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Reference

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Towards Mechanistic Understanding of Mercury Availability and Toxicity to Aquatic Primary Producers

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Abstract: The present article reviews current knowledge and recent progress on the bioavailability and toxicity of mercury to aquatic primary producers. Mercury is a ubiquitous toxic trace element of global concern. At the base of the food web, primary producers are central for mercury incorporation into the food web. Here, the emphasis is on key, but still poorly understood, processes governing the interactions between mercury species and phytoplankton, and macrophytes, two representatives of primary producers. Mass transfer to biota surface, adsorption to cell wall, internalization and release from cells, as well as underlying toxicity mechanisms of both inorganic mercury and methylmercury are discussed critically. In addition, the intracellular distribution and transformation processes, their importance for mercury toxicity, species-sensitivity differences and trophic transfer are presented. The mini-review is illustrated with examples of our own research.

Keywords: Bioavailability · Macrophytes · Methylmercury · Mercury · Phytoplankton · Speciation

Introduction

Mercury (Hg) is a priority contaminant of global concern.[1] In most aquatic ecosystems, the main sources of Hg are diffuse atmospheric deposition and point sources related to industrial activities.[1] Inorganic mercury (Hg⁰) and methylmercury (CH₃Hg) are toxic to aquatic organisms, but CH₃Hg is strongly biomagnified in the food web (Fig. 1), thus ultimately representing the main threat for humans through fish consumption.[3] As a trace metal, Hg is inherently persistent. Once entered into aquatic ecosystems, Hg undergoes different transformation processes encompassing photoreduction, oxidation, methylation and demethylation, complexation by dissolved ligands and adsorption to colloids and particles.[3–5] Consequently Hg is distributed under a variety of chemical species (Fig. 1) with differing reactivity. In surface water, Hg is present under several inorganic and organic ligand complexes, hydroxo- (Hg(OH)⁺, Hg(OH)₂, Hg(OH)₃⁻, CH₃HgOH) and chloro- (HgCl⁺, HgClOH, HgCl₂, HgCl₃⁻, HgCl₄²⁻, CH₃HgCl) complexes are predominant, but their proportion changes as a function of pH and chloride concentration.[6] Given the very strong tendency of Hg⁺ to form complexes, the estimated free Hg⁺ concentration is extremely low e.g. below 10⁻²⁷ M to 10⁻²⁸ M.[8] In addition, in surface waters, the chemical speciation seems to be controlled by the complexes formed with fulvic and humic-
like dissolved organic matter (DOM) (Fig. 2). Therefore examination of the chemical speciation, (rarely addressed) in addition to the measurement of total Hg concentrations, would improve the understanding of the different processes at the medium-biota interfaces.

The current activities in our laboratory focus on the study of the interactions of different Hg species with two major groups of primary producers: phytoplankton and macrophytes. Phytoplankton accounts for half of the primary productivity on the Earth and, as a result, sustains the largest ecosystem on our planet. Macrophytes contribute to the primary productivity in shallow waters including rivers, marshes, ponds and lakes. What is more, the primary producers are at the basis of trophic webs, providing a support to high trophic level consumers and as such represent the main pathway of Hg incorporation into the food webs. Indeed phytoplankton and macrophytes were shown to be the major entry points of Hg into a fish food web in water bodies impacted by a chlor-alkali plant discharge, with a bioconcentration factor of CH$_3$Hg reaching 10$^8$ and greater. It is therefore of the upmost importance to understand underlying mechanisms of the interactions of Hg species with primary producers, its internal handling and effects, as well as its transfer from primary producers to higher trophic levels. The fate and effects of Hg to these two primary producers, as well as their role in its transformation processes in the environment were recently reviewed.

Understanding the basic mechanisms of Hg bioavailability and toxicity to primary producers is part of an ongoing initiative to understand some of the key processes controlling the fate and impact of vital and toxic trace elements and engineered nanoparticles in aquatic ecosystems. This mini-review deals with the chemo- and biodynamic aspects of Hg$^{II}$ and CH$_3$Hg interactions with phytoplankton and macrophytes and is illustrated with examples from our own research as well as the literature.

**Interactions of Mercury with Primary Producers**

Key chemo- and biodynamic processes governing the interactions of Hg$^{II}$ and CH$_3$Hg with primary producers comprise: (i) transport of different forms of Hg from the medium to the biointerface (Fig. 3) (e.g. by diffusion); (ii) interactions with various organic and inorganic compounds forming complexes; (iii) adhesion of different sites of the biota surface (e.g. cell wall); (iv) transport across the membrane (e.g. internalization); distribution and transformation of Hg species inside the cell; following the interactions with intracellular components, Hg species can affect the cellular processes at different levels (e.g. genomic, proteomic and physiological levels); release from the cells or further translocation via intracellular (symplast) or paracellular transport (apoplast) in pluricellular organisms.

In the case of macrophytes, their exposure to Hg in the aquatic environment can occur either by their roots or directly by their shoots, most frequently by both. However, submerged species usually show higher Hg accumulation than emerging plants found at the same sites. Accumulated Hg can be translocated from the roots or from the cells or further translocation via intracellular (symplast) or paracellular transport (apoplast) in pluricellular organisms.

**Diffusion towards Biointerfaces**

To enter in contact with primary producers mercury species should first diffuse from the bulk medium to the biointerface. The diffusion flux is given by Eqn. (1):

$$J_{\text{diff}} = D c_b \left( \frac{1}{r^2} + \frac{1}{\delta} \right)$$

where $r$ is the radius of the cell, $\delta$ is the thickness of the unstirred boundary layer, $D$ is the diffusion coefficient of Hg species, $c_b$ is the Hg concentration in the medium. Since the Hg concentration in surface waters is vanishingly low, very small diffusional flux could be expected. Consequently diffusion limitation of Hg uptake by primary producers could take place. In such a case, Hg complexes are anticipated to contribute to Hg fluxes towards...
cell surfaces, depending on their mobility and lability. However, there is a lack of experimental evidence supporting such chemodynamic considerations for Hg. The experiments performed with artificial membranes demonstrated that lipophilic HgCl₂ uptake is controlled by the mass transport of Hg from the bulk medium to the membrane since the permeability coefficient in medium was about one order of magnitude lower than that through the membrane. However, in most laboratory experiments, even those performed at environmentally relevant concentrations, the transport of metal across the biological membrane is estimated to be the rate-limiting step (Fig. 4), therefore the internalization flux can be directly related to the concentration of any metal species in equilibrium e.g. HgCl₂ or CH₃HgCl.

**Mercury Adsorption and Internalization**

The cell wall is that most of the phytoplankton species and macrophytes possess in addition to the cytoplasmic membrane represents a supplementary protective barrier. Cell wall composition can vary and may be formed of cellulose in green algae and macrophytes, peptidoglycan in cyanobacteria, and silica frustule in diatoms. Moreover cell walls contain polysaccharides and structural proteins, rich in hydroxyl-, carboxyl-, phosphate- and thiol-groups binding Hg. Indeed, about 41% of Hg⁴⁺ and 27% of CH₃Hg were reversibly adsorbed to Chlorella pyrenoidosa walls[25] and about 88% to the cell walls of the green alga Chlamydomonas reinhardtii[26], indicating the important adsorbing role of the cell walls, in particular in Hg⁴⁺ binding. However the experimental distinction between Hg adsorbed to the cell wall and Hg transported inside the cells is operational and is based on the extraction by using different reagents: for example mixture of EDTA/cysteine for E. nuttallii[25] and cysteine for C. reinhardtii.[26]

The precise mechanisms of Hg internalization by primary producers are not yet elucidated in detail, but several mechanisms were proposed: (i) simple passive diffusion of neutral lipophilic complexes, (ii) facilitated transport (e.g. via channel mediated diffusion), (iii) active transport (e.g. through the essential trace metal transporters) and (iv) indirect transport of Hg⁴⁺ and CH₃Hg bound to amino acids or thiol[8,12,19,27].

The passive diffusion of HgCl₂ and CH₃HgCl through algal membranes was deduced to be the central mechanism of Hg uptake by the diatom Thalassiosira weissflogii,[28] since Hg⁴⁺ and CH₃Hg internalization fluxes were linearly correlated with the overall octanol–water partition coefficients, K of Hg in the exposure solutions.

Hg⁴⁺ and CH₃Hg form neutral lipophilic complexes: HgCl₂ with K of 3.3 and CH₃HgCl with K of 1.7[8,28]. However no uptake or toxicity was detected upon exposure to (CH₃)₂Hg characterized with much higher K of 182 Hg⁴⁺ with K of 4.15, demonstrating that lipophilicity of the Hg species is not the only factor governing Hg internalization.

Various evidence exists demonstrating that the CH₃Hg internalization could take place by active transport. For example, CH₃Hg uptake rate in Selenastrum capricornutum was inhibited by chemical uncouplers such as carbonyl cyanide m-chlorophenylhydrazone (CCCP) or 2,4-dinitrophenol, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (dianion) and parquat.[27] Metabolically dependent transport of Hg was further supported by the decrease of CH₃Hg uptake by algae exposed to CH₃Hg in the dark or exposed to γ irradiation.[29] Heat-killed diatoms were shown to contain less CH₃Hg and Hg⁴⁺ in their cytoplasm compared to living cells, further suggesting a metabolically controlled uptake of both Hg species by the diatoms.[30] By contrast to CH₃Hg, no unequivocal evidence exists for facilitated transport of inorganic Hg into other phytoplankton species. Hg uptake through essential metal transporters was demonstrated in methylating organisms, with the transport of Hg-cysteine complexes or neutral HgCl₂ being in competition with zinc for uptake.[31] In macrophytes, unintentional transport of Hg⁴⁺ by Cu transport system was proposed as a major route for Hg⁴⁺ internalization in E. nuttallii.[32] The hypothesis of Hg uptake via high affinity Cu transporters was further supported by transcriptomic analysis revealing decrease of EnCOPT1 gene expression at increasing Hg⁴⁺ concentrations.[32] Thus the possible involvement of Hg⁴⁺ binding to membrane transporters, is not as straightforward as for other metals.[15,33]

Similarly to other trace metals, the above-mentioned processes at the biota-medium interface can be influenced by:[33] (i) the characteristics of the cell wall and biological membrane; (ii) the reactivity of the species towards the biological membranes; (iii) the water quality parameters, such as pH and water hardness; (iv) the presence and concentrations of micronutrients and toxic trace metals; (v) the presence of different ligands of natural (e.g. DOM) or anthropogenic origins affecting Hg speciation. However the influence of these modifying factors need still to be ex-
The uptake of methionine-Hg by the diatom *C. reinhardtii* was studied in our laboratory. – 40% of the total Hg was bound to the cell walls, whereas 60% was in the cell sap, suggesting that both Hg species do not share the same transport system. High concentrations of seleno-l-methionine decreased CH$_3$Hg, but enhanced Hg$_2^+$ uptake in the diatom *C. pseudomana* while no effect of selenium or selenate was detected. To explain Hg uptake was observed, suggesting that the uptake of methionine-Hg complex is faster than that of Hg$_2^+$ alone. Further investigations are thus clearly needed to understand the mechanisms behind selenomethionine effects.

**Mercury Intracellular Transformations**

To minimize non-specific binding of Hg$^0$ to physiologically important biomolecules and thus prevent toxic effects, phytoplankton and macrophytes were reported to increase glutathione (GSH) cellular content, synthesize phytochelatins (PCs) and/or form metacinnabar β-HgS. Furthermore CH$_3$Hg accumulated in the cytoplasm of diatoms was about four times higher than in the presence of the transphilic one. By contrast, 8 mg/L DOM promoted Hg uptake in aquatic invertebrates and bacteria. Low molecular weight DOM fractions enhanced Hg accumulation in plankton, while high molecular weight reduced it.

Very few studies explored systematically the effect of different water quality parameters, including pH and micro- and macronutrients, on the uptake of Hg$^0$ and CH$_3$Hg, thus their role as modifying factors is still to be elucidated. The decrease of pH from 6.5 to 5.5 was shown to increase the HgCl$_2$ uptake by *C. reinhardtii* by 40%. Little or no effect of major water quality cations was observed on the intracellular content in shoots of *E. nuttallii* exposed to 200 ng/L of HgCl$_2$ and 2500-fold excess of Fe$^{3+}$, Mg$^{2+}$, Na$^+$, K$^+$ or Ni$^{2+}$ (Fig. 5). However, significant inhibition of Hg$^0$ accumulation was found in the presence of Cu$^{2+}$. Under comparable conditions no effect of Cu and other tested ions on the CH$_3$Hg uptake was observed, suggesting that both Hg species do not share the same transport system. High concentrations of seleno-l-methionine decreased CH$_3$Hg, but enhanced Hg$_2^+$ uptake in the diatom *C. pseudomana* while no effect of selenium or selenate was detected. To explain Hg$_2^+$ uptake increase, the authors hypothesized that the uptake of methionine-Hg$^0$ complex is faster than that of Hg$_2^+$ alone. Further investigations are thus clearly needed to understand the mechanisms behind selenomethionine effects.

**Mercury Cellular Distribution**

Understanding intracellular distribution of Hg$^0$ and CH$_3$Hg is pivotal for the assessment of their toxicity, species-sensitivity differences, trophic transfer and assimilation. Indeed the ‘reactivity’ of Hg distributed in various cellular fractions is different. Hg$^0$ bound to ‘organelles and heat-denaturated proteins’ is expected to induce stress effects in phytoplankton, whereas the fractions ‘granules and heat-stable proteins’ are expected to sequester and detoxify Hg. The differences in species sensitivity of three phytoplankton species were shown to correlate with the proportion of Hg$^0$ in the fraction containing mitochondria and chloroplasts. By contrast intracellular CH$_3$Hg was mainly bound to heat-stable proteins. Higher cellular Hg content was measured in the less sensitive organism *T. weissflogii*, while the lowest accumulation corresponded to the most sensitive species *C. autotrophica*, demonstrating that intracellular fate of Hg is a key factor for understanding interspecies differences. Hg distribution between cell wall, cell sap or membranes of the macrophyte *E. nuttallii* was studied in our laboratory. – 40% of the total Hg was bound to the cell walls, whereas 60% was in the cell sap, supposedly in the vacuole, of shoots exposed to 200 ng/L Hg$^0$ or 30 ng/L CH$_3$Hg. However CH$_3$Hg accumulated in the cytoplasm of diatoms was about 4 times more efficiently assimilated by zooplankton in comparison to Hg$^0$, which was bound to cell membranes. Similarly Hg accumulation and trophic transfer were comparable for four phytoplankton species with different cell walls and correlated with the cystolic CH$_3$Hg and Hg$^0$ fractions.

**Mercury Release from Cells**

Excretion of accumulated Hg does not seem to play significant role in the decrease of mercury accumulation in primary producers. Due to its strong intracellular binding, once assimilated Hg$^0$ remained within *C. reinhardtii*. To decrease cellular accumulation of Hg$^0$, some primary producers reduced intracellular Hg to volatile elementary Hg$^0$. High volatiliza-
tion rates were measured in *Euglena gracilis* exposed to 5 µM HgCl₂.[44] Production of the gaseous Hg₂⁺ was also demonstrated to be species dependent with rates decreasing in the order *C. autotrophica* > *I. galbana* ~ *T. weissflogii*.[45]

**Mercury Toxicity towards Primary Producers**

The mode of toxic action of Hg involves binding to –SH functional groups of essential biomolecules (e.g. enzymes), displacement of essential ions from such groups, or modification of their conformation, as well as binding to active groups of ADP or ATP.[50] At molecular level, the alteration of the electron transport activity in photosystems II, the increase of reactive oxygen species (ROS) concentrations and oxidative stress, the modification of nutrient metabolism was demonstrated in a variety of primary producers, from cyanobacteria to higher plants.[3,4,50] Exposure to high (µM) Hg²⁺ concentrations reduced electron transport in photosystems II and I of cyanobacteria *Nostoc muscorum*,[53] and *Synechococcus*,[54] as well as decreased the quantum yield of photosynthesis and altered photosystem II photochemistry in *S. platensis*. [55] Hg²⁺ increased the lifetime of chlorophyll fluorescence by blocking the photosynthetic electron chain in *T. weissflogii*, whereas comparable concentrations of CH₃HgCl did not induce any effect.[56] Hg²⁺ was also shown to substitute the Mg²⁺ in chlorophyll molecules[57] and to inhibit the dark reduction of plastoquinone.[58] Nanomolar concentrations of Hg²⁺ were found to affect the photosystem of six microalgal species,[59] suggesting that alteration of photosynthesis machinery might be a plausible mechanism of Hg toxicity. However its significance for CH₃HgCl is still to be proved.

Inorganic mercury was found to affect the nutrient metabolism in primary producers. Hg²⁺ at µM concentrations reduced phosphate and nitrate uptake by *Vallisneria spiralis* and *Azolla pinnata*,[60] probably by binging –SH groups of cysteine-rich nitrate reductase and inhibiting its activity.[61] Hg²⁺ altered the homeostasis of polyamines and the activity of ornithine decarboxylase and arginine decarboxylasein water hyacinth *Eichhornia crassipes*.[62] The disturbance of this homeostasis could negatively affect cell growth or even could lead to cell death.[63] No similar studies were published for CH₃HgCl.

Mercury was reported to increase ROS content and to induced oxidative stress in the green alga *C. reinhardtii* exposed to µM of HgCl₂.[64] Both Hg²⁺ and CH₃HgCl induced lipid peroxidation in *C. reinhardtii*.[65] and affected membrane integrity.[66] However, different membrane damage mechanisms were proposed for Hg²⁺ and CH₃HgCl: Hg²⁺ was postulated to act directly on the plasma membrane, whereas CH₃HgCl to disturb organelle metabolism in the cytoplasm.[66] The generation of oxidative stress, reflected in increased lipid peroxidation in response to Hg exposure was also reported for several macrophyte species.[67-69] The stress was related to the alteration of the activity of class III peroxidases, superoxide dismutase, catalase, or lipoygenase, involved in the regulation of ROS cellular level.[68,69]

It is recognized that the mechanisms underlying Hg effects on phytoplankton and macrophytes are dependent on the Hg exposure concentrations. Nonetheless, almost all the reported work, described above, has been done at environmentally unrealistic concentrations 10⁻³ to 10⁸ times higher than Hg concentrations in water, suggesting that primary producers will thus very likely not be impacted by ambient mercury concentrations at the population level in the environment. Nevertheless too few data are available for ambient water conditions to be conclusive.

Recent development of omics-approaches in our laboratory have shed new light on the Hg⁴⁺ effect on macrophytes.[32]

Whole transcriptome response of *E. nuttallii* exposed to increasing HgCl₂ concentrations from ca.1 nM to 5 µM revealed up-regulation of proteins (e.g. chaperones) known for their stress response function. A modification of reserve metabolism, notably sugar-catabolizing proteins, putatively caused by the inhibition of production of energy reserves by photosynthesis (Table 1). Down-regulation of metal transporters and genes related to homeostasis also appeared to most probably control and reduce accumulation of Hg²⁺.[122] These results support the involvement of oxidative stress and effects on protein structure as toxicity mechanism of Hg²⁺, and further highlighted that even exposure to 1 nM resulted in significant changes in the metabolic production of energy and adaptation of the nutrition pathways as well as the induction of a protective response. On the other hand it also suggested that at environmental concentrations of Hg the stress level experienced by macrophytes is probably very low. These transcriptomic results are consistent with proteomic analysis demonstrating the small stress level affecting photosynthesis and therefore energy pathways as well as an adaptation of cell structure, especially through lignification in *E. nuttallii* exposed to Hg²⁺.[124] The capabilities of the next generation sequencing to determine the effects of Hg⁴⁺ and CH₃HgCl on the gene expression pattern and signature are currently explored for the green microalga *C. reinhardtii*.

**Conclusion and Outlook**

Important advances in the understanding of Hg⁴⁺ and CH₃HgCl bioavailability and toxicity to aquatic primary producers, such as phytoplankton and macrophytes were achieved. The interactions of Hg with primary producers are governed by linked chemodynamic and biodynamic processes. However the understanding of these linkages is still partial and obtained with experiments with single organism exposed to contaminant present at concentrations several orders of magnitude higher than those encountered in ambient waters. Indeed, understanding these interactions in the presence of multiple stressors and contaminant mixtures, assessment of Hg effects with phytoplankton communities rather than individual species represent examples of future research priorities. The development of the new stable isotope-based methods[22] and effect-oriented tools, such as biosensors[70,71] and –omics tools[122] would provide further impetus to the understanding of key interactions between Hg and primary producers under environmental conditions.

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Table 1. MIPS functional categories whose genes were significantly enriched ($p < 0.05$; FDR $< 0.05$) in a dose-dependent manner (fold change $> 2$) in response of *E. nuttallii* to Hg treatment (adapted from ref. [32])

| Functional Category | Up-regulated                                                                 | Down-regulated                                                                 |
|---------------------|------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| 01 Metabolism       | 01.02.03 sulfur metabolism                                                    | 01.01.03 assimilation of ammonia, metabolism of the glutamate group           |
|                     | 01.05.02 sugar, glucoside, polyol and carboxylate metabolism                 | 01.02 nitrogen, sulfur and selenium metabolism                                |
|                     | 01.05.02.07 sugar, glucoside, polyol and carboxylate catabolism              | 01.05.05 C-1 compound metabolism                                              |
|                     | 01.20 secondary metabolism                                                  | 01.05.05.04 C-1 compound anabolism                                            |
|                     | 01.20.01 metabolism of primary metabolic sugar derivatives                   | 01.05.05.07 C-1 compound catabolism                                           |
|                     | 01.20.01.07 metabolism of glycosides                                          | 01.05.07 C-3 compound metabolism                                              |
| 02 Energy           | 02.10 tricarboxylic-acid pathway (citrate cycle, Krebs cycle, TCA cycle)     |                                                                                |
| 10 Cell cycle and DNA processing | 10.03 cell cycle                                                               | 20.01 transported compounds (substrates)                                     |
|                     | 10.03.02 meiosis                                                              | 20.01.01 ion transport                                                        |
|                     | 10.03.05 cell cycle dependent cytoskeleton reorganization                     | 20.01.01.01 cation transport (H$^+$, Na$^+$, K$^+$, Ca$^{2+}$, NH$_4^+$, etc.) |
|                     | 10.03.05.01 spindle pole body/centrosome and microtubule cycle               | 20.01.01.01 heavy metal ion transport (Cu$^+$, Fe$^{3+}$, etc.)               |
|                     | 10.03.05.03 cell cycle dependent actin filament reorganization                | 20.01.01.07 anion transport                                                   |
| 14 Protein fate (folding, modification, destination) | 14.01 protein folding and stabilization                                       | 20.01.03 C-compound and carbohydrate transport                               |
|                     | 14.10 assembly of protein complexes                                           | 20.01.15 electron transport                                                   |
| 16 Protein with binding function or cofactor requirement | 16.02 peptide binding                                                         | 20.03 transport facilities                                                    |
|                     | 16.06 motor protein binding                                                   | 20.09.18.07 non-vesicular cellular import                                     |
| 18 Regulation of Metabolism and protein function | 18.02.01.02 enzyme inhibitor                                                  |                                                                                |
| 20 Cellular transport, transport facilities and transport routes |                                                                                |                                                                                |
| 30 Cellular communication/signal transduction mechanism | 30.01 cellular signalling                                                     | 34.01 homeostasis                                                            |
|                     | 30.01.09 second messenger mediated signal transduction                       | 34.01.01 homeostasis of cations                                               |
| 32 Cell rescue, defense and virulence | 32.01 stress response                                                         | 34.01.01.01 homeostasis of metal ions (Na$^+$, K$^+$, Ca$^{2+}$ etc.)       |
|                     | 32.01.05 heat shock response                                                  |                                                                                |
| 34 Interaction with the environment | 34.03 membrane excitability                                                  |                                                                                |
|                     | 34.03.01 synaptic transmission                                               |                                                                                |
|                     | 34.03.03 regulation, generation and propagation of action potential          |                                                                                |
|                     | 34.05.02 motor activity                                                      |                                                                                |
|                     | 34.11 cellular sensing and response to external stimulus                     |                                                                                |
|                     | 34.11.09 temperature perception and response                                 |                                                                                |
| 42 Biogenesis of cellular components | 42.05 centrosome                                                            |                                                                                |
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