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

Perrine Dranguet, Rebecca Flück, Nicole Regier, Claudia Cosio, Séverine Le Faucheur, and Vera I. Slaveykova*

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 (Hg^{II}) and methylmercury (CH_3Hg) are toxic to aquatic organisms, but CH_3Hg is strongly biomagnified in the food web (Fig. 1), thus ultimately representing the main threat for humans through fish consumption.[2] 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 chemical forms, with elementary Hg (Hg^0), Hg^{II} and CH_3Hg as predominant species.[3,6] The concentrations of Hg species decrease in the order of Hg^{II}>Hg^0~CH_3Hg.[7] In addition, Hg^{II} and CH_3Hg are bound to particulate (>0.45 µm), colloidal (0.45 µm – 1 kDa) and truly dissolved fractions (<1 kDa). Among the dissolved inorganic and organic ligand complexes, hydroxo- ($Hg(OH)^+$, $Hg(OH)_2$, $Hg(OH)_3^-$, CH_3HgOH) and chloro- ($HgCl^+$, $HgClOH$, $HgCl_2$, $HgCl_3^-$, $HgCl_4^{2-}$, CH_3HgCl) complexes are predominant, but their proportion changes as a function of pH and chloride concentration.[6] Given the very strong tendency of Hg^{II} to form complexes, the estimated free Hg^{2+} 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-

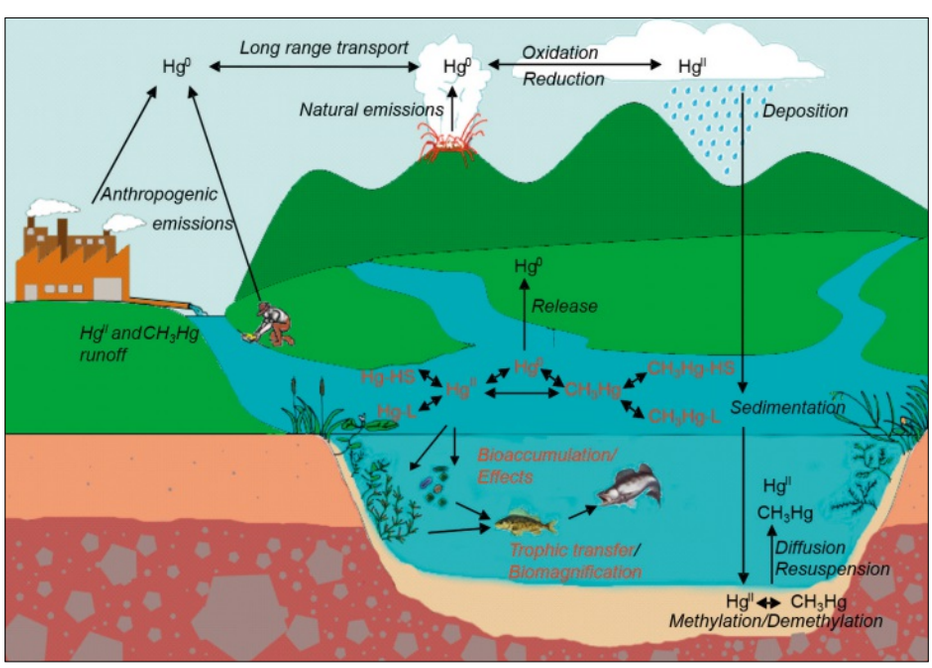

Fig. 1. Mercury biogeochemical cycle in aquatic environment. Major steps involve: (i) release of mercury (Hg^0, Hg^{II}) from natural (rock, soils, volcanoes) or anthropogenic sources (artisanal gold mining, fossil fuel combustion, chlor-alkali plants), (ii) dispersion in gaseous form (Hg^0) through the atmosphere, (iii) dry or wet deposition of Hg^{II} on land and surface waters, (iv) sedimentation, (v) bioconversion into CH_3Hg, (vi) trophic transfer and bioaccumulation (Hg^{II} and CH_3Hg) and biomagnification (CH_3Hg) in the food web or (vii) evasion (Hg^0) to the atmosphere. The processes which are the subject of the present overview, are given in red.

*Correspondence: Prof. V. I. Slaveykova
Environmental Biogeochemistry and Ecotoxicology
Institute F.-A. Forel, Earth and Environmental Sciences
Faculty of Sciences, University of Geneva
10 route de Suisse
CH-1290 Versoix
Tel.: +41 22 379 03 35
E-mail: vera.slaveykova@unige.ch

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.[10] Macrophytes contribute to the primary productivity in shallow waters including rivers, marshes, ponds and lakes.[11] 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.[8,12] Indeed phytoplankton and macrophytes were shown to be the major entry points of CH_3Hg in a fish food web in water bodies impacted by a chlor-alkali plant discharge,[13] with a bioconcentration factor of CH_3Hg reaching 10^4 and greater.[12] 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.[8,12,14]

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[15,16] and engineered nanoparticles[17,18] in aquatic ecosystems. This mini-review deals with the chemo- and biodynamic aspects of Hg^{II} and CH_3Hg 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_3Hg 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) adsorption to 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.

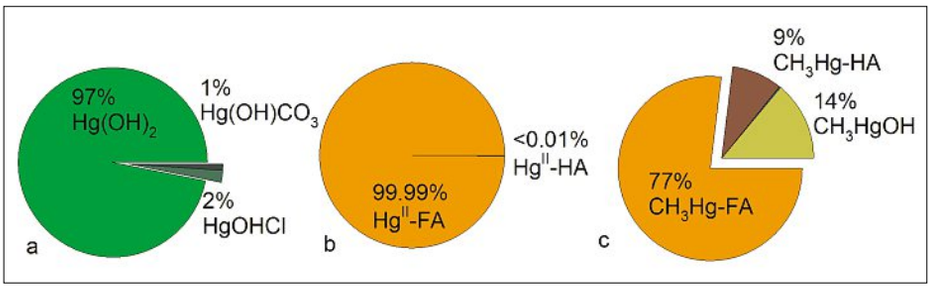

Fig. 2. Distribution of Hg species in a model lake water contaminated with Hg and containing 0.35 mg/L [NO_3^-], 9.14 mg/L [Cl^-], 43.64 mg/L [SO_4^{2-}], 6.2 mg/L [Na^+], 1.59 mg/L [K^+], 40.7 mg/L [Ca^{2+}], 5.9 mg/L [Mg^{2+}] at pH 8.3 and a dissolved organic matter concentration of 0.25 mg/L humic and 2.25 mg/L fulvic acids. The water composition corresponds to that measured in G3 sampling point of Lake Geneva in October 2013. Speciation was computed with the WHAM/Model VII chemical equilibrium program with updated formation constants for OH^-, Cl^-, NH_4^+, PO_4^{3-} and CO_3^{2-}.[9] (a) 3.1×10^{-10} M [Hg^{II}], only inorganic complexes are considered; (b) 3.1×10^{-10} M [Hg^{II}], DOM is taken into account; (c) 3.1×10^{-12} M [CH_3Hg], DOM is taken into account. The complexes formed with fulvic and humic acids are the prevailing forms of Hg^{II} and CH_3Hg, as well as chloride and hydroxide complexes. Estimated free mercury concentration $Hg^{2+} < 10^{-30}$ M and free methylmercury concentration $CH_3Hg^{2+} < 10^{-15}$ M.

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):[8,21]

$$J_{diff} = Dc_b\left(\frac{1}{r}+\frac{1}{\delta}\right) \quad (1)$$

where r is the radius of the cell, δ 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

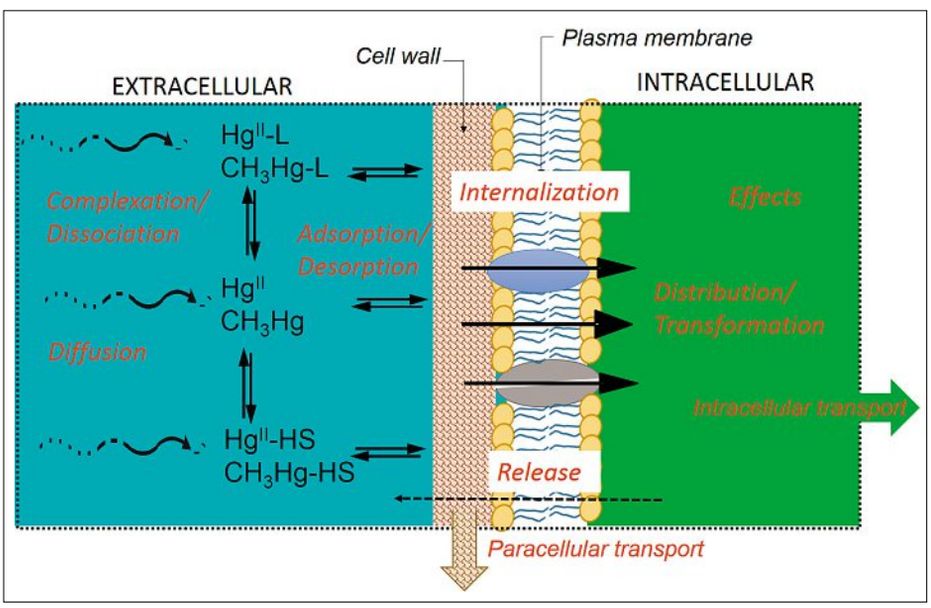

Fig. 3. Key processes determining the interactions of inorganic (Hg^{II}) and methylated Hg (CH_3Hg) forms with primary producers. Charges are omitted for simplicity.

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 $HgCl_2^0$ 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.[8] 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.* $HgCl_2$ or CH_3HgCl.

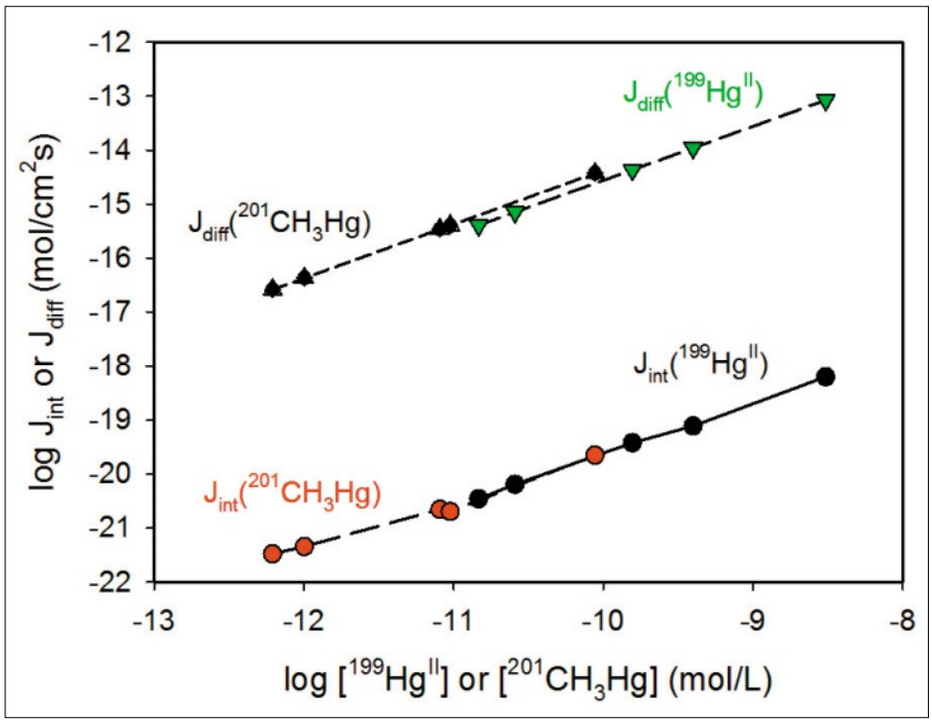

Fig. 4. Experimentally determined internalization and calculated diffusive fluxes to the green alga *Chlamydomonas reinhardtii* exposed to a mixture of $^{199}Hg^{II}$ and $^{201}CH_3Hg$. The internalization fluxes were estimated from the intracellular concentrations at 48 h determined by double stable isotope spiking.[22] Linear increase of the intracellular content over time was assumed. Single alga surface area is 3.2×10^{-6} cm^2. Diffusive flux were calculated by using Eqn. (1). Diffusion coefficients are 8.47×10^{-6} cm^2/s for Hg^{2+}[23] and 1.3×10^{-5} cm^2/s for CH_3HgCl,[24] the radius of the cell, *r* is 5×10^{-4} cm, the thickness of the unstirred boundary layer, δ is 8×10^{-4} cm.[8] Initial Hg concentrations at the biota surface was taken as 0. Chemical speciation in the exposure medium taken into account is: 75.9% $HgCl_2$, 15.7% HgClOH, 6.4% $HgCl_3^-$, 89.5% CH_3HgCl and 10.5% CH_3HgOH (complex fractions equal or below 1% are omitted).[22] If $HgCl_2$ and CH_3HgCl species were bioavailable, then the diffusive flux would not be limiting under these conditions.

Mercury Adsorption and Internalization

The cell wall 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 Hg^{II} and 27% of CH_3Hg were reversibly adsorbed to *Elodea nuttallii* cell walls[25] and about 88% to the cell walls of the green alga *Chlamydomonas reinhardtii*,[26] demonstrating the important adsorbing role of the cell walls, in particular in Hg^{II} 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. vi*a channel mediated diffusion), (iii) active transport (*e.g.* through the essential trace metal transporters) and (iv) indirect transport of Hg^{II} and CH_3Hg bound to amino acids or thiols.[8,12,19,27]

The passive diffusion of $HgCl_2^0$ and CH_3HgCl^0 through algal membranes was deduced to be the central mechanism of Hg uptake by the diatom *Thalassiosira weissflogii*,[28] since Hg^{II} and CH_3Hg internalization fluxes were linearly correlated with the overall octanol–water partition coefficients, K_{ow} of Hg in the exposure solutions. Hg^{II} and CH_3Hg form neutral lipophilic complexes: $HgCl_2^0$ with K_{ow} of 3.3 and CH_3HgCl^0 with K_{ow} of 1.7.[8,28] However no uptake or toxicity was detected upon exposure to $(CH_3)_2Hg$ characterized with much higher K_{ow} of 182 or Hg^0 with K_{ow} of 4.15, demonstrating that lipophilicity of the Hg species is not the only factor governing Hg internalization.

Various evidence exists demonstrating that the CH_3Hg internalization could take place by active transport. For example CH_3Hg 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 (diuron) and paraquat.[27] Metabolically dependent transport of Hg was further supported by the decrease of CH_3Hg uptake by algae exposed to CH_3Hg in the dark or exposed to γ irradiation.[29] Heat-killed diatoms were shown to contain less CH_3Hg and Hg^{II} in their cytoplasm compared to living cells, further suggesting a metabolically controlled uptake of both Hg species by the diatoms.[30] By contrast to CH_3Hg, 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 $HgCl_2$ being in competition with zinc for uptake.[31] In macrophytes, unintentional transport of Hg^{II} by Cu transport system was proposed as a major route for Hg^{II} internalization in *E. nuttallii*.[19] The hypothesis of Hg uptake *via* high affinity Cu transporters was further supported by transcriptomic analysis revealing decrease of *EnCOPT1* gene expression at increasing Hg^{II} concentrations.[32] Thus the possible involvement of Hg^{2+} 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-

plored. Below we will focus on the effect of DOM, pH, trace metals and nutrients, factors that were considered for primary producers.

There is quite inconclusive and contradictory evidence on the role of DOM on Hg uptake. DOM decreases Hg uptake in algae by strongly binding of Hg by reduced sulfur sites on the DOM[27,34–36] and thus reducing potentially bioavailable Hg fraction. In addition, the DOM concentration and quality were shown to affect the degree to which it inhibits algal Hg uptake. For example, CH_3Hg content in the diatom *Cyclotella meneghiniana* decreased in the presence of DOM; however the effect was more pronounced in the presence of the highly aromatic hydrophobic fraction of DOM than in the presence of the transphilic one.[35] By contrast, 8 mg/L DOM promoted Hg uptake in aquatic invertebrates[37] and bacteria.[38] Low molecular weight DOM fractions enhanced Hg accumulation in plankton, while high molecular weight reduced it.[39]

Very few studies explored systematically the effect of different water quality parameters, including pH and micro- and macronutrients, on the uptake of Hg^{II} and CH_3Hg, 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 $HgCl_2^0$ uptake by *C. reinhardtii* by 40%.[26] 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^{2+}, Mg^{2+}, Na^+, K^+ or Ni^{2+} (Fig. 5). However, significant inhibition of Hg^{II} accumulation was found in the presence of Cu^+.[19] Under comparable conditions no effect of Cu and other tested ions on the CH_3Hg uptake was observed, suggesting that both Hg species do not share the same transport system. High concentrations of seleno-L-methionine decreased CH_3Hg, but enhanced Hg^{II} uptake in the diatom *T. pseudonana*,[40] while no effect of selenite or selenate was detected. To explain Hg^{II} uptake increase, the authors hypothesized that the uptake of methionine-Hg^{II} complex is faster than that of Hg^{II} alone. Further investigations are thus clearly needed to understand the mechanisms behind selenomethionine effects.

Mercury Cellular Distribution

Understanding intracellular distribution of Hg^{II} and CH_3Hg 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.[41,42] Hg 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[42] were shown to correlate with the proportion of Hg^{II} in the fraction containing mitochondria and chloroplasts. By contrast intracellular CH_3Hg was mainly bound to heat-stable proteins.[42] Higher cellular Hg content was measured in the less sensitive organism *T. weisflogii*, while the lowest accumulation corresponded to the most sensitive species *C. autotrophica*,[43] 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^{II} or 30 ng/L CH_3Hg.[19] Furthermore CH_3Hg accumulated in the cytoplasm of diatoms was about four times more efficiently assimilated by zooplankton in comparison to Hg^{II}, which was bound to cell membranes.[28] Similarly Hg accumulation and trophic transfer were comparable for four phytoplankton species with different cell walls and correlated with the cytosolic CH_3Hg and Hg^{II} fractions.[30]

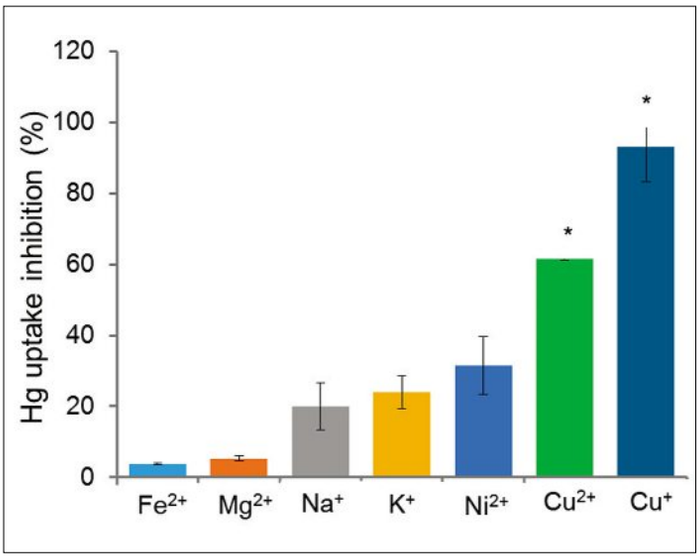

Fig. 5. Effect of different competitors on accumulation of Hg^{II} in shoots of *E. nuttallii* (mean ± s.d., N=3). Macrophytes were exposed 24 h to 200 ng/L of $HgCl_2$ in presence of 500 µg/L Fe^{2+}, Mg^{2+}, Na^+, K^+, Ni^+, Cu^{2+} or Cu^+. Values are expressed as percentage of inhibition of Hg accumulation in *E. nuttallii* exposed to Hg only. Asterisks indicate significant differences to Hg only treated plants. *p<0.001, adapted from ref. [19]

Mercury Intracellular Transformations

To minimize non-specific binding of Hg^{II} 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 *meta*cinnabar β-HgS.[44–46] However at lower Hg concentrations and longer exposure time, PCs do not seem to have a noticeable role in diatoms.[41] Therefore PCs role in Hg detoxification under environmentally relevant conditions remains to be confirmed. In addition, CH_3Hg seems to be a poor inducer of phytochelatins.[47] Primary producers can also sequester Hg as β-HgS.[46,48] The formation of β-HgS was found in a variety of algal species, from chlorophytes to diatoms with highest percentage formed in *T. weissflogii* and much lower in *C. autotrophica* and *I. galbana*.[43] Reduction and demethylation of Hg are other intracellular transformations observed for both phytoplankton and macrophytes. Algal Hg reduction rates were shown to depend on exposure concentrations, but not on the light conditions.[44,45] The diatom *T. weissflogii* exposed to 5 nM Hg^{II} produced comparable amount of Hg^0 in the light and in the dark conditions.[45] The mechanisms by which primary producers reduce Hg are still to be elucidated.

Double stable isotope spiking demonstrated enrichment of intracellular $^{201}Hg^{II}$ originating from $^{201}CH_3Hg$ spike indicating the intracellular demethylation in *C. reinhardtii* when exposed to a mixture of pM $^{199}Hg^{II}$ and $^{201}CH_3Hg$.[22] The macrophyte *E. nuttallii* was also shown to demethylate CH_3Hg in Hg^{II}, which is further reduced to volatile Hg^0.[12] Methylation was also observed in the macrophyte *I. aquatic* after 4-day exposure to $HgCl_2$, where CH_3Hg concentration increased significantly in the shoots after additional 96-hour exposure in non-spiked medium.[49] Phytoplankton Hg methylation was also investigated, but no evidence has yet been found.[22,46]

Mercury Release from Cells

Excretion of accumulated Hg does not seem to play significant role in the decrease of mercury accumulation in primary producers.[8,50] Due to its strong intracellular binding, once assimilated Hg^{II} remained within *C. reinhardtii*.[26] To decrease cellular accumulation of Hg^{II}, some primary producers reduced intracellular Hg to volatile elementary Hg^0.[48,51,52] High volatiliza-

tion rates were measured in *Euglena gracilis* exposed to 5 μM Hg^{II}.[44] Production of the gaseous Hg^0 was also demonstrated to be species dependent with rates decreasing in the order *C. autotrophica> I. galbana ~ T. weissflogii*.[43]

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.[8,14,50] Exposure to high (μM) Hg^{II} 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] Hg^{II} increased the lifetime of chlorophyll fluorescence by blocking the photosynthetic electron chain in *T. weissflogii*, whereas comparable concentrations of CH_3Hg did not induce any effect.[56] Hg^{II} was also shown to substitute the Mg in chlorophyll molecules[57] and to inhibit the dark reduction of plastoquinone.[58] Nanomolar concentrations of Hg^{II} 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 CH_3Hg is still to be proved.

Inorganic mercury was found to affect the *nutrient metabolism* in primary producers. Hg^{II} 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] Hg^{II} altered the homeostasis of polyamines and the activity of ornithine decarboxylase and arginine decarboxylasein water hyacinth *Eichornia crassipe*.[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 CH_3Hg.

Mercury was reported to *increase ROS content* and to *induced oxidative stress* in the green alga *C. reinhardtii* exposed to μM of Hg^{II}.[64] Both Hg^{II} and CH_3Hg induced lipid peroxidation in *C. reinhardtii*[65] and affected membrane integrity.[66] However, different membrane damage mechanisms were proposed for Hg^{II} and CH_3Hg: Hg^{II} was postulated to act directly on the plasma membrane, whereas CH_3Hg 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 lipoxygenase, 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 10^6 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 Hg^{II} effect on macrophytes.[32] Whole transcriptome response of *E. nuttallii* exposed to increasing $HgCl_2$ 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 Hg^{II}.[32] These results support the involvement of oxidative stress and effects on protein structure as toxicity mechanism of Hg^{II}, 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 Hg^{II}.[24] The capabilities of the next generation sequencing to determine the effects of Hg^{II} and CH_3Hg 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 Hg^{II} and CH_3Hg 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[32] would provide further impetus of 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 01.05.02 sugar, glucoside, polyol and carboxylate metabolism 01.05.02.07 sugar, glucoside, polyol and carboxylate catabolism 01.20 secondary metabolism 01.20.01 metabolism of primary metabolic sugar derivatives 01.20.01.07 metabolism of glycosides	01.01.03 assimilation of ammonia, metabolism of the glutamate group 01.02 nitrogen, sulfur and selenium metabolism 01.05.05 C-1 compound metabolism 01.05.05.04 C-1 compound anabolism 01.05.05.07 C-1 compound catabolism 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 10.03.02 meiosis 10.03.05 cell cycle dependent cytoskeleton reorganization 10.03.05.01 spindle pole body/centrosome and microtubule cycle 10.03.05.03 cell cycle dependent actin filament reorganization	
14 Protein fate (folding, modification, destination)	14.01 protein folding and stabilization 14.10 assembly of protein complexes	
16 Protein with binding function or cofactor requirement	16.02 peptide binding 16.06 motor protein binding 16.17.01 calcium binding 16.21.08 Fe/S binding	
18 Regulation of Metabolism and protein function	18.02.01.02 enzyme inhibitor	
20 Cellular transport, transport facilities and transport routes		20.01 transported compounds (substrates) 20.01.01 ion transport 20.01.01.01 cation transport (H^+, Na^+, K^+, Ca^{2+} , NH_4^+, *etc.*) 20.01.01.01.01 heavy metal ion transport (Cu^+, Fe^{3+}, *etc.*) 20.01.01.07 anion transport 20.01.03 C-compound and carbohydrate transport 20.01.15 electron transport 20.03 transport facilities 20.09.18.07 non-vesicular cellular import
30 Cellular communication/signal transduction mechanism	30.01 cellular signalling 30.01.09 second messenger mediated signal transduction	
32 Cell rescue. defense and virulence	32.01 stress response 32.01.05 heat shock response	32.07 detoxification 32.07.05 detoxification by export
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	34.01 homeostasis 34.01.01 homeostasis of cations 34.01.01.01 homeostasis of metal ions (Na^+, K^+, Ca^{2+} *etc.*)
42 Biogenesis of cellular components	42.05 centrosome	

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