Role of phytoplankton in aquatic mercury speciation and transformations

Cossart, Thibaut; Garcia-Calleja, Javier; Santos, João; Kalahroodi, Elaheh Lotfi; Worms, Isabelle; Pedrero, Zoyne; Amouroux, David; Slaveykova, Vera

How to cite

COSSART, Thibaut et al. Role of phytoplankton in aquatic mercury speciation and transformations. In: Environmental chemistry, 2022. doi: 10.1071/EN22045

This publication URL: https://archive-ouverte.unige.ch/unige:163838
Publication DOI: 10.1071/EN22045

Role of phytoplankton in aquatic mercury speciation and transformations

Thibaut Cossart[1,§], Javier Garcia-Calleja[2,§], João P. Santos [1,§], Elaheh Lotfi Kalahroodi[2], Isabelle A. M. Worms [1], Zoyne Pedrero[2], David Amouroux [2], Vera I. Slaveykova [1*]

[1] Environmental Biogeochemistry and Ecotoxicology, Department F.-A. Forel for Environmental and Aquatic Sciences, Earth and Environmental Sciences, Faculty of Sciences, University of Geneva, Uni Carl Vogt, Bvd Carl-Vogt 66, CH-1211 Geneva 4, Switzerland.

[2] Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Institut des Sciences Analytiques et de Physico-chimie pour l'Environement et les Matériaux, Pau, France.

*corresponding author: vera.slaveykova@unige.ch

[§] equal contribution

Environmental Context

Understanding mercury transformations in the aquatic environment is of utmost importance for the improvement of mercury biogeochemical modelling and sound environmental risk assessment. In such a context, we discuss critically the advancement in the knowledge on the role of the phytoplankton (algae and cyanobacteria) in mercury cycling and transformations in the aquatic environment. Important research advances revealed that different microalgal species and cyanobacteria contribute: to biotic reduction of inorganic mercury to elemental mercury; to demethylation of methylmercury and transformation of inorganic mercury into metacinnabar; and to production of different biomolecules which can contribute to abiotic mercury reduction.

Thibaut Cossart is a Ph.D. student in Environmental Science at the University of Geneva, Switzerland. He got a MSc in marine biodiversity and biomolecules from the University of Toulon, France. His research is focused on the interactions between phytoplankton and mercury. He highlighted the role of phytoplankton in the biogeochemical cycle of mercury by (i) evaluating the biotic transformations performed by a cyanobacterium and natural phytoplankton communities, (ii) determining the effects of thiols bioligands on the bioaccumulation of Hg species by cyanobacteria.

João P. Santos holds a BSc in Biology and a MSc in Ecology, Environment and Landscape, and a MSc in Marine Sciences from the University of Porto, Portugal. He worked with the nitrogen cycle communities until he started his Ph.D. Currently, he is a Ph.D student in the Slaveykova's lab at the University of Geneva, Switzerland. He studies the interaction of diatoms and natural phytoplankton communities with different mercury species, and explores their capability to accumulate, transform and detoxify mercury.

Dr. Isabelle A.M. Worms is a senior scientist of environmental biogeochemistry and ecotoxicology at the University of Geneva. She is a biochemist with a MSc in molecular chemistry. Since her Ph.D., her research interests include (i) understanding of the bioavailability of trace metals; (ii) developing of AF4-ICP-MS to assess the role of natural organic matter on the binding and dispersion of trace metals in surface water; and (iii) identifying key processes involved in nanoparticle stability using quantitative approaches.

Dr. David Amouroux is a research director at the French CNRS appointed at IPREM, CNRS-UPPA. He is responsible of the Research Unit for Environmental Chemistry and Microbiology. He is an environmental and analytical chemist, specifically interested in the cycling and reactivity of contaminants in the environment. Some of his research lines include: (i) transformations and transfer of mercury; selenium and other metal(loid)s at aquatic environment interfaces and (ii) development of analytical and experimental methods using stable isotopes of trace elements to investigate biogeochemical mechanisms in the environment.

Javier García Calleja is a Ph.D. student in the Amouroux lab at the IPREM, CNRS- UPPA, Pau, France. He holds a BSc in chemistry and a MSc in analytical and bioanalytical chemistry from the University of Oviedo, Spain. His research includes (i) development of mathematical approaches based on isotope pattern deconvolution for studying Hg compound reactivity in *in situ* Hg incubation experiments and (ii) characterization of bioligands involved in Hg speciation in phytoplankton by hyphenated techniques based on elemental and mass spectrometry.

Dr. Elaheh Lotfi Kalahroodi is a postdoctoral researcher in Environmental Geochemistry at the Umeå University in Sweden. Her research is focused on studies the biogeochemical mechanisms of organic and inorganic contaminants in the environment through isotope fractionation of stable non-traditional isotopes. She evaluates and optimizes methodologies to trace and quantify the spread of Hg from contaminated sediment sites to surrounding sediment and pelagic and benthic biota using Hg stable isotope measurements.

Dr. Zoyne Pedrero ZAYAS is a research scientist at the French CNRS appointed at IPREM, CNRS-UPPA. She is analytical chemist and her work principally focuses on metal speciation (mainly Hg and Se) by hyphenated chromatography-based separation mass spectrometry techniques and isotopic analyses in living organisms. Her research interest comprises, among others, the interaction of Hg and Se in biota as wells as pollution sources and metabolic processes tracking.

Dr. Vera I. Slaveykova is a full professor at University of Geneva and president of the School of Earth and Environment Sciences. She works on the development of concepts and tools for a better understanding of the fundamental processes governing the behavior and impact of trace elements and nanoparticles in the aquatic environements. Her current research interests and portfolio include (i) speciation and bioavailability of trace elements, and nanoparticles in the aquatic environment; (ii) aquatic toxicology of inorganic contaminants and nanoparticles; transcriptomics and metabolomics.

Abstract

Phytoplankton may directly influence biogeochemical cycling and transformations of mercury (Hg) through biotic transformations of the accumulated metal via methylation/demethylation and reduction/oxidation, and indirectly, through the excretion of low and high molecular weight ligands, likely triggering or influencing different abiotic transformation pathways as well as the transformations carried by bacteria. However, unlike the extensive work already done on the role of bacteria in Hg transformations, the current knowledge about the influence of phytoplankton (algae and cyanobacteria) on such processes is still limited.

Critical evaluation of the existing advances in the research topic revealed that different microalgal species and cyanobacteria contribute to the biotic reduction of inorganic mercury (iHg or Hg^{II}) into elemental Hg (Hg^0), monomethylmercury (MeHg) demethylation, and transformation of iHg into metacinnabar. The low and high molecular weight biomolecules released by phytoplankton can complex Hg species and contribute to abiotic mercury reduction. Despite these advances, the underlying mechanisms and their importance in the aquatic environment are to be explored and detailed. The development of the novel molecular, stable isotope-based, and multi-omics approaches would provide further impetus for the understanding of the key interactions between Hg species and phytoplankton. Such understanding will be of utmost importance for the improvement of the Hg biogeochemical modelling, mitigation strategies, and rational environmental risk assessment in the changing aquatic environment.

Keywords: Mercury cycling, methylmercury, speciation, methylation, demethylation, reduction, oxidation, algae, cyanobacteria

1. Introduction

Mercury is naturally present in the environment, however, since the industrial revolution, anthropogenic activities have increased the global Hg emissions by a factor of 2-15 and disturbed the Hg biogeochemical cycle (Driscoll *et al.* 2013, Ariya *et al.* 2015, Asaduzzaman *et al.* 2019, Branfireun *et al.* 2020), leading to a significant increase in the concentration of various Hg compounds in the aquatic environment.

In aquatic environments, mercury is commonly found as oxidized Hg^{II} (iHg), reduced elemental mercury (Hg^0),and monomethyle mercury (MeHg, MMHg, CH_3Hg^+) with their relative abundances being controlled by numerous chemical, physical and biological processes (Branfireun *et al.* 2020). Mercury species interact with different biotoc and abiotic constituents forming complexes with inorganic ligands such as the hydroxide, chloride anions (Powell *et al.* 2005), low molecular weight thiols (Hsu-Kim and Sedlak 2005) and dissolved organic matter (DOM) (Ravichandran 2004, Wang *et al.* 2015, Jiang *et al.* 2017, Liem-Nguyen *et al.* 2017, Klapstein and O'Driscoll 2018, Lavoie *et al.* 2019, Poulin *et al.* 2019). Among all chemical functional groups present in DOM, mercury binds preferentially the –SH groups (Ravichandran 2004). Given the very strong tendency of iHg to form complexes, the estimated value of the mercury free ion concentrations in surface waters is $< 10^{-26}$ mol L^{-1} (Le Faucheur *et al.* 2014). In addition, Hg species are subject of various transformations, which results in a distribution that is usually follows the order of $iHg>Hg^0\sim MeHg$ in freshwater and $iHg>Hg^0>MeHg$ in seawater (Le Faucheur *et al.* 2014). The major transformation pathways involve the reduction/oxidation of iHg/ Hg^0 ,and the methylation/demethylation of iHg/MeHg. iHg and MeHg can accumulate in the aquatic organisms and MeHg biomagnifies along the food webs, presenting a hazard to higher consumers, including humans (Sheehan *et al.* 2014, Yang *et al.* 2020). The bioconcentration of Hg by phytoplankton represents one of the main entry steps of Hg into the food web (Dranguet *et al.* 2014, Le Faucheur *et al.* 2014, Wu *et al.* 2019). The knowledge of various transformation processes determined by photochemical, chemical, and biologically mediated reactions (such as those performed by phytoplankton) is also crucial for understanding the global Hg^0 fluxes (Jiskra *et al.* 2021).

Exploring mercury transformation pathways in the aquatic environment is an active research area. Extensive studies have been already done on the role of bacteria in Hg transformations, as comprehensively reviewed by (Hsu-Kim *et al.* 2013). Several recent reviews deal with specific transformation mechanisms and influencing factors, including advances in the knowledge regarding the methylation (Paranjape and Hall 2017, Gallorini and Loizeau 2021, Wang *et al.* 2021), demethylation (Barkay and Gu 2022), production/degradation of MeHg in the cryosphere (Ghimire *et al.* 2019), biotic and abiotic degradation of MeHg (Du *et al.* 2019) and photochemical transformation of Hg species (Luo *et al.* 2020). The role of phytoplankton (algae and cyanobacteria) in Hg cycling (i. e., alteration of Hg redox state, Hg scavenging, the potential for methylation), as well as the description of the cellular and molecular targets involved in the toxicity of Hg in phototrophs, were thoroughly discussed previously (Grégoire and Poulain 2014, Beauvais-Flück *et al.* 2018). Hg bioavailability to phytoplankton (Dranguet *et al.* 2014, Le Faucheur *et al.* 2014) and its toxicity to primary producers were also reviewed (Nuzzi 1972, Wu and Wang 2011, Chen *et al.* 2014, Dranguet *et al.* 2014, Beauvais-Flück *et al.* 2017, Beauvais-Flück *et al.* 2018). In this context, the importance of phytoplankton in aquatic mercury transformations was always questioned but never systematically addressed.

In the present review, we focussed on the possible controls exerted by phytoplankton on the key transformations of mercury in the aquatic environment (Fig. 1). In particular, we critically discuss (i) the role of biomolecules released by phytoplankton in Hg speciation, (ii) different abiotic transformation pathways triggered or influenced by phytoplankton and (iii) biotic transformation pathways and cellular speciation of Hg species.

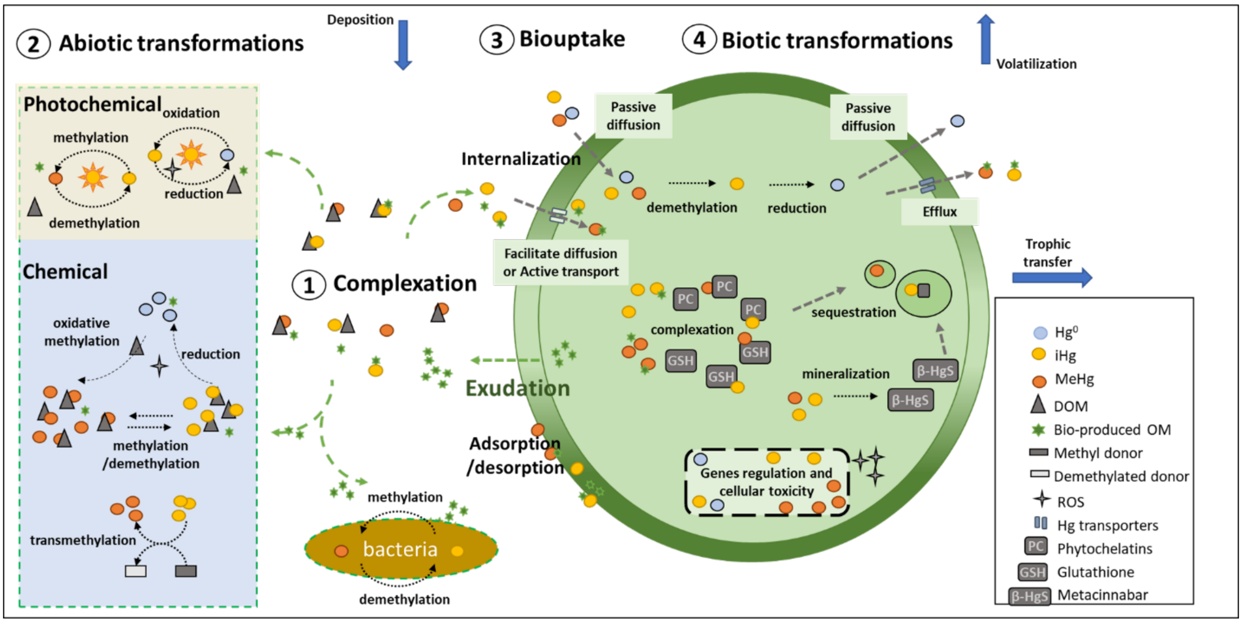

Figure 1. Conceptual view of main processes involved in aquatic Hg biouptake and biotic transformations by phytoplankton, and abiotic transformations influenced or triggered by phytoplankton species.

2. Mercury complexation by biomolecules produced by the phytoplankton

Phytoplankton produces various small molecules, including fatty acids, carboxylic acids, amino acids, and extracellular polymeric substances (EPS, such as polysaccharides, nucleic acid, and proteins) (Seymour *et al.* 2017). EPS represent up to 25% of natural organic matter in freshwaters, especially during algal blooms (Wilkinson *et al.* 1997, Shou *et al.* 2018). The EPS components comprise diverse anionic groups (e.g., –SH, $-NH_2$, –COOH), thus providing metal-binding properties (Babiak and Krzemińska 2021). Phytoplankton is also known to release small thiols with strong capacities to bind metals in their ambient environment (Liu *et al.* 2020), which are expected to affect the speciation and thus Hg abiotic and biotic transformations. The nature and concentration of such small biomolecules vary with the algal species and environmental factors (Mangal *et al.* 2019a). For example, the diatom *Phaeodactylum tricornutum* has been reported to release cysteine-like exudates, whereas the coccolithophore *Emiliana huxleyi* excreted both glutathione- and cysteine-like compounds (Vasconcelos *et al.* 2002). The effects of biomolecules released by algae on Hg speciation (Fig. 1 ❶) have been examined in the field, where dissolved organic carbon and algal exudates are the predominant ligands of Hg in the rivers and lakes of Long Island Sound, USA (Lamborg *et al.* 2004). Six thiols (mercaptoacetic acid, cysteine, homocysteine, N-acetyl-cysteine, mercaptoethane-sulfonate, and glutathione) were detected with total concentrations of 7-153 nM in boreal lake waters (Bouchet and Björn 2014, Liem-Nguyen *et al.* 2015). Recent studies have shown that several green algae excrete some ligands, particularly thiol-containing ligands that form strong complexes with Hg species and thus modify Hg speciation and bioavailability (Mangal *et al.* 2016, Ly *et al.* 2017, Mangal *et al.* 2019a).

The interaction of Hg species with EPS is poorly documented although recognized to have a unique molecular character (Mangal *et al.* 2016, Ly *et al.* 2017). EPS were shown to complex significantly different metals, including Hg (Naveed *et al.* 2019). Indeed Hg was found to bind to protein-like material produced by *Chloroccochus* (molecular weight, MW > 3.5 kDa) (Song *et al.* 2014), and EPS from activated sludge (MW > 3.5 kDa) presumably by electrostatic interactions (Zhang *et al.* 2013). Biomolecules released by several microalgae with apparent molecular mass > 1kDa, likely prevented the induction of Hg microbial biosensor, whereas the presence of smaller biomolecules (MW 0.3 kDa-1 kDa) allowed the induction of Hg controlled fluorescence (Mangal *et al.* 2019a). Nevertheless, further studies are necessary to explore the relative importance of the biomolecules released by the phytoplankton species, including thiols and EPS, in Hg speciation and their role in its transformations in aquatic environments.

3. Abiotic transformation pathways of mercury triggered or influenced by phytoplankton

In oxic waters, Hg species can be subjected to a series of abiotic transformations (Fig. 1 ❷), such as reduction/oxidation and methylation/demethylation, as influenced or not by incident light. The extent of these transformations depends on the environmental factors and biological activity of aquatic living microorganisms (Hsu-Kim *et al.* 2013, Grégoire and Poulain 2014).

Photochemical reactions are responsible for the ***abiotic reduction*** of iHg to Hg^0 and Hg^0 oxidation to iHg (Vost *et al.* 2012). The extent of these reactions depends on the intensity of ultraviolet (UV) radiation (Black *et al.* 2012) and the ambient water composition (Lalonde *et al.* 2001, Whalin *et al.* 2007). Although the importance of each reaction is not resolved yet, it is recognized that their kinetics is strongly affected by Hg speciation, which in turn is modulated by the nature and concentrations of ligands present in surface waters (Zhang and Hsu-Kim 2010, Tai *et al.* 2014). For example, it was shown that the biogenic DOM produced by the marine diatom *Chaetoceros* sp. was involved in the photoreduction of iHg (Lanzillotta *et al.* 2004). A recent review highlighted that the ***photooxidation*** of Hg^0 is mainly mediated by reactive oxygen species (ROS) (Luo *et al.* 2020). The ROS generation can also occur by the absorption of UV-B radiation by humic and fulvic-like DOM resulting in various photochemical transformations involving oxygen. High production of dissolved gaseous mercury (DGM) was correlated to the high concentration of DOM, in particular thiols binding sites (Ariya *et al.* 2015). On the other hand, the Hg redox cycle in oxic surface waters is mainly dominated by photochemical iHg reduction (Amyot *et al.* 1997).

Abiotic methylation can occur through transmethylation with organometallic species (methylated Pb, I, etc.) and methyl- donors such as methylcobalamin, but these pathways are not considered predominant compared to biotic methylation (Weber 1993, Celo *et al.* 2006). Indeed, experimentation in productive or organic-rich natural waters, incubation in filtered water enriched with iHg at environmental levels demonstrated no production of MeHg even if some methylation was detected in some particular conditions (Monperrus *et al.* 2007, Alanoca *et al.* 2016). Physico-chemical parameters of the aquatic environment greatly impact the extent of abiotic methylation. For example, in Canadian Lakes, DOM having molecular sizes lower than 5 kDa and between 30 kDa and 300 kDa have been reported to mitigate abiotic methylation occurring through solar irradiation (Siciliano *et al.* 2005).

Demethylation occurs by multiple and complex processes, which can be mediated by different biotic and abiotic mechanisms (Barkay and Gu 2022). Two abiotical processes are commonly evocated: (i) photodemethylation, which is believed to be responsible for a significant part of MeHg degradation in surface waters (Hammerschmidt and Fitzgerald 2010, Zhang and Hsu-Kim 2010); and (ii) chemical demethylation, most likely due to e.g. reaction with H_2S or sulfide minerals (Jonsson *et al.* 2016, Kanzler *et al.* 2018) and selenoamino acids (Khan and Wang 2010). The photodemethylation of MeHg and dimethylmercury is well-described and considered to be central in the MeHg degradation in surface waters (Barkay and Gu 2022). The extent of MeHg photodemethylation was observed to depend on the type of solar radiation, the concentration of DOM and the structure of MeHg binding sites, which in turn could influence the generation of free radicals and ROS (e.g. $^{\bullet}OH$ or $^1O_2$) (Sellers *et al.* 1996, Hammerschmidt and Fitzgerald 2006, Lehnherr and Louis 2009, Luo *et al.* 2020). However, there are still some controversies about the role of DOM in photodemethylation. For example, low DOM concentration promoted, whereas very high DOM concentration inhibited MeHg photodemethylation by DOM (Klapstein and O'Driscoll 2018). However, using Hg compound-specific stable isotope analysis it was shown in situ that MeHg photodegradation in natural waters mostly occurred in waters exposed to UV radiation and was modulated by the DOM level (Bouchet *et al.* 2022). The photodemethylation of MeHg to iHg was shown to increase in the presence of fulvic acids while this process was limited in the presence of humic acids (Luo *et al.* 2020). Labile Fe and photochemically produced ROS were shown to play a role in MeHg photodecomposition (Hammerschmidt and Fitzgerald 2010), although demonstrated to be not compulsory as thiol and phenyl may be the major moieties in DOM-mediated MeHg photodegradation (Zhang *et al.* 2018).

The production of biomolecules by phytoplankton was also shown to affect the Hg transformations ***indirectly*** by affecting mercury methylation/demethylation by bacteria. For example, organic matter derived from phytoplankton is considered "a fuel" for methylating organisms and shapes the community structures of periphytic biofilms (Xing *et al.* 2018). The symbiotic presence of *Chlorella* increased the methylation by *Geobacter sulfurreducens* PCA (Zhao *et al.* 2021). However, the biomolecules released by *Chlorella* had only limited effects on iHg methylation by *G.sulfurreducens* PCA but significantly increased the MeHg production by *D Desulfovibrio desulfuricans* (Yin *et al.* 2022). A clear positive correlation between the activity of methylating microorganisms in sediments and algal productivity was proven in several aqueous systems (Bravo *et al.* 2017, Ortega *et al.* 2018, Wu *et al.* 2022). However, further studies are needed since the quality and quantity of the produced biomolecules are species-specific and dependent on the environmental conditions.

4. Biotic transformation pathways mediated by phytoplankton

Biotic transformations of Hg are considered as intracellular processes (Hsu-Kim *et al.* 2013), therefore the ***uptake*** (Fig. 1③) of iHg and MeHg species by phytoplankton is an important first step in the overall transformation processes. Nonetheless, the uptake pathways and their kinetics are still not well understood for phytoplankton species. Evidence are supporting passive diffusion of uncharged complexes (Bienvenue *et al.* 1984, Mason *et al.* 1996, Kim *et al.* 2014), facilitated diffusion (Wang *et al.* 2004, Le Faucheur *et al.* 2011, Moreno *et al.* 2014) as well as possible active transport pathways (Miles *et al.* 2001, Moye *et al.* 2002, Pickhardt and Fisher 2007). Assimilation of MeHg by diatoms, chlorophyte, dinoflagellate and coccolithophore by passive diffusion is considered the most plausible uptake mechanism considering the high surface-area-to-volume ratio of algal cells. However, the uptake of MeHg by dinoflagellate *Prorocentrum minimum* was suggested to be an active process (Lee and Fisher 2016).

The biouptake of Hg by aquatic microorganisms is recognized to be affected by the complexation by DOM and different ligands (Chiasson-Gould *et al.* 2014, Le Faucheur *et al.* 2014, Bravo *et al.* 2017, Grégoire *et al.* 2018). Nevertheless, the influence of the EPS on the mercury species' biouptake is still poorly understood. EPS produced by 5 algae (*Scenedesmus obliquus*, *Chlorella* *vulgaris*, *Chlamydomonas reinhardtii*, *Euglena gracilis,* and *Euglena mutabilis*) were shown to reduce the Hg gene lux induction used as a surrogate for the uptake for modified *E. coli* but in a way which depends on the species and molecular mass of the EPS: low molecular weight fractions likely participate to bacterial Hg uptake, whereas high molecular weight fractions decrease the uptake (Mangal *et al.* 2019b). Thiol ligands, such as 2-mercaptoethanol, dithiothreitol, and glutathione reduced the uptake of MeHg by a cyanobacterium, *Nostoc calcicole* (Pant *et al.* 1995). MeHg uptake by a green alga *Selenastrum capricornutum* was decreased in the presence of cysteine, mercaptoacetic acid, 2-mercaptopropionic acid, glutathione, *N*-acetyl-L-cysteine and *N*-acetyl-penicillamine (Skrobonja *et al.* 2019).

Phytoplankton was reported to trigger different mercury transformation processes (Fig. 1 ④), including reduction, demethylation, and sequestration of Hg as β-HgS. The **reduction of iHg** to gaseous Hg^0 has been demonstrated in laboratory experiments with several phytoplankton species (Mason *et al.* 1995, Poulain *et al.* 2004, Kelly *et al.* 2006, Poulain *et al.* 2007, Morelli *et al.* 2009, Oh *et al.* 2011, Grégoire and Poulain 2014, 2016). The volatilization rates varied between the algal species, Hg concentration and exposure duration (Devars *et al.* 2000, Morelli *et al.* 2009). Early works demonstrated that the exposure of various phytoplankton species to very high iHg concentrations resulted to Hg^0 production, observations often linked to the detoxification mechanisms (Ben-Bassat *et al.* 1972, Ben-Bassat and Mayer 1975, Macka *et al.* 1978, Wilkinson *et al.* 1989, Kelly *et al.* 2007). Diatom *T. weissflogii* was shown to produce DGM under light and dark conditions suggesting that biological rather than photochemical processes or photosynthetic metabolites mediate this transformation (Morelli *et al.* 2009). Production of DGM was observed for 3 other diatoms species (Wu and Wang 2014), suggesting an important role of intracellular thiols. The reduction of iHg was, however, observed in cultures of *Chlorella vulgaris,* but the organic matter released or obtained after cell degradation was shown to reduce more iHg than living algal cells themselves (Liang *et al.* 2022). Nevertheless, the fundamental mechanisms involved in the biological reduction process remain poorly understood.

Recent advances revealed the activation of a *MerR*-like transcription factor, Slr0701 when cyanobacterium *Synechocystis* sp. PCC6803 is exposed to iHg (Singh *et al.* 2019). The activation of this transcription factor promotes the expression of the mercuric reductase, *MerA*-like coded by the Slr1849 gene, which allows the reduction of iHg into the volatile form Hg^0 (Boyd and Barkay 2012, Singh *et al.* 2019). This process of Hg reduction could enhance the Hg tolerance of this cyanobacterium. The reduction of iHg to Hg^0 followed by its volatilization is the process responsible for the evasion of Hg from both terrestrial and aquatic systems (Gonzalez-Raymat *et al.* 2017). Hg^0 production by phytoplankton has been thus evidenced in the field (Grégoire and Poulain 2014) and the formation of DGM was correlated with phytoplankton dynamics and blooms (Poulain *et al.* 2004, Poulain *et al.* 2007). It was also shown that phototrophic bacteria use iHg as an electron sink to maintain redox homeostasis to produce Hg^0 (Grégoire and Poulain 2016). However, it is still unclear whether algae and cyanobacteria are directly involved in DGM production or mediate this process by triggering bacterial activity or releasing biogenic organic ligands.

Hg ***methylation/demethylation*** by phytoplankton was investigated in the laboratory. For instance, the potential MeHg demethylation was seen in the different species of algae and cyanobacteria (Bravo *et al.* 2014, Franco *et al.* 2018, Li *et al.* 2022, Yin *et al.* 2022) however, no evidence of methylation was found. Indeed, a specific gene cluster (*hgcAB*) used as a proxy for the microorganism's capability to methylate iHg (Gilmour *et al.* 2013), is not found in phytoplankton species. Similarly, no methylation was observed by pico-nanoplankton from a eutrophic lake (Cossart *et al.* 2021). Although there is no direct evidence that phytoplankton microorganisms can methylate Hg itself, numerous studies have highlighted the importance of algae in MeHg production (Lázaro et al., 2019). Several studies have reported a positive correlation between phototrophic productivity and an increase in MeHg (Tsui *et al.* 2010, Lázaro *et al.* 2013, Xing *et al.* 2018, Lazaro *et al.* 2019). Strong links have been uncovered between methylation rates in open oceans and the presence of nano- and pico-phytoplankton (Heimbürger *et al.* 2010). iHg methylation rates were measured at the maximum chlorophyll depth (i.e. maximum phytoplankton biomass) in oxic surface seawater and were shown to be influenced by pelagic microorganism abundance and activities (phyto- and bacterioplankton)(Monperrus *et al.* 2007). iHg methylation in the water column was shown to account for around 47% of the MeHg present in polar marine waters (Lehnherr *et al.* 2011). The Hg methylation rates have been linked to the presence of thiols produced by phytoplankton species and the decomposition of algal-derived organic matter (Bravo *et al.* 2017, Bouchet *et al.* 2018, Zhao *et al.* 2021).

Demethylation in oxic surface waters has been reported to be partially biologically mediated besides being induced by solar radiation (Whalin *et al.* 2007). Reduction and demethylation of Hg were also demonstrated in the diatom *T. weissflogii* (Devars *et al.* 2000, Morelli *et al.* 2009) and green alga *C. reinhardii* (Bravo *et al.* 2014). However, the transformation yields and demethylation rate constants are still to be elucidated and quantified. Very recently, the demethylation capacity of 15 algae species was investigated, and 6 out of 15 species (dinoflagellates, chrysophytes, and diatoms) tested were able to demethylate MeHg (Li *et al.* 2022). The demethylation was also demonstrated in natural pico-nanoplankton communities from a eutrophic lake (Cossart *et al.* 2021) as well as in productive coastal waters (Sharif *et al.* 2014) and maximum phytoplankton biomass depth of marine waters (Monperrus *et al.* 2007). Overall, the current understanding of methylation and demethylation of Hg species by phytoplankton is rather limited. Yet, recent results have highlighted phytoplankton direct or indirect implications for both processes.

Phytoplankton species could control ***intracellular Hg speciation*** and thus affect the intracellular transformations by cytosolic ligands. It has been shown that the quantity and the quality of the intracellular metabolites, which could interact with Hg species, are altered by iHg exposure (Mangal *et al.* 2022). Glutathione (GSH) content, which is the most prevalent thiol in algae, was found to increase in algae exposed to iHg (Howe and Merchant 1992, Devars *et al.* 2000, Morelli *et al.* 2009). MeHg exposure was also seen to induce the synthesis of GSH in *Thalassiosira weissflogii*, but it was iHg that contributed to higher levels of other thiol compounds, such as cysteine and phytochelatins (PCs) (Wu and Wang 2012, 2013). More recently, GSH was identified as the main low molecular weight binding ligand to iHg and MeHg in the cytosolic fraction of cyanobacterium *Synechocystis* sp. PCC 6803 (Garcia-Calleja *et al.* 2021). PCs enzymatically produced from glutathione are additional thiols used by algae to counteract Hg negative effects (Mehra *et al.* 1996). For example, the PC_{2-3} have been reported to be synthesized by *T. weissfloggi* when exposed to 5 and 150 nM Hg while MeHg seems to be a poor inducer (Howe and Merchant 1992, Ahner and Morel 1995, Knauer *et al.* 1998, Morelli *et al.* 2009). A comparison of diatom *T. weissflogii* with the green alga *Chlorella autotrophica* revealed that PCs induction is highly dependent on the phytoplanktonic species with higher biological responses seen in *T. weissflogii*, and low PCs induction observed for *C. autotrophica* (Wu and Wang 2014). The sequestration of iHg bound to PCs was identified in the microalga *Chlorella sorokiniana* exposed to high iHg concentrations (Gómez-Jacinto *et al.* 2015). However, the role of these thiols in cellular transformations of iHg and MeHg still needs to be confirmed under lower environmentally realistic exposure Hg concentrations.

Hg sequestration as β-cinabar (HgS) has been demonstrated as a detoxification mechanism in a variety of cyanobacteria *Limnothrix planctonica*, *Synechococcus leopoldiensis*, and *Phormidium limnetica* (Kelly *et al.* 2006, Kelly *et al.* 2007). Green algae *Chlorella autotrophica*, flagellate *Isochrysis galbana*, and marine diatom *Thalassiosira weissflogii* could transform iHg into metacinnabar (β-HgS) (Wu and Wang 2014). Sunlight was also shown to facilitate the transformation of Hg to less bioavailable species, such as β-HgS and Hg-PCs (Liang *et al.* 2022). Overall, phytoplankton can sequester high quantities of Hg as a detoxification strategy without apparent harmful effects. The tolerance to Hg species toxicity has been related to the capacity of the phytoplankton to capture Hg in subcellular compartments as vacuoles which serve as a sink for mineralized forms or low molecular weight thiol compounds. Nevertheless, no information has been ever provided at environmentally relevant concentrations or even in real environmental conditions in various aquatic systems.

The examples presented above demonstrated the important role of thiol compounds in intracellular handling of iHg and MeHg and the existing research gaps in understanding the underlying mechanisms and interplay between iHg and MeHg transformation and thiol pathways. However, the recent developments allowing direct quantification of both low and high molecular weight thiols and their Hg complexes (Pedrero *et al.* 2011, Pedrero Zayas *et al.* 2014, Garcia-Calleja *et al.* 2021) open new opportunities for exploring the effect of intra- and extracellular ligands in Hg uptake and biotic transformation. On the other hand, the exposures of green alga *C. reinhardtii* to low (5 nM) and high (50 nM) iHg and MeHg concentrations induced metabolic perturbations in amino acid and nucleotide synthesis and degradation, fatty acids, carbohydrates, tricarboxylic acids, antioxidants and photorespiration (Slaveykova *et al.* 2021).

5. Conclusion and perspectives

Important research advances confirmed that phytoplankton could affect Hg speciation and transformations directly, e.g. via biotic transformations of the accumulated mercury species and/or indirectly via the release of low and high molecular weight molecules which could complex mercury and affect both abiotic and biotic transformations of Hg compounds. The up-to-date studies revealed that different microalgal species and cyanobacteria contribute to iHg biotic reduction into Hg^0, MeHg demethylation and transformation of iHg into metacinnabar, as well as produce different biomolecules which can contribute to abiotic mercury reduction. Nevertheless, numerous questions remain open concerning the underlying mechanisms of Hg species interactions with phytoplankton in terms of their uptake mechanisms and cellular handling, including the release of biomolecules, which can be the focus of future research. The mechanisms behind the potential transformations of mercury species in aquatic environment mediated by the phytoplankton are still not fully understood and further research studies are needed. The role of the phytoplankton in biotic transformations of mercury species and their significance compared to other microorganisms such as bacteria are overlooked and need to be further explored especially combining both phototrophic and heterotrophic microrogansims in specific experiments. Therefore, studies that quantitatively examine different transformation processes and identify the phytoplankton species or groups of species able to demethylate or reduce mercury in situ are highly sought.

The development of the Hg stable isotope fractionation approach opens up the possibility to decipher the contribution of interconnected abiotic and biotic transformation (Kritee *et al.* 2013) and to track further the processes controlling origin and cycling of Hg before its incorporation in the foodweb (Bouchet *et al.* 2022). Since the biotic transformations are considered prevailing, the development of the novel -omics approaches would provide key information on the interactions between Hg and phytoplankton species (Beauvais-Flück *et al.* 2016, Beauvais-Flück *et al.* 2018, Slaveykova *et al.* 2021, Mangal *et al.* 2022). These approaches can be used to design the strategies to understand the mechanisms of Hg-induced metabolic perturbations and to explore their relationship with cellular transformations of Hg species. A combination of the chromatography-based separation with mass spectrometry detection for quantification of both low and high molecular weight cellular molecules and their Hg complexes (Garcia-Calleja et al., 2021) with the cellular effects could contribute to uncover the relationship between transformations and toxicological outcomes of Hg exposure. Furthermore, the relationship between different cellular transformation processes and different detoxification mechanisms merits deeper insight. Identifying specific organelles and cellular compartments where Hg species accumulate and can be transformed could be also of added value, given the importance of cellular speciation and distribution in the toxicity, detoxification and trophic transfer of mercury (Wu and Wang 2011). In addition to the mechanistics studies with model microorganisms, the above-mentioned approaches can be used to address key questions on the interactions between Hg species and the phytoplankton community and, to improve the current understanding of their contributions to Hg species transformations in natural environment.

The understanding of the cellular transformations and speciation is central for the elucidation of the role of phytoplankton in Hg biogeochemical cycle and is of high importance to better predict the long-term changes in Hg bioavailability to food webs. Indeed, a global circulation 3D model of MeHg in seawater showed that diatoms and picocyanobacterium *Synechococcus* sp. are the most important phytoplankton categories for the transfer of MeHg from seawater to herbivorous zooplankton, contributing 35% and 25%, respectively (Zhang *et al.* 2020). Given the interconnection between the global change and biogeochemical cycling of mercury (Chetelat *et al.* 2022), a deeper understanding of the mercury transformation processes triggered by phytoplankton, measured both in the laboratory and in situ and the development of mechanistic models coupling primary production, Hg transport, abiotic and biotic transformations and climate models would allow projections under various climate change scenarios at a global scale. This will further constrain the efficiency of the measures taken by the Minamata convention to reduce mercury emissions. This is important given continuous anthropogenic Hg inputs to aquatic ecosystems and considerable shifts in the phytoplankton dynamics predicted with global change.

Data Availability Statement

Data sharing is not applicable as no new data were generated or analyzed during this study.

Conflicts of Interest

The authors declare no conflicts of interest.

Declaration of Funding

This work was supported by the Swiss National Science Foundation project No 175721 and Agence Nationale de la Recherche, project No 17-CE34-0014-01.

References

Ahner BA, Morel FMM (1995) Phytochelatin Production In Marine-Algae. 2. Induction By

Various Metals. *Limnology and Oceanography* **40** (4), 658-665. 10.4319/lo.1995.40.4.0658

Alanoca L, Amouroux D, Monperrus M, Tessier E, Goni M, Guyoneaud R, Acha D, Gassie C, Audry S, Garcia ME, Quintanilla J, Point D (2016) Diurnal Variability and Biogeochemical Reactivity of Mercury Species in an Extreme High-Altitude Lake Ecosystem of the Bolivian Altiplano. *Environmental Science and Pollution Research* **23** (7), 6919-6933. 10.1007/s11356-015-5917-1

Amyot M, Gill GA, Morel FMM (1997) Production and Loss of Dissolved Gaseous Mercury in Coastal Seawater. *Environmental Science & Technology* **31** (12), 3606-3611. 10.1021/es9703685

Ariya PA, Amyot M, Dastoor A, Deeds D, Feinberg A, Kos G, Poulain A, Ryjkov A, Semeniuk K, Subir M, Toyota K (2015) Mercury Physicochemical and Biogeochemical Transformation in the Atmosphere and at Atmospheric Interfaces: A Review and Future Directions. *Chemical Reviews* **115** (10), 3760-3802. 10.1021/cr500667e

Asaduzzaman A, Riccardi D, Afaneh AT, Cooper CJ, Smith JC, Wang F, Parks JM, Schreckenbach G (2019) Environmental Mercury Chemistry – In Silico. *Accounts of Chemical Research* **52** (2), 379-388. 10.1021/acs.accounts.8b00454

Babiak W, Krzemińska I (2021) Extracellular Polymeric Substances (EPS) as Microalgal Bioproducts: A Review of Factors Affecting EPS Synthesis and Application in Flocculation Processes. *Energies* **14** (13), 4007. 10.3390/en14134007

Barkay T, Gu B (2022) Demethylation─ The Other Side of the Mercury Methylation Coin: A Critical Review. *ACS Environmental Au* **2**, 77–97. 10.1021/acsenvironau.1c00022

Beauvais-Flück R, Slaveykova V, Cosio C (2018) Molecular Effects of Inorganic and Methyl Mercury in Aquatic Primary Producers: Comparing Impact to A Macrophyte and A Green Microalga in Controlled Conditions. *Geosciences* **8** (11), 393. 10.3390/geosciences8110393

Beauvais-Flück R, Slaveykova VI, Cosio C (2016) Transcriptomic and Physiological Responses of the Green Microalga *Chlamydomonas reinhardtii* during Short-Term Exposure to Subnanomolar Methylmercury Concentrations. *Environmental Science & Technology* **50** (13), 7126-7134. 10.1021/acs.est.6b00403

Beauvais-Flück R, Slaveykova VI, Cosio C (2017) Cellular Toxicity Pathways of Inorganic and Methyl Mercury in the Green Microalga *Chlamydomonas reinhardtii*. *Scientific Reports* **7** (1), 8034-8034. 10.1038/s41598-017-08515-8

Ben-Bassat D, Mayer AM (1975) Volatilization of Mercury by Algae. *Physiologia Plantarum* **33** (2), 128-132. 10.1111/j.1399-3054.1975.tb03779.x

Ben-Bassat D, Shelef G, Gruner N, Shuval HI (1972) Growth of Chlamydomonas in a Medium Containing Mercury. *Nature* **240** (5375), 43-4. 10.1038/240043a0

Bienvenue E, Boudou A, Desmazes JP, Gavach C, Georgescauld D, Sandeaux J, Sandeaux R, Seta P (1984) Transport of Mercury Compounds Across Bimolecular Lipid Membranes: Effect of Lipid Composition, pH and Chloride Concentration. *Chemico- Biological Interactions* **48**, 91-101. 10.1016/0009-2797(84)90009-7

Black FJ, Poulin BA, Flegal AR (2012) Factors Controlling the Abiotic Photo-Degradation of Monomethylmercury in Surface Waters. *Geochimica et Cosmochimica Acta* **84**, 492-507. 10.1016/j.gca.2012.01.019

Bouchet S, Björn E (2014) Analytical Developments For The Determination of Monomethylmercury Complexes With Low Molecular Mass Thiols by Reverse Phase Liquid Chromatography Hyphenated to Inductively Coupled Plasma Mass Spectrometry. *Journal of Chromatography A* **1339**, 50-58. 10.1016/j.chroma.2014.02.045

Bouchet S, Goñi-Urriza M, Monperrus M, Guyoneaud R, Fernandez P, Heredia C, Tessier E, Gassie C, Point D, Guédron S, Achá D, Amouroux D (2018) Linking Microbial Activities and Low-Molecular-Weight Thiols to Hg Methylation in Biofilms and Periphyton from High-Altitude Tropical Lakes in the Bolivian Altiplano. *Environmental Science &*

Technology **52** (17), 9758-9767. 10.1021/acs.est.8b01885

Bouchet S, Tessier E, Masbou J, Point D, Lazzaro X, Monperrus M, Guédron S, Acha D, Amouroux D (2022) In Situ Photochemical Transformation of Hg Species and Associated Isotopic Fractionation in the Water Column of High-Altitude Lakes from the Bolivian Altiplano. *Environmental Science & Technology* **56** (4), 2258-2268. 10.1021/acs.est.1c04704

Boyd E, Barkay T (2012) The Mercury Resistance Operon: From an Origin in a Geothermal Environment to an Efficient Detoxification Machine. *Frontiers in Microbiology* **3** (349) 10.3389/fmicb.2012.00349

Branfireun BA, Cosio C, Poulain AJ, Riise G, Bravo AG (2020) Mercury Cycling in Freshwater Systems - An Updated Conceptual Model. *Sci Total Environ* **745**, 140906. 10.1016/j.scitotenv.2020.140906

Bravo AG, Bouchet S, Tolu J, Bjorn E, Mateos-Rivera A, Bertilsson S (2017) Molecular Composition of Organic Matter Controls Methylmercury Formation in Boreal Lakes. *Nature Communications* **8** 10.1038/ncomms14255

Bravo AG, Le Faucheur S, Monperrus M, Amouroux D, Slaveykova VI (2014) Species-Specific Isotope Tracers to Study the Accumulation and Biotransformation of Mixtures of Inorganic and Methyl Mercury by The Microalga *Chlamydomonas reinhardtii*. *Environ Pollut* **192**, 212-5. 10.1016/j.envpol.2014.05.013

Celo V, Lean DR, Scott SL (2006) Abiotic Methylation of Mercury in the Aquatic Environment. *Sci Total Environ* **368** (1), 126-37. 10.1016/j.scitotenv.2005.09.043

Chen HW, Wu YY, Li YX, Huang WJ (2014) Methylmercury Accumulation and Toxicity to Cyanobacteria: Implications of Extracellural Polymeric Substances and Growth Properties. *Water Environmental Research* **86** (7), 626-34. 10.2175/106143014x13975035525465

Chetelat J, McKinney MA, Amyot M, Dastoor A, Douglas TA, Heimburger-Boavida LE, Kirk J, Kahilainen KK, Outridge PM, Pelletier N, Skov H, St Pierre K, Vuorenmaa J, Wang FY

(2022) Climate Change and Mercury in the Arctic: Abiotic Interactions. *Science of The Total Environment* **824** 10.1016/j.scitotenv.2022.153715

Chiasson-Gould SA, Blais JM, Poulain AJ (2014) Dissolved Organic Matter Kinetically Controls Mercury Bioavailability to Bacteria. *Environmental Science & Technology* **48** (6), 3153-3161. 10.1021/es4038484

Cossart T, Garcia-Calleja J, Worms IAM, Tessier E, Kavanagh K, Pedrero Z, Amouroux D, Slaveykova VI (2021) Species-Specific Isotope Tracking of Mercury Uptake and Transformations by Pico-Nanoplankton in an Eutrophic Lake. *Environmental Pollution* **288**, 117771. 10.1016/j.envpol.2021.117771

Devars S, Avilés C, Cervantes C, Moreno-Sánchez R (2000) Mercury Uptake and Removal by *Euglena gracilis*. *Archives of Microbiology* **174**, 175-180.

Dranguet P, Flück R, Regier N, Cosio C, Le Faucheur S, Slaveykova VI (2014) Towards Mechanistic Understanding of Mercury Availability and Toxicity to Aquatic Primary Producers. *CHIMIA International Journal for Chemistry* **68** (11), 799-805. 10.2533/chimia.2014.799

Driscoll CT, Mason RP, Chan HM, Jacob DJ, Pirrone N (2013) Mercury as a Global Pollutant: Sources, Pathways, and Effects. *Environ Sci Technol* **47** (10), 4967-83. 10.1021/es305071v

Du HX, Ma M, Igarashi Y, Wang DY (2019) Biotic and Abiotic Degradation of Methylmercury in Aquatic Ecosystems: A Review. *Bulletin of Environmental Contamination and Toxicology* **102** (5), 605-611. 10.1007/s00128-018-2530-2

Franco MW, Mendes LA, Windmöller CC, Moura KAF, Oliveira LAG, Barbosa FAR (2018) Mercury Methylation Capacity and Removal of Hg Species from Aqueous Medium by Cyanobacteria. *Water, Air, & Soil Pollution* **229** (4) 10.1007/s11270-018-3782-5

Gallorini A, Loizeau JL (2021) Mercury Methylation in Oxic Aquatic Macro-environments: A Review. *Journal of Limnology* **80** (2) 10.4081/jlimnol.2021.2007

Garcia-Calleja J, Cossart T, Pedrero Z, Santos JP, Ouerdane L, Tessier E, Slaveykova VI, Amouroux D (2021) Determination of the Intracellular Complexation of Inorganic and Methylmercury in Cyanobacterium *Synechocystis* sp. PCC 6803. *Environmental Science & Technology* **55** (20), 13971-13979. 10.1021/acs.est.1c01732

Ghimire PS, Tripathee L, Zhang QG, Guo JM, Ram K, Huang J, Sharma CM, Kang SC (2019) Microbial Mercury Methylation in the Cryosphere: Progress and Prospects. *Science of The Total Environment* **697** 10.1016/j.scitotenv.2019.134150

Gilmour CC, Podar M, Bullock AL, Graham AM, Brown SD, Somenahally AC, Johs A, Hurt RA, Jr., Bailey KL, Elias DA (2013) Mercury Methylation by Novel Microorganisms from New Environments. *Environ Sci Technol* **47** (20), 11810-20. 10.1021/es403075t

Gómez-Jacinto V, García-Barrera T, Gómez-Ariza JL, Garbayo-Nores I, Vílchez-Lobato C (2015) Elucidation of the Defence Mechanism in Microalgae *Chlorella sorokiniana* Under Mercury Exposure. Identification of Hg–Phytochelatins. *Chemico-Biological Interactions* **238**, 82-90. 10.1016/j.cbi.2015.06.013

Gonzalez-Raymat H, Liu G, Liriano C, Li Y, Yin Y, Shi J, Jiang G, Cai Y (2017) Elemental Mercury: Its Unique Properties Affect its Behavior and Fate in the Environment. *Environmental Pollution* **229**, 69-86. 10.1016/j.envpol.2017.04.101

Grégoire DS, Poulain AJ (2014) A Little Bit of Light Goes a Long Way: The Role of Phototrophs on Mercury Cycling. *Metallomics* **6** (3), 396-407. 10.1039/c3mt00312d

Grégoire DS, Poulain AJ (2016) A Physiological Role for Hg^{II} During Phototrophic Growth. *Nature Geoscience* **9** (2), 121-125. 10.1038/ngeo2629

Grégoire DS, Poulain AJ, Ivanova EP (2018) Shining Light on Recent Advances in Microbial Mercury Cycling. *Facets* **3** (1), 858-879. 10.1139/facets-2018-0015

Hammerschmidt CR, Fitzgerald WF (2006) Photodecomposition of Methylmercury in an Arctic Alaskan Lake. *Environmental Science & Technology* **40** (4), 1212-1216. 10.1021/es0513234

Hammerschmidt CR, Fitzgerald WF (2010) Iron-mediated Photochemical Decomposition of Methylmercury in an Arctic Alaskan Lake. *Environ Sci Technol* **44** (16), 6138-43. 10.1021/es1006934

Heimbürger L-E, Cossa D, Marty J-C, Migon C, Averty B, Dufour A, Ras J (2010) Methyl Mercury Distributions in Relation to The Presence of Nano- and Picophytoplankton in an Oceanic Water Column (Ligurian Sea, North-Western Mediterranean). *Geochimica et Cosmochimica Acta* **2010**, 5549-5559.

Howe G, Merchant S (1992) Heavy Metal-activated Synthesis of Peptides in *Chlamydomonas reinhardtii*. *Plant Physiology* **98**, 127-136.

Hsu-Kim H, Kucharzyk KH, Zhang T, Deshusses MA (2013) Mechanisms Regulating Mercury Bioavailability for Methylating Microorganisms in the Aquatic Environment: A Critical Review. *Environmental Science & Technology* **47** (6), 2441-2456. 10.1021/es304370g

Hsu-Kim H, Sedlak DL (2005) Similarities between Inorganic Sulfide and the Strong Hg(II)-Complexing Ligands in Municipal Wastewater Effluent. *Environmental Science & Technology* **39** (11), 4035-4041. 10.1021/es050013i

Jiang T, Skyllberg U, Björn E, Green NW, Tang J, Wang D, Gao J, Li C (2017) Characteristics of Dissolved Organic Matter (DOM) and Relationship With Dissolved Mercury in Xiaoqing River-Laizhou Bay Estuary, Bohai Sea, China. *Environmental Pollution* **223**, 19-30. 10.1016/j.envpol.2016.12.006

Jiskra M, Heimburger-Boavida LE, Desgranges MM, Petrova MV, Dufour A, Ferreira-Araujo B, Masbou J, Chmeleff J, Thyssen M, Point D, Sonke JE (2021) Mercury Stable Isotopes Constrain Atmospheric Sources to The Ocean. *Nature* **597** (7878), 678-682. 10.1038/s41586-021-03859-8

Jonsson S, Mazrui NM, Mason RP (2016) Dimethylmercury Formation Mediated by Inorganic and Organic Reduced Sulfur Surfaces. *Scientific Reports* **6** (1), 1-7. 10.1038/srep27958

Kanzler CR, Lian P, Trainer EL, Yang X, Govind N, Parks JM, Graham AM (2018) Emerging

Investigator Series: Methylmercury Speciation and Dimethylmercury Production in Sulfidic Solutions. *Environmental Science: Processes & Impacts* **20** (4), 584-594. 10.1039/C7EM00533D

Kelly D, Budd K, Lefebvre DD (2006) Mercury Analysis of Acid- And Alkaline-Reduced Biological Samples: Identification of Meta-Cinnabar as the Major Biotransformed Compound in Algae. *Applied and Environmental Microbiology* **72** (1), 361-367. 10.1128/AEM.72.1.361-367.2006

Kelly DJ, Budd K, Lefebvre DD (2007) Biotransformation of Mercury in pH-stat Cultures of Eukaryotic Freshwater Algae. *Archives of Microbiology* **187** (1), 45-53. 10.1007/s00203-006-0170-0

Khan MAK, Wang F (2010) Chemical Demethylation of Methylmercury by Selenoamino Acids. *Chemical Research in Toxicology* **23** (7), 1202-1206. 10.1021/tx100080s

Kim H, Van Duong H, Kim E, Lee BG, Han S (2014) Effects of Phytoplankton Cell Size and Chloride Concentration on The Bioaccumulation of Methylmercury in Marine Phytoplankton. *Environ Toxicol* **29** (8), 936-41. 10.1002/tox.21821

Klapstein SJ, O’Driscoll NJ (2018) Methylmercury Biogeochemistry in Freshwater Ecosystems: A Review Focusing on DOM and Photodemethylation. *Bulletin of Environmental Contamination and Toxicology* **100** (1), 14-25. 10.1007/s00128-017-2236-x

Knauer K, Ahner B, Xue HB, Sigg L (1998) Metal and Phytochelatin Content in Phytoplankton from Freshwater Lakes with Different Metal Concentrations. *Environmental Toxicology and Chemistry* **17** (12), 2444-2452. 10.1002/etc.5620171210

Kritee K, Blum JD, Reinfelder JR, Barkay T (2013) Microbial Stable Isotope Fractionation of Mercury: A Synthesis of Present Understanding and Future Directions. *Chemical Geology* **336**, 13-25. 10.1016/j.chemgeo.2012.08.017

Lalonde JD, Amyot M, Kraepiel AM, Morel FM (2001) Photooxidation of Hg(0) in Artificial and Natural Waters. *Environ Sci Technol* **35** (7), 1367-72. 10.1021/es001408z

Lamborg CH, Fitzgerald WF, Skoog A, Visscher PT (2004) The Abundance and Source of Mercury-Binding Organic Ligands in Long Island Sound. *Marine Chemistry* **90** (1-4 SPEC. ISS.), 151-163. 10.1016/j.marchem.2004.03.014

Lanzillotta E, Ceccarini C, Ferrara R, Dini F, Frontini FP, Banchetti R (2004) Importance of The Biogenic Organic Matter in Photo-Formation of Dissolved Gaseous Mercury in a Culture of the Marine Diatom Chaetoceros sp. *Science of The Total Environment* **318** (1-3), 211-221. 10.1016/S0048-9697(03)00400-5

Lavoie RA, Amyot M, Lapierre J-F (2019) Global Meta-Analysis on the Relationship Between Mercury and Dissolved Organic Carbon in Freshwater Environments. *Journal of Geophysical Research: Biogeosciences* **124** (6), 1508-1523. 10.1029/2018JG004896

Lazaro WL, Diez S, Bravo AG, da Silva CJ, Ignacio ARA, Guimaraes JRD (2019) Cyanobacteria as Regulators of Methylmercury Production in Periphyton. *Sci Total Environ* **668**, 723-729. 10.1016/j.scitotenv.2019.02.233

Lázaro WL, Guimarães JRD, Ignácio ARA, Da Silva CJ, Díez S (2013) Cyanobacteria Enhance Methylmercury Production: A Hypothesis Tested in the Periphyton of Two Lakes in the Pantanal Floodplain, Brazil. *Science of The Total Environment* **456-457**, 231-238. 10.1016/j.scitotenv.2013.03.022

Le Faucheur S, Campbell PG, Fortin C, Slaveykova VI (2014) Interactions Between Mercury and Phytoplankton: Speciation, Bioavailability, and Internal Handling. *Environ Toxicol Chem* **33** (6), 1211-24. 10.1002/etc.2424

Le Faucheur S, Fortin C, Campbell PGC (2011) Acidification Increases Mercury Uptake by a Freshwater Alga, *Chlamydomonas reinhardtii*. *Environmental Chemistry* **8**, 612-622.

Lee CS, Fisher NS (2016) Methylmercury Uptake by Diverse Marine Phytoplankton. *Limnol Oceanogr* **61** (5), 1626-1639. 10.1002/lno.10318

Lehnherr I, Louis VLS (2009) Importance of Ultraviolet Radiation in the Photodemethylation of Methylmercury in Freshwater Ecosystems. *Environmental Science & Technology* **43** (15), 5692-5698. 10.1021/es9002923

Lehnherr I, St. Louis VL, Hintelmann H, Kirk JL (2011) Methylation of Inorganic Mercury in Polar Marine Waters. *Nature Geoscience* **4** (5), 298-302. 10.1038/ngeo1134

Li Y, Li D, Song B, Li Y (2022) The Potential of Mercury Methylation and Demethylation by 15 Species of Marine Microalgae. *Water Research* **215** 10.1016/j.watres.2022.118266

Liang X, Zhu N, Johs A, Chen H, Pelletier DA, Zhang L, Yin X, Gao Y, Zhao J, Gu B (2022) Mercury Reduction, Uptake, and Species Transformation by Freshwater Alga *Chlorella vulgaris* under Sunlit and Dark Conditions. *Environ Sci Technol* 10.1021/acs.est.1c06558

Liem-Nguyen V, Bouchet S, Björn E (2015) Determination of Sub-Nanomolar Levels of Low Molecular Mass Thiols in Natural Waters by Liquid Chromatography Tandem Mass Spectrometry after Derivatization with p-(Hydroxymercuri) Benzoate and Online Preconcentration. *Analytical Chemistry* **87** (2), 1089-1096. 10.1021/ac503679y

Liem-Nguyen V, Skyllberg U, Björn E (2017) Thermodynamic Modeling of the Solubility and Chemical Speciation of Mercury and Methylmercury Driven by Organic Thiols and Micromolar Sulfide Concentrations in Boreal Wetland Soils. *Environmental Science & Technology* **51** (7), 3678-3686. 10.1021/acs.est.6b04622

Liu F, Tan Q-G, Weiss D, Crémazy A, Fortin C, Campbell PGC (2020) Unravelling Metal Speciation in the Microenvironment Surrounding Phytoplankton Cells to Improve Predictions of Metal Bioavailability. *Environmental Science & Technology* **54** (13), 8177-8185. 10.1021/acs.est.9b07773

Luo H, Cheng Q, Pan X (2020) Photochemical Behaviors of Mercury (Hg) Species in Aquatic Systems: A Systematic Review on Reaction Process, Mechanism, and Influencing Factors. *Science of The Total Environment* **720**, 137540. 10.1016/j.scitotenv.2020.137540

Ly QV, Maqbool T, Hur J (2017) Unique Characteristics of Algal Dissolved Organic Matter and Their Association with Membrane Fouling Behavior: A Review. *Environmental Science and Pollution Research* **24** (12), 11192-11205. 10.1007/s11356-017-8683-4

Macka W, Wihlidal H, Stehlik G, Washuttl J, Bancher E (1978) Metabolic Studies of Hg-203 on *Chlamydomonas reinhardi*. *Experientia* **34** (5), 602-3. 10.1007/BF01936983

Mangal V, Donaldson ME, Lewis A, Saville BJ, Guéguen C (2022) Identifying *Euglena gracilis* Metabolic and Transcriptomic Adaptations in Response to Mercury Stress. *Frontiers in Environmental Science* **10** 10.3389/fenvs.2022.836732

Mangal V, Phung T, Nguyen TQ, Guéguen C (2019a) Molecular Characterization of Mercury Binding Ligands Released by Freshwater Algae Grown at Three Photoperiods. *Frontiers in Environmental Science* **6** 10.3389/fenvs.2018.00155

Mangal V, Stenzler BR, Poulain AJ, Guéguen C (2019b) Aerobic and Anaerobic Bacterial Mercury Uptake is Driven by Algal Organic Matter Composition and Molecular Weight. *Environmental Science & Technology* **53** (1), 157-165. 10.1021/acs.est.8b04909

Mangal V, Stock NL, Gueguen C (2016) Molecular Characterization of Phytoplankton Dissolved Organic Matter (DOM) and Sulfur Components Using High Resolution Orbitrap Mass Spectrometry. *Analytical and Bioanalytical Chemistry* **408** (7), 1891-1900. 10.1007/s00216-015-9295-9

Mason RP, Morel FMM, Hemond HF (1995) The Role of Microorganisms in Elemental Mercury Formation in Natural Waters. *Water Air And Soil Pollution* **80**, 775-787. 10.1007/BF01189729

Mason RP, Reinfelder JR, Morel FMM (1996) Uptake, Toxicity, and Trophic Transfer of Mercury in a Coastal Diatom. *Environmental Science and Technology* **30**, 1835-1845. 10.1021/es950373d

Mehra RK, Miclat J, Kodati R, Abdullah R, Hunter TC, Mulchandani P (1996) Optical Spectroscopic and Revers-Phase HPLC Analysis of Hg^{2+} Binding to Phytochelatins. *Biochem J* **314**, 73-82.

Miles CJ, Moye HA, Phlips EJ, Sargent B (2001) Partitioning of Monomethylmercury Between Freshwater Algae and Water. *Environmental Science & Technology* **35** (21), 4277-4282.

Doi 10.1021/Es010792c

Monperrus M, Tessier E, Amouroux D, Leynaert A, Huonnic P, Donard OFX (2007) Mercury Methylation, Demethylation and Reduction Rates in Coastal and Marine Surface Waters of the Mediterranean Sea. *Marine Chemistry* **107** (1), 49-63. 10.1016/j.marchem.2007.01.018

Morelli E, Ferrara R, Bellini B, Dini F, Di Giuseppe G, Fantozzi L (2009) Changes in the Non-Protein Thiol Pool and Production of Dissolved Gaseous Mercury in The Marine Diatom *Thalassiosira weissflogii* under Mercury Exposure. *Science of The Total Environment* **408** (2), 286-293. Doi 10.1016/J.Scitotenv.2009.09.047

Moreno F, Garcia-Barrera T, Gomez-Jacinto V, Gomez-Ariza JL, Garbayo-Nores I, Vilchez-Lobato C (2014) Antagonistic Interaction of Selenomethionine Enantiomers on Methylmercury Toxicity in The Microalgae *Chlorella sorokiniana*. *Metallomics* **6** (2), 347-55. 10.1039/c3mt00296a

Moye HA, Miles CJ, Phlips EJ, Sargent B, Merritt KK (2002) Kinetics and Uptake Mechanisms for Monomethylmercury Between Freshwater Algae and Water. *Environmental Science and Technology* **36**, 3550-3555. 10.1021/es011421z

Naveed S, Li CH, Lu XD, Chen SS, Yin B, Zhang CH, Ge Y (2019) Microalgal Extracellular Polymeric Substances and Their Interactions with Metal(loid)s: A Review. *Critical Reviews in Environmental Science and Technology* **49** (19), 1769-1802. 10.1080/10643389.2019.1583052

Nuzzi R (1972) Toxicity of Mercury to Phytoplankton. *Nature* **237** (5349), 38-40. 10.1038/237038a0

Oh S, Kim M-K, Lee Y-M, Zoh K-D (2011) Effect of Abiotic and Biotic Factors on the Photo-Induced Production of Dissolved Gaseous Mercury. *Water, Air, & Soil Pollution* **220** (1-4), 353-363. 10.1007/s11270-011-0759-z

Ortega SH, Catalan N, Bjorn E, Grontoft H, Hilmarsson TG, Bertilsson S, Wu PP, Bishop K, Levanoni O, Bravo AG (2018) High Methylmercury Formation in Ponds Fueled by Fresh

Humic and Algal Derived Organic Matter. *Limnology and Oceanography* **63**, S44-S53. 10.1002/lno.10722

Pant A, Srivastava SC, Singh SP (1995) Factors Regulating Methyl Mercury Uptake in a Cyanobacterium. *Ecotoxicology and Environmental Safety* **32** (1), 87-92. 10.1006/eesa.1995.1089

Paranjape AR, Hall BD (2017) Recent advances in the Study Of Mercury Methylation in Aquatic Systems. *Facets* **2** 10.1139/facets-2016-0027

Pedrero Z, Mounicou S, Monperrus M, Amouroux D (2011) Investigation of Hg Species Binding Biomolecules in Dolphin Liver Combining GC and LC-ICP-MS with Isotopic Tracers. *Journal of Analytical Atomic Spectrometry* **26** (1), 187-194. 10.1039/c0ja00154f

Pedrero Zayas Z, Ouerdane L, Mounicou S, Lobinski R, Monperrus M, Amouroux D (2014) Hemoglobin as a Major Binding Protein for Methylmercury in White-Sided Dolphin Liver. *Analytical and Bioanalytical Chemistry* **406** (4), 1121-1129. 10.1007/s00216-013-7274-6

Pickhardt PC, Fisher NS (2007) Accumulation of Inorganic and Methylmercury by Freshwater Phytoplankton in Two Contrasting Water Bodies. *Environmental Science and Technology* **41**, 125-131. Doi 10.1021/Es060966w

Poulain AJ, Amyot M, Findlay D, Telor S, Barkay T, Hintelmann H (2004) Biological and Photochemical Production of Dissolved Gaseous Mercury in a Boreal Lake. *Limnology and Oceanography* **49** (6), 2265-2275. 10.4319/lo.2004.49.6.2265

Poulain AJ, Garcia E, Amyot M, Campbell PGC, Raofie F, Ariya PA (2007) Biological and Chemical Redox Transformations of Mercury in Fresh And Salt Waters of the High Arctic During Spring and Summer. *Environmental Science & Technology* **41** (6), 1883-1888. Doi 10.1021/Es061980b

Poulin BA, Ryan JN, Tate MT, Krabbenhoft DP, Hines ME, Barkay T, Schaefer J, Aiken GR (2019) Geochemical Factors Controlling Dissolved Elemental Mercury and

Methylmercury Formation in Alaskan Wetlands of Varying Trophic Status. *Environmental Science & Technology* **53** (11), 6203-6213. 10.1021/acs.est.8b06041

Powell K, Brown P, Byrne R, Gadja T, Hefter G, Sjöberg S, Wanner H (2005) Chemical Speciation of Environmentally Significant Heavy Metals with Inorganic Ligands. Part 1: The Hg^{2+} - Cl^{-}, OH^{-}, CO_3^{2-}, SO_4^{2-}, and PO_4^{3-} Aqueous Systems *Pure and applied chemistry* **77**, 739-800.

Ravichandran M (2004) Interactions Between Mercury and Dissolved Organic Matter--A Review. *Chemosphere* **55** (3), 319-31. 10.1016/j.chemosphere.2003.11.011

Sellers P, Kelly CA, Rudd JWM, MacHutchon AR (1996) Photodegradation of Methylmercury in Lakes. *Nature* **380** (6576), 694-697. 10.1038/380694a0

Seymour JR, Amin SA, Raina J-B, Stocker R (2017) Zooming in on the Phycosphere: The Ecological Interface for Phytoplankton–Bacteria Relationships. *Nature Microbiology* **2** (7), 17065. 10.1038/nmicrobiol.2017.65

Sharif A, Monperrus M, Tessier E, Bouchet S, Pinaly H, Rodriguez-Gonzalez P, Maron P, Amouroux D (2014) Fate of Mercury Species in the Coastal Plume of the Adour River Estuary (Bay of Biscay, SW France). *Science of The Total Environment* **496**, 701-713. 10.1016/j.scitotenv.2014.06.116

Sheehan MC, Burke TA, Navas-Acien A, Breysse PN, McGready J, Fox MA (2014) Global Methylmercury Exposure from Seafood Consumption and Risk of Developmental Neurotoxicity: A Systematic Review. *Bulletin of the World Health Organization* **92**, 254-269F. 10.2471/BLT.12.116152

Shou W, Kang F, Lu J (2018) Nature and Value of Freely Dissolved EPS Ecosystem Services: Insight into Molecular Coupling Mechanisms for Regulating Metal Toxicity. *Environmental Science & Technology* **52** (2), 457-466. 10.1021/acs.est.7b04834

Siciliano SD, O'Driscoll NJ, Tordon R, Hill J, Beauchamp S, Lean DRS (2005) Abiotic Production of Methylmercury by Solar Radiation. *Environmental Science & Technology*

39 (4), 1071-1077. DOI 10.1021/es048707z

Singh DK, Lingaswamy B, Koduru TN, Nagu PP, Jogadhenu PSS (2019) A Putative merR Family Transcription Factor Slr0701 Regulates Mercury Inducible Expression of MerA in the Cyanobacterium *Synechocystis* sp. PCC6803. *MicrobiologyOpen* **8** (9), e00838. 10.1002/mbo3.838

Skrobonja A, Gojkovic Z, Soerensen AL, Westlund P-O, Funk C, Björn E (2019) Uptake Kinetics of Methylmercury in a Freshwater Alga Exposed to Methylmercury Complexes with Environmentally Relevant Thiols. *Environmental Science & Technology* **53** (23), 13757-13766. 10.1021/acs.est.9b05164

Slaveykova VI, Majumdar S, Regier N, Li W, Keller AA (2021) Metabolomic Responses of Green Alga *Chlamydomonas reinhardtii* Exposed to Sublethal Concentrations of Inorganic and Methylmercury. *Environmental Science & Technology* **55** (6), 3876-3887. 10.1021/acs.est.0c08416

Song WJ, Pan XL, Mu SY, Zhang DY, Yang X, Lee DJ (2014) Biosorption of Hg(II) onto Goethite with Extracellular Polymeric Substances. *Bioresource Technology* **160**, 119-122. 10.1016/j.biortech.2013.12.052

Tai C, Li Y, Yin Y, Scinto LJ, Jiang G, Cai Y (2014) Methylmercury Photodegradation in Surface Water of the Florida Everglades: Importance of Dissolved Organic Matter-Methylmercury Complexation. *Environmental Science & Technology* **48** (13), 7333-7340. 10.1021/es500316d

Tsui MT, Finlay JC, Balogh SJ, Nollet YH (2010) In situ Production of Methylmercury Within a Stream Channel in Northern California. *Environ Sci Technol* **44** (18), 6998-7004. 10.1021/es101374y

Vasconcelos MTSD, Leal MFC, van den Berg CMG (2002) Influence of the Nature of The Exudates Released by Different Marine Algae on The Growth, Trace Metal Uptake, and Exudation of *Emiliania huxleyi* in natural Seawater. *Marine Chemistry* **77** (2-3), 187-210.

10.1016/S0304-4203(01)00087-1

Vost EE, Amyot M, O'Driscoll NJ (2012) Photoreactions of Mercury in Aquatic Systems. In 'Environmental Chemistry and Toxicology of Mercury'. (Eds GL Liu, Y Cai, NJ O'Driscoll) pp. 193-218. (Wiley: Hoboken)

Wang K, Liu GL, Cai Y (2021) Possible Pathways for Mercury Methylation in Oxic Marine Waters. *Critical Reviews in Environmental Science and Technology*, 1-19. 10.1080/10643389.2021.2008753

Wang W, Wong RSK, Wang J, Yen Y (2004) Influences of Different Selenium Species on the Uptake and Assimilation of Hg(II) and Methylmercury by Diatoms and Green Mussels. *Aquatic Toxicology* **68**, 39-50. 10.1016/j.aquatox.2004.02.003

Wang Y, Li Y, Liu G, Wang D, Jiang G, Cai Y (2015) Elemental Mercury in Natural Waters: Occurrence and Determination of Particulate Hg(0). *Environmental Science & Technology* **49** (16), 9742-9749. 10.1021/acs.est.5b01940

Weber JH (1993) Review of Possible Paths for Abiotic Methylation of Mercury(II) in the Aquatic Environment. *Chemosphere* **26** (11), 2063-2077. 10.1016/0045-6535(93)90032-Z

Whalin L, Kim EH, Mason R (2007) Factors Influencing the Oxidation, Reduction, Methylation and Demethylation of Mercury Species in Coastal Waters. *Marine Chemistry* **107** (3), 278-294. 10.1016/j.marchem.2007.04.002

Wilkinson KJ, Joz-Roland A, Buffle J (1997) Different Roles of Pedogenic Fulvic Acids and Aquagenic Biopolymers on Colloid Aggregation and Stability in Freshwaters. *Limnology and Oceanography* **42** (8), 1714-1724. 10.4319/lo.1997.42.8.1714

Wilkinson SC, Goulding KH, Robinson PK (1989) Mercury Accumulation and Volatilization in Immobilized Algal Cell Systems. *Biotechnology Letters* **11** (12), 861-864. 10.1007/bf01026841

Wu P, Kainz MJ, Bravo AG, Åkerblom S, Sonesten L, Bishop K (2019) The Importance of Bioconcentration into The Pelagic Food Web Base for Methylmercury Biomagnification:

A Meta-Analysis. *Science of The Total Environment* **646**, 357-367. 10.1016/j.scitotenv.2018.07.328

Wu Y, Wang W-X (2012) Thiol Compounds Induction Kinetics in Marine Phytoplankton During and After Mercury Exposure. *Journal of Hazardous Materials* **217-218**, 271-278. 10.1016/j.jhazmat.2012.03.024

Wu Y, Wang W-X (2013) Differential Acclimation of a Marine Diatom to Inorganic Mercury and Methylmercury Exposure. *Aquatic Toxicology* **138-139**, 52-59. 10.1016/j.aquatox.2013.04.012

Wu Y, Wang W-X (2014) Intracellular Speciation and Transformation of Inorganic Mercury in Marine Phytoplankton. *Aquatic Toxicology* **148**, 122-129. 10.1016/j.aquatox.2014.01.005

Wu Y, Wang WX (2011) Accumulation, Subcellular Distribution and Toxicity of Inorganic Mercury and Methylmercury in Marine Phytoplankton. *Environmental Pollution* **159** (10), 3097-105. 10.1016/j.envpol.2011.04.012

Wu ZY, Li ZK, Shao B, Zhang YY, He W, Lu YR, Gusvitskii K, Zhao YX, Liu YR, Wang XJ, Tong YD (2022) Impact of Dissolved Organic Matter and Environmental Factors on Methylmercury Concentrations Across Aquatic Ecosystems Inferred from a Global Dataset. *Chemosphere* **294** 10.1016/j.chemosphere.2022.133713

Xing Z, Zhao T, Bai W, Yang X, Liu S, Zhang L (2018) Temporal and Spatial Variation in the Mechanisms Used by Microorganisms to Form Methylmercury in the Water Column of Changshou Lake. *Ecotoxicol Environ Saf* **160**, 32-41. 10.1016/j.ecoenv.2018.05.018

Yang L, Zhang Y, Wang F, Luo Z, Guo S, Strähle U (2020) Toxicity of Mercury: Molecular Evidence. *Chemosphere* **245**, 125586. 10.1016/j.chemosphere.2019.125586

Yin X, Wang L, Liang X, Zhang L, Zhao J, Gu B (2022) Contrary Effects of Phytoplankton *Chlorella vulgaris* and its Exudates on Mercury Methylation by Iron- and Sulfate-Reducing Bacteria. *Journal of Hazardous Materials* **433**, 128835. 10.1016/j.jhazmat.2022.128835

Zhang DY, Lee DJ, Pan XL (2013) Desorption of Hg(II) and Sb(V) on Extracellular Polymeric Substances: Effects of pH, EDTA, Ca(II) and Temperature Shocks. *Bioresource Technology* **128**, 711-715. 10.1016/j.biortech.2012.10.089

Zhang T, Hsu-Kim H (2010) Photolytic Degradation of Methylmercury Enhanced by Binding to Natural Organic Ligands. *Nature Geoscience* **3** (7), 473-476. 10.1038/ngeo892

Zhang X, Li Y, Feng G, Tai C, Yin Y, Cai Y, Liu J (2018) Probing the DOM-mediated photodegradation of Methylmercury by Using Organic Ligands with Different Molecular Structures as the DOM Model. *Water Research* **138**, 264-271. 10.1016/j.watres.2018.03.055

Zhang Y, Soerensen AL, Schartup AT, Sunderland EM (2020) A Global Model for Methylmercury Formation and Uptake at the Base of Marine Food Webs. *Global Biogeochemical Cycles* **34** (2), e2019GB006348. 10.1029/2019GB006348

Zhao Q, Wang J, OuYang S, Chen L, Liu M, Li Y, Jiang F (2021) The Exacerbation Of Mercury Methylation by *Geobacter sulfurreducens* PCA in a Freshwater Algae-Bacteria Symbiotic System Throughout the Lifetime of Algae. *J Hazard Mater* **415**, 125691. 10.1016/j.jhazmat.2021.125691