of bottom sediments and of sediments in the shallower part of the lake near the shores, but even these sources were ruled out. Shallow

surface sediments are subject to high level of oxygen, which makes the creation of an anoxic environments difficult. Such anoxic conditions are necessary for MeHg production because all known methylators are obligate anaerobes (Gilmour et al., 2013). Even if anoxic condition could be reached and maintained, the solar irradiation to which shallow sediments are subject decomposes MeHg quite rapidly (Seller et al., 1996), long before MeHg bearing particles could reach the sampling point chosen for this research (e.g. SHL2), located several kilometers away from all shores. SHL2 is also situated in the deepest part of the lake (e.g. 309 m), which makes the resuspension of sediment aggregates form such a depth to the surface layers unlikely. Even the deepest depth sampled (e.g. 100 m) is 200 m from the bottom sediments, which is still a great distance to overcame for both dissolved MeHg (that could be demethylated) and sediment resuspension. This is why we choose the 100 m depth to study the hypolimnion. In the light of these reasons and in order to explain the MeHg concentrations found in the lake suspended particles, an in-situ production must be present inside the *lake snow*.

IV.1.2. *Lake snow* as an anoxic substratum for Hg methylators

As mentioned before, all known methylators are obligate anaerobes, which means that for MeHg to be produced in-situ inside *lake snow,* an anoxic substratum must be present in the particles and aggregates along the water column.

In order to test this possibility, settling particles were collected in a film of polyacrylamide gel to determine the dissolved oxygen (DO) concentrations inside particles macro-aggregates (≥100 µm) and around micro-aggregates (<100 µm) high density regions, using a micro-probe with a 50 µm tip. All analyzed aggregates showed hypoxic conditions and a single macro-aggregate showed a fully anoxic interior (0.22 mg/L). This macro-aggregate was characterized by a very dark color and was among the biggest aggregates found. In order to explain the lack of fully anoxic macro-aggregates in the collected settling particles, the following hypothesis was considered: fully anoxic aggregates tend to be highly organic and stay in the suspended phase more than less organic aggregates.

This hypothesis is in accordance with the work of Stabel (1987) and of Grossart and Simon (1998), who found that settling velocities appear to be low for high organic material. Highly organic micro-aggregates stay in the water column for long period of time which enable them to reach complete and sustained anoxia and start methylating, as the size increases aggregates tend to sink faster and did not reach complete anoxia. Considering this, sediment traps may not be the ideal sampling technique to investigate the ability of *lake snow* to methylate mercury as they tend to under collect aggregates that have low settling velocities.

Furthermore, SEM analysis on the settling particles made possible to detect, in almost the entirety of the particles analyzed, bacteria embedded in regions covered by a film of OM, which could represent

an OM mineralization process. This process could, in time, allow the creation of an anoxic niche inside the particles, which in turn could create the right substratum for MeHg production.

IV.1.3. Behavior and importance of *lake snow* in the limnic MeHg cycle

In addition to the Hg and DO analysis on the particulate phase, water samples were collected to investigate the levels of MeHg into the dissolved phase (Chapter II, 4.3). The MeHg concentrations found in the water samples proved to be quite homogenous along the water column, showing little to no difference with depth. Moreover, the MeHg stock of the dissolved phase of the water column proved to be higher than the MeHg stock of the particulate phase. This is mainly due to the very low concentrations of particles in the water column of Lake Geneva making the suspended particles MeHg less important in the total MeHg budget of the water column. To explain the MeHg concentrations found in the dissolved phase several possible sources were discussed: i) diffusion from the bottom sediments; ii) lateral diffusion from the shallow sediments of the shores and iii) input from the watershed via tributaries water. The first two possible sources were ruled out for reasons similar to the ones used to discredit the sources for particulate MeHg. Diffusion from the bottom sediments probably cannot overcome the distance between the bottom and the sampling depths, which is testified also by the lack of a MeHg gradient with depth. Lateral diffusion from the shores is not plausible due to the strong photo-degradation carried out by the solar irradiation and, of course, to the sheer distance between the shores and the sampling point. MeHg input from the tributaries water was also ruled out due to the differences found in the average concentrations of MeHg. As discussed in the second chapter, MeHg average concentrations in rivers from comparable settings found in literature, showed values of 0.04 ng/L while the average MeHg concentrations found in the water samples is around 0.63 ng/L. The tributaries concur in the MeHg concentrations of the water column but most probably they are not the main source of it. Our results and these values from the literature strongly suggest that the main source of MeHg into the dissolved phase of the water column is represented by the particulate phase. Suspended particles and aggregates with low settling velocities that can remain for long period of time in the water column steadily releasing MeHg in the dissolved phase. This could have important consequences in the MeHg cycle in a limnic environment.

In order to better understand the importance of these low settling velocities aggregates in the limnic system and their consequence in the interaction with the food chain, we propose the following schematic model (figure IV.1) where we summarize our findings and the cycle of mercury in limnic water column.

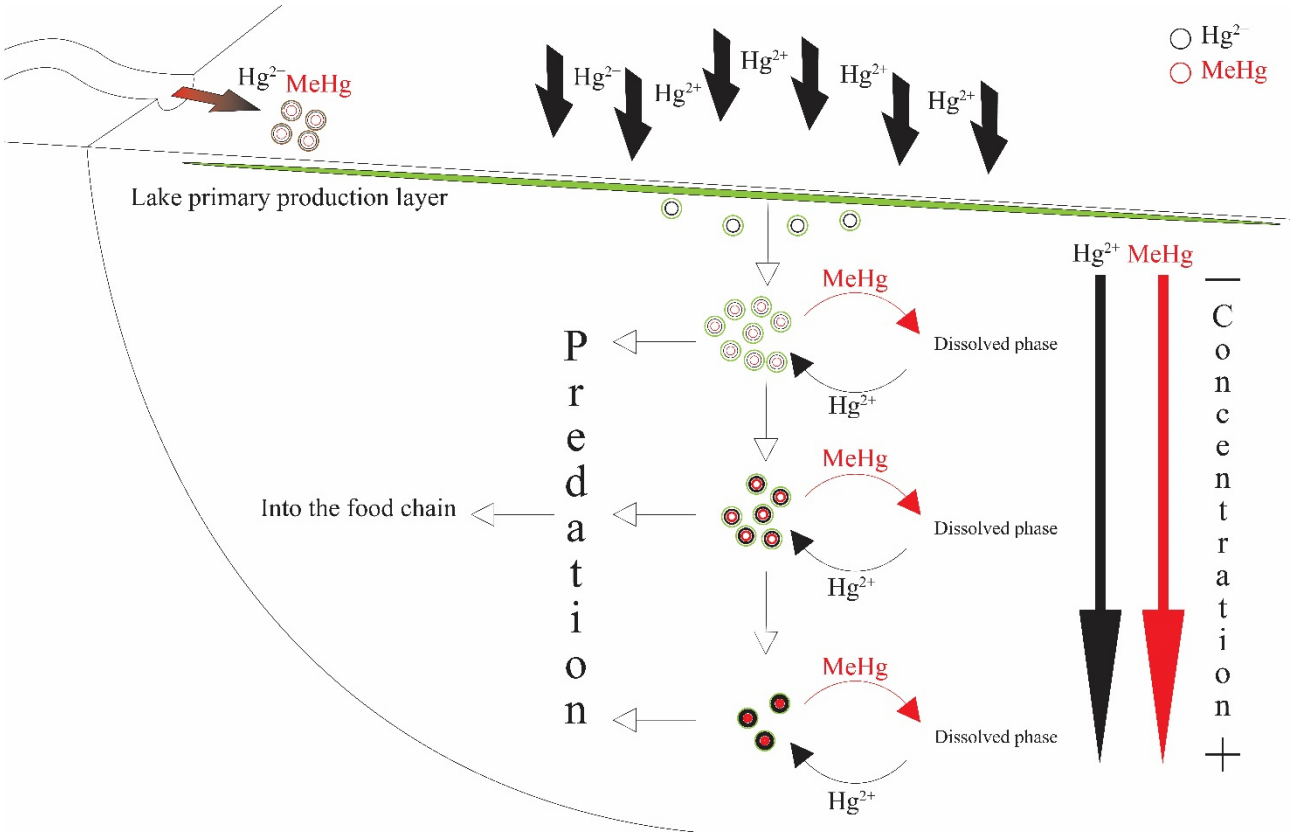

Figure IV.8: Schematic model of the role of low settling velocities aggregates in the water column of a deep lake (Lake Geneva). The highly organic aggregates are mainly produced in the lake primary production layer after the death of phytoplankton, and are already enriched in THg due to the tendency of phytoplankton to accumulate Hg (Le Faucheur et al., 2014). As they begin to settle MeHg production takes place and MeHg concentrations will increase with increasing depth. The aggregates scavenge Hg^{2+} from the dissolved phase and at the same time MeHg will slowly diffuse from the aggregates into the water column. The particulate concentrations showed to be decreasing with depth highlighting the process of predation by zooplankton and fish, which represent the main entry point for MeHg into the food chain.

Highly organic, low settling velocities aggregates are mainly formed after the death of phytoplankton in the primary production layer of the lake. Due to the tendency of phytoplankton to retain Hg (Le Faucheur et al., 2014), these aggregates are already enriched in THg even before starting their descent. As they begin to settle, the bacteria populations present in the aggregates start to mineralize the OM and consume dissolved oxygen, leading to the formation of an anoxic micro-environment, essential to sustain methylation of Hg. These anoxic micro-environments could be maintained in an oxic macro-environment (i.e. water column) thanks to a process highlighted by Alldredge and Cohen (1987). They found oxygenic photosynthesis at the surface of *marine snow* driven by healthy phytoplankton that create an inverse oxygen gradient keeping the DO from the surrounding water to insert in the aggregate. Even at night, when oxygenic photosynthesis ceases, Alldredge and Cohen (1987) found that a boundary layer depleted of oxygen extended from the surface of the aggregate up to 800 µm into the surrounding water. They observed that the oxygen gradient was steeper in this boundary layer and more gradual in the inside of the aggregate, slowing the oxygen insertion into the interior of the aggregate. As these organic aggregates slowly sink along the water column, they tend to: i) increase their THg and MeHg concentrations; ii) interact with the surrounding water (water-particulate exchanges) and iii) decrease their concentration in the water column. As the aggregates

descent into the water column, MeHg is continuously produces inside their anoxic micro-environments, increasing the MeHg concentrations in the aggregates with depth. At the same time, water-particulate interactions take place with MeHg slowly diffusing from the aggregates to the dissolved phase and the aggregates scavenging Hg from the surrounding water, as indicated by the partitioning coefficient (K_d^p MeHg< K_d^p THg). These processes increase the level of inorganic Hg and fuel further methylation. The decrease in particles concentrations in the hypolimnion could be linked to an increase of the settling velocity of the particles, around 3 times faster in the aphotic zone compared to the euphotic zone (Stabel, 1987), and to predation from zooplankton and fish (Finlay and Berninger, 1984; Miracle, 1974; Rienmann, 1985). *Lake snow* predation from the lake biota is an important entry point of MeHg into the limnic food chain in system where the water column is deeper enough for *lake snow* to develop an anoxic micro-environment and to produce MeHg. As for shallow limnic systems, *lake snow* probably does not have the same importance in the MeHg cycle because of the water column depth, which limits the time for the anoxic micro-niche to form, and for the increased importance of sediment resuspension, which remobilize the bottom sediments (and the MeHg therein) into the water column. The shallower the lake the more important are the bottom sediments as a MeHg production compartment (bottom sediments could be easily reached by lake biota), while the importance of water column is difficult to determine due to the interference of particles from the bottom sediments (i.e. sediment resuspension).

As presented in the introduction, this thesis took place in the bigger framework of a research project composed by 3 main modules. As this thesis covered the first two modules the third one would have investigated the genetic expressions of the microorganism in the settling particles hopefully highlighting the possible methylators for the *lake snow* of Lake Geneva. This module has been taken over by a group of workers that conducted analysis on both bottom sediment and settling particles of Lake Geneva (Capo et al. 2022). Their work, now under revision, found different microorganism populations in the bottom sediments and in the settling particles. While in the bottom sediments they found a dominance of the *hgcA* genes from the *Desulfobacterota* phylum, which is in agreement with other works on lake systems, settling particles showed *hgc*$^+$ genes from *Firmicutes* phylum as the potential main methylators in settling particles micro-niche.

Overall, *lake snow*, and specifically highly organic aggregates, plays an important role in the Hg cycle of a deep lake. The capability to develop an anoxic micro-environment and to transport Hg and MeHg along the water column, makes *lake snow* an important hotspot for Hg methylation. The comparison between our results regarding MeHg concentrations (chapter II) and available data on the values of bottom sediments of Lake Geneva Díez et al. (2016) showed, in average, an increase in MeHg concentrations in *lake snow* between 10.3% (February) to 77% (July) compared to the bottom sediments collected the same month. This difference in MeHg concentrations is of great importance.

Wu et al. (2019) showed that the MeHg concentration in the seston (all particulate matter in the water column) predicted 63% of the variability of the fish MeHg. Based on this evidence, it is very likely that *lake snow*, and the water column, are more important for the MeHg insertion into the food chain than the bottom sediments, especially in deep water column, where *lake snow* has time to develop the conditions for Hg methylation and the bottom sediments are more distant from the habitats of most fish. Fish consumption is the primary pathway for human exposure to MeHg, which is a major health concern (Fitzgerald and Lamborg, 2014), it is therefore important to determine the entry points of MeHg into the food chain and to understand how the in-situ specific physico-chemical parameters contribute to in this uptake and in the limnic cycle of Hg and MeHg.

IV.1.4. Anthropogenic forcing on freshwater MeHg cycle

Climate change will have a major disruptive effect on the global mercury cycle, leading to significant changes in freshwater environments.

Assessing the impact of climate change on freshwater systems worldwide is not straightforward, due to the complex natural and socio-economic backlashes generated. However, several studies have attempted to understand how the key parameters (e.g. temperature, hydrological cycles, land use) will affect the freshwater environments, especially lakes.

Global projections from atmospheric circulation models predict a temperature increase of 2 to 4.5 °C (Paranjape and Hall, 2017), which will in turn increase the temperature of lakes worldwide. Woolway et al. (2020) found that lake temperatures around the world have been increasing at a rate of 0.34 °C per decade, with cold winter lakes (mean air temperature < 0.4 °C) warming more rapidly than warm winter lakes. Moreover, Butcher et al. (2015) using LISSS, a one-dimensional dynamic thermal simulation model applied to different hydroclimatic regions of the U.S., found that surface water of lakes increased in temperature by about 77% of the total increase in the overlaying air, while bottom waters only increased by about 30% (for deep lake). The increased temperature determined by climate change will affect several key processes that in turn may change the MeHg production in limnic environments. Increased temperature will increase the melting of ice and decrease the duration of ice cover on northern lakes, delaying the formation of ice caps (Butcher et al., 2015; Paranjape and Hall, 2017; Woolway et al., 2020). The increase of ice melting will affect the hydrological cycle of lakes especially those who are fed by a glacier. Increased melting will result in less ice covering, which will expose more land to be eroded and flooded by the rivers and the precipitations releasing particulate Hg, and more water input into the lakes, which will enlarge the tributaries with the risk of forming new inundated wetlands-like regions along the course of the rivers which will yield increased quantities of MeHg into the lakes (Paranjape and Hall, 2017).

These floodings will also mobilize more DOM (dissolved organic matter) and POM (particulate organic matter) into the lakes, enhancing the microbial population, Hg bioavailability and MeHg production. Moreover, higher concentrations of DOM will block out most of the solar radiation, decreasing substantially the photo-demethylation process (Klapstein and O'Driscoll, 2018; Luo et al., 2020; Paranjape and Hall, 2017). Moreover, as more OM enter the lakes, concentrations of nutrients will eventually increase, changing the trophic status of many lakes, especially shallower ones, as a consequence of increase in limnic primary production (e.g. blooms of algae and cyanobacteria) (Meerhoff et al., 2022). This could result in an increase in MeHg production due to the higher concentrations of labile OM. Periods of severe drought are expected among the hydrologic fluctuation, decreasing the input of water into the lakes and in turn, increasing the water temperature of the water bodies, concentrations of nutrients and Hg. The abundancy of nutrient and Hg, coupled with higher water temperature, will increase microorganisms population and activity and increasing the possibility for an hypoxic layer to be created in the water body of the lakes, enhancing MeHg production (Paranjape and Hall, 2017; Woolway et al., 2020). However, Hg methylation could be counterbalanced by a decrease in DOM and Hg inputs from the watersheds during the periods of drought, which will increase photo-demethylation and decrease bioavailable Hg for the microorganism communities (Klapstein and O'Driscoll, 2018; Luo et al., 2020; Paranjape and Hall, 2017).

Increasing lakes surface temperatures will also increase the thermal resistance to mixing of the entire water column. Projected trends show that lakes are moving towards extended periods of stratification due to an earlier onset of the thermal stratification and its prolonged presence through fall (Butcher et al., 2015; Woolway et al., 2020). This enhanced stability of the water column of lakes could lead to more and longer blooms of cyanobacteria, which would increase the turbidity of the water and the POM in the lake water column. This in turn will decrease the solar radiation in the water column, decreasing photo-demethylation, increasing microorganisms activity and oxygen depletion in the deeper layers of the water column (Butcher et al., 2015). Maberly et al. (2020) defined 9 lake thermal regions based on similarities on latitude and annual mean temperature pattern, and they found that, depending on the level of severity of the greenhouse concentration in the atmosphere, 12% to 66% of lakes worldwide will change to a lower latitude thermal region by 2080-2099, with an alarmingly reduction in the number of lakes in the uppermost thermal region of 79% in the worst-case scenario. Projections on the alterations of lake mixing regimes showed that by 2080-2099 around 17% of all lakes will change from dimictic to monomictic and monomictic lakes could change to oligomictic and meromictic (Woolway et al., 2020).

It is rather speculative to project these global changes into a specific site, however it is possible to infer some effect in the case of Lake Geneva.

Lake Geneva is a warm monomictic peri-alpine lake, which main tributary is the Rhone, a glacier-fed river. Starting with this information, it is possible to infer that one of the main variables in the hydrological evolution of the lake will be the fate of the Rhone. As previously presented, the increasing temperature of the atmosphere will prolong the melting period of the Rhone glacier, increasing its discharge. Beniston et al. (2011) analyzed several projections of the impact of the climate change on the Alps glaciers and on the climate of the northern Europe. They found that, in the next decades, summers will tend to become hotter and with less precipitation in average, while winter will be warmer and present higher precipitation in total. This will decrease the ice and snow cover of the glaciers below 2000 m and increase them above this altitude. Glaciers will have more ice but only above 2000 m, which will decrease their overall volume and surface area. The reduced ice cover of the glacier and the increase discharge of the Rhone will mobilize previously untouched sediments and soils, bringing to the lake DOM, POM and particulate Hg. This process will end when all the ice below 2000 m has melted, switching to a new equilibrium where the Rhone will decrease its overall discharge volume showing periods of low flow or even total dryness during late summer and fall (Beniston et al., 2011), with an increase in the importance of rainfall in its regime, now mainly fed by glaciers. Regarding the lake itself, prolonged stratification from early spring to late fall could extend the periods of blooms of cyanobacteria and algae, following the projections determined by Butcher et al. (2015) and Woolway et al. (2020), which in turn will increase the input of fresh OM into the water column, increasing the concentration of *lake snow* and giving to the microorganism more substratum and nutrients to carry out their processes (including MeHg production). Regarding the biota, and specifically the fish population, metabolic allometric scaling theory predicts that higher temperatures will shift the fish population to smaller body sizes, increasing THg concentrations in those fish (Eagles-Smith et al., 2018). Moreover, low energy content in food and preys will lead to an increase in consumption to meet basal energy requirements, which will increase the exposition to MeHg even if the concentration in the food and preys remains the same (Eagles-Smith et al., 2018). In the case of Lake Geneva, it is difficult to define if the quality of food will decrease sufficiently to induce major MeHg exposition to the fish population; however, an increase in temperature of the surface waters could change the thermal optimum for many species of fish, bringing them closer to the base of the metalimnetic mixed zone (Butcher et al., 2015). In this scenario, fish in Lake Geneva will be closer to the MeHg rich hypolimnetic *lake snow* and, considering the increase in POM, they could be exposed to higher concentrations of MeHg than the ones observed in our work (chapter II). This effect will be further enhanced in the case of a decrease of the oxygen content in the hypolimnion due to the extended stratification period.

IV.1.5. Future research needs

More studies need to be carried out to further investigate the importance of *lake snow* into the Hg cycle in limnic environments. OM specific characterization could highlight the degree of mineralization with depth and the sources of the OM (allochtonous or autochthonous), and its quality in terms of food for the biota, to assess the quantities necessary for fish to meet their metabolic needs. Furthermore, analysis on the gene cluster *hgcAB* to highlight the kind of methylators present in the *lake snow* to pinpoint the substratum that they need, this could have important repercussions in the understanding of the climate change in MeHg production, as microorganisms with different temperature optimum could produce more or less depending on the temperatures that we should. Moreover, studies need to be done to see the importance of the *lake snow* in other systems, especially those where the water column is less deep, to check the relative importance between the water column and the bottom sediments.

The devised sampling and analytical techniques used in all the experiment presented in this thesis worked as expected, enabling the sampling and analysis of natural material as undisturbed as possible, regarding the redox conditions, which were the most pressing requirements for these experiments. Avoiding as much as possible any induced anoxic condition was paramount in the determination of the natural concentrations of MeHg on *lake snow*. Nevertheless, these sampling and analytical setups presented some limitations that needed to be addressed in order to increase their utility in the analysis process. The continuous flow filtration used to sample the suspended particles in the lake water column was essentially useless for any other kind of analysis apart THg and MeHg determination due to the impossibility to remove the particles from the filter after the sampling. A different kind of sampling technique that keeps the advantages (no induced redox alteration and capability to sample suspended matter) and add the possibility to use the same sampled material for other analysis (e.g. OM characterization and DNA and RNA analysis), must be designed and tested, to reach a standardized sampling technique. Similarly, an alternative sampling technique must be conceived to collect material for the DO analysis. Sediment traps proved to be not the ideal solution to collect *lake snow* aggregates due to their low settling velocities, thus a sampling technique capable of doing that could be useful to investigate the DO concentrations across all the aggregates in the water column. Moreover, the polyacrylamide gel, while useful as previously discussed, has a major limitation, once inside the gel an aggregates cannot easily be removed from it which makes very difficult to use the same sample for multiple analysis. A possible solution could be to design a microcosm in which lake aggregates are been resuspended continuously in a tank of lake water with the same physico-chemical conditions and after an incubation period investigate all the necessary parameters (MeHg, THg, DO, OM, DNA, RNA) on the aggregates removed from the microcosm.

IV.2. General Conclusion and perspectives

In conclusion, all the finding presented and discussed in this thesis highlight the importance of *lake snow* in the cycle of Hg in a deep limnic environment. Similar to *marine snow*, *lake snow* has shown the ability to sustain hypoxic and anoxic micro-environments, where MeHg production could take place. *Lake snow* has also showed to bear MeHg concentrations higher than bottom sediments, which indicates the water column as another important hotspot for MeHg production. The relative importance of MeHg production from *lake snow* is greater the longer the water column of the lake. The deeper the lake, the longer the residence time of the *lake snow* in the water column, considering the same settling velocities, and in turn the more time the microorganism populations have to produce MeHg. Therefore, the longer the water column the higher the concentrations of MeHg with depth, and the more time the aquatic biota has to predate on the *lake snow*. Predation in the water column on *lake snow* is probably one of the most important inputs of MeHg into the limnic food chain, which in turn leads to the human diet. This hotspot of MeHg in the habitat of most aquatic biota needs to be further analyzed because of its importance in the limnic Hg cycles that could have important repercussion in the remediation processes in polluted systems. Furthermore, shallower systems need to be investigated to better link in-situ parameters (e,g. water column depth) to the relative importance of *lake snow* on their Hg cycle. Overall, this thesis provided new data in favor of the hypothesis that *lake snow* is indeed an important MeHg production micro-environment like *marine snow*, and an important step in the Hg cycle in limnic systems. Furthermore, we highlighted sampling and analytical necessities that need to be addressed to develop future standardized sampling and analytical techniques, allowing research to produce comparable data in order to investigate all kind of limnic systems.

IV.3. References

Alldredge, A. L., and Cohen, Y., 1987, Can Microscale Chemical Patches Persist in the Sea? Microelectrode Study of Marine Snow, Fecal Pellets: Science, v. 235, no. 4789, p. 689-691.

Beniston, M., Stoffel, M., and Hill, M., 2011, Impacts of climatic change on water and natural hazards in the Alps: Can current water governance cope with future challenges? Examples from the European “ACQWA” project: Environmental Science & Policy, v. 14, no. 7, p. 734-743.

Butcher, J. B., Nover, D., Johnson, T. E., and Clark, C. M., 2015, Sensitivity of lake thermal and mixing dynamics to climate change: Climatic Change, v. 129, no. 1-2, p. 295-305.

Datalakes, 2022, Lake Geneva physical-chemical parameters, accessed 15 April 2022, https://www.datalakes-eawag.ch/.

Díez, E. G., Loizeau, J.-L., Cosio, C., Bouchet, S., Adatte, T., Amouroux, D., and Bravo, A. G., 2016, Role of Settling Particles on Mercury Methylation in the Oxic Water Column of Freshwater Systems: Environmental Science & Technology, v. 50, no. 21, p. 11672-11679.

Eagles-Smith, C. A., Silbergeld, E. K., Basu, N., Bustamante, P., Diaz-Barriga, F., Hopkins, W. A., Kidd, K. A., and Nyland, J. F., 2018, Modulators of mercury risk to wildlife and humans in the context of rapid global change: Ambio, v. 47, no. 2, p. 170-197.

Finlay, B. J., and Berninger, U.-G., 1984, Coexistence of congeneric ciliates (karyorelictida: loxodes) in relation to food resources in two freshwater lakes: Journal of Animal Ecology, v. 53, p. 929-943.

Fitzgerald, W. F., and Lamborg, C. H., 2014, Geochemistry of Mercury in the Environment, In: Treatise on Geochemistry (Second Edition): Oxford, Elsevier,, p. 91-129.

Gilmour, C. C., Podar, M., Bullock, A. L., Graham, A. M., Brown, S. D., Somenahally, A. C., Johs, A., Hurt, R. A., Bailey, K. L., and Elias, D. A., 2013, Mercury Methylation by Novel Microorganisms from New Environments: Environmental Science & Technology, v. 47, no. 20, p. 11810-11820.

Grossart, H., and Simon, M., 1998, Significance of limnetic organic aggregates (lake snow) for the sinking flux of particulate organic matter in a large lake: Aquatic Microbial Ecology, v. 15, p. 115-125.

Klapstein, S. J., and O'Driscoll, N. J., 2018, Methylmercury Biogeochemistry in Freshwater Ecosystems: A Review Focusing on DOM and Photodemethylation: Bulletin of Environmental Contamination and Toxicology, v. 100, no. 1, p. 14-25.

Le Faucheur, S., Campbell, P. G., Fortin, C., and Slaveykova, V. I., 2014, Interactions between mercury and phytoplankton: speciation, bioavailability, and internal handling: Environ Toxicol Chem, v. 33, no. 6, p. 1211-1224.

Luo, H., Cheng, Q., and Pan, X., 2020, Photochemical behaviors of mercury (Hg) species in aquatic systems: A systematic review on reaction process, mechanism, and influencing factor: Science of The Total Environment, v. 720, p. 137540.

Maberly, S. C., O'Donnell, R. A., Woolway, R. I., Cutler, M. E. J., Gong, M., Jones, I. D., Merchant, C. J., Miller, C. A., Politi, E., Scott, E. M., Thackeray, S. J., and Tyler, A. N., 2020, Global lake thermal regions shift under climate change: Nature Communications, v. 11, no. 1.

Meerhoff, M., Audet, J., Davidson, T. A., De Meester, L., Hilt, S., Kosten, S., Liu, Z., Mazzeo, N., Paerl, H., Scheffer, M., and Jeppesen, E., 2022, Feedback between climate change and eutrophication: revisiting the allied attack concept and how to strike back: Inland Waters, v. 12, no. 2, p. 187-204.

Miracle, M. R., 1974, Niche structure in freshwater zooplankton: A principal components approach: Ecology, v. 55, p. 1306-1316.

Paranjape, A. R., and Hall, B. D., 2017, Recent advances in the study of mercury methylation in aquatic systems: FACETS, v. 2, no. 1, p. 85-119.

Rienmann, B., 1985, Potential Importance of Fish Predation and Zooplankton Grazing on Natural Populations of Freshwater Bacteria: Applied and Environmental Microbiology, v. 50, p. 187-193.

Seller, P., Kelly, C. A., Rudd, J. W. M., and Machutchon, A. R., 1996, Photodegradation of methylmercury in lakes: Nature, v. 380, no. 6576, p. 694-697.

Stabel, H.-H., 1987, Settling velocity and residence time of particles in Lake Constance: Schweiz. Z. Hydrol., v. 49, p. 284-293.

Woolway, R. I., Kraemer, B. M., Lenters, J. D., Merchant, C. J., O'Reilly, C. M., and Sharma, S., 2020, Global lake responses to climate change: Nature Reviews Earth & Environment, v. 1, no. 8, p. 388-403.

Wu, P., Kainz, M. J., Bravo, A. G., Åkerblom, S., Sonesten, L., and Bishop, K., 2019, The importance of bioconcentration into the pelagic food web base for methylmercury biomagnification: A meta-analysis: Science of The Total Environment, v. 646, p. 357-367.