SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 33, No. 6, pp. 1225–1237, 2014

Printed in the USA

Global Mercury Partnership

EFFECTS OF MACROPHYTES ON THE FATE OF MERCURY IN AQUATIC SYSTEMS

CLAUDIA COSIO,* REBECCA FLÜCK, NICOLE REGIER, and VERA I. SLAVEYKOVA
Institute F.-A. Forel, Earth and Environmental Sciences, Faculty of Sciences, University of Geneva, Versoix, Switzerland

(Submitted 8 August 2013; Returned for Revision 16 October 2013; Accepted 27 November 2013)

Abstract: Vegetated and shallow areas such as wetlands and salt marshes, as well as freshwater lakes and rivers, have been identified as hotspots for Hg methylation. The presence of aquatic macrophytes, the predominant primary producers in shallow waters, plays an important but still poorly understood role in the fate of Hg in these environments. The present review focuses on the influences of macrophytes on Hg speciation and distribution in sediments, the rhizosphere, and the water column; on Hg transformation; and on Hg release to the environment, including transfer to the trophic web. Future research will require an improved understanding of the mechanisms and the factors controlling these aspects as well as a broader general view. Thus, the main gaps in knowledge are also discussed. *Environ Toxicol Chem* 2014;33:1225–1237. © 2013 SETAC

Keywords: Methylmercury Bioaccumulation Biomagnification Physical effect Rhizosphere

INTRODUCTION

Macrophytes comprise a vast diversity of aquatic photosynthetic organisms that are visible to the naked eye. They encompass large algae (chlorophyta, xanthophyta, and rhodophyta), bryophytes (mosses, hornworts, and liverworts), and vascular plants including both aquatic spermatophytes (flowering plants) and pteridophytes (ferns) [1]. In a wide range of littoral ecosystems (i.e., rivers, marshes, ponds, and lakes), macrophytes represent the predominant group of organisms within the euphotic zone, in terms of biomass and primary production [2].

As primary producers, macrophytes play a role as food source to the detrital and herbivore food webs [3–5]. Additionally, the aquatic vegetation offers numerous microhabitats and refuges for periphyton, invertebrate, fish, water birds, and other fauna [4–6]. Macrophytes also provide oxygen to the sediments and the water column and are involved in biogeochemical cycling of trace elements and nutrients [7,8]. Moreover, macrophytes in shallow water environments have been shown to directly and indirectly influence the availability and the fate of mercury (Hg) species as well as its dispersion (Figure 1) [9–12].

Mercury is a global pollutant of primary concern because of its volatility and long-range transport [13]. Both inorganic Hg (IHg) and organic (e.g., methylmercury [MeHg] and dimethylmercury) forms are toxic and disturb ecosystems; however, MeHg is biomagnified in the food web, whereas IHg is not [13,14]. The first entry point of Hg into the food web is generally accumulation in primary producers. Areas with shallow water and vegetation—notably salt marshes, wetlands, and mangroves—have been described as hotspots of Hg methylation, whereas other shallow and vegetated freshwater environments might also be of concern [2,15–20].

The present review summarizes the available literature concerning the role of macrophytes in Hg fate (Figure 1). Specific emphasis is given to the different processes at the macrophyte/sediment and macrophyte/water interfaces, to Hg bioaccumulation and transformation in macrophytes, and to Hg release from macrophytes. Major gaps in existing knowledge are highlighted, and suggestions for future research are provided.

EFFECTS OF ROOTS

Effects on rhizospheric sediments

Sediments are the main sink for Hg in aquatic ecosystems, and MeHg is thought to be mainly produced in the sediments. Macrophytes influence Hg fate in rhizospheric sediments by affecting pH and redox potential (Eh) and by the exudation of organic ligands. These factors indeed play important roles in Hg mobilization and availability to the below-ground organs in the sediments [9,21]. Two recent studies considered the rhizospheric sediments of *Phragmites australis* and *Juncus maritimus*—2 emergent macrophytes from salt marshes—and the effect of their presence on Hg distribution [19,22]. In the rhizosphere of *P. australis*, pH decreased in summer to 6.2 and increased in autumn to 8.0; however, no correlation with increase in biomass or Hg accumulation was found. In *J. maritimus*, the pH tended to decrease in autumn to 5.8, when Hg concentrations in rhizospheric sediments increased and shoot biomass was the smallest [21]. In general, a decreased pH is expected to increase mobility and availability of Hg by reducing complexation with, for example, organic matter or sulfides. Nevertheless this effect is observed at a lower pH than 5 [23]. This might explain why both studies show no significant correlation of pH change with Hg bioaccumulation or translocation in plants [19,22].

In the same studies, Eh increased during autumn in the rhizosphere of *P. australis*, and *J. maritimus* when the concentration of Hg in plants was highest, supporting the importance of this parameter for Hg bioavailability [9,21]. The ability to increase Eh of sediments is correlated to the capacity of macrophytes to transport oxygen, which differs among species [22]. Oxygen released from roots affects not only the Eh but also the biogeochemical dynamics of sulfur in sediments that is known to interact with Hg biogeochemical cycle [24]. For example, in the rhizosphere of *Spartina*, the precipitation of metacinnabar β-HgS decreased Hg availability for both methylation and accumulation [25]. Sulfide tends to precipitate with Hg at values of Eh less than −150 mV, whereas for Eh

* Address correspondence to Claudia.Cosio@unige.ch.
Published online 24 December 2013 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.2499

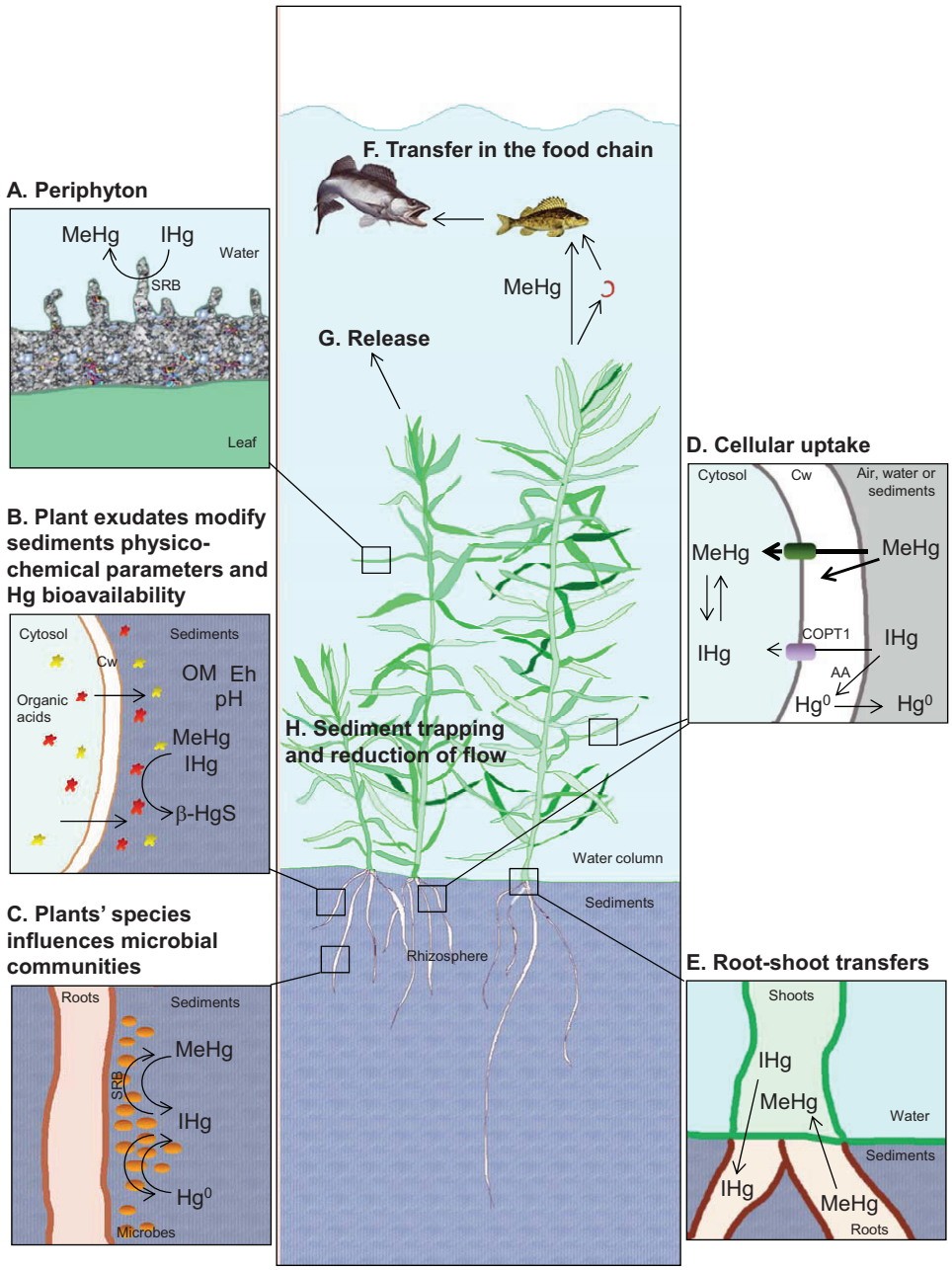

Figure 1. Main indirect and direct effects of macrophytes on Hg fate in the aquatic environment: Hg is methylated by the periphyton associated with macrophytes (**A**) and chelated by plant exudates (organic acids: yellow and red stars; **B**). Macrophytes modify the physicochemical parameters of sediments (**B**) and affect microbial communities composition (microbes: orange dots; **C**). Macrophytes accumulate both inorganic and organic Hg by passive diffusion and through carrier-mediated mechanisms, notably Cu transporters, such as copper transporter 1 COPT1 (colored boxes). Mercury is then reduced, methylated, or demethylated intracellularly (**D**). Mercury accumulated in macrophytes is further transported to other organs, acropetally for methylmercury (MeHg) and basipetally for inorganic mercury (IHg; **E**). Eventually, Hg moves into the food web through herbivores (**F**) or is released to the environment by volatilization or during decay (**G**). Trapping of sediment and reduction of water flow caused by the presence of macrophyte beds also increase Hg retention (**H**). (**A**, **C**, and **D**) affect Hg speciation. (**B**–**D**) affect Hg bioavailability. (**D**–**H**) affect the cycle of Hg. AA = ascorbic acid; Cw = cell wall; Eh = redox potential; OM = organic matter content; SRB = sulfate-reducing bacteria. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

greater than +200 mV, sulfate is the predominant form, with iron and manganese occurring as insoluble hydrous oxides, hence favoring binding of Hg to organic ligands [21]. Values of Eh between −150 and 200 mV are considered favorable for Hg mobility [21]. Therefore, Eh values measured in *J. maritimus* rizosphere (−150 and 260 mV) and for *P. australis* (250–500 mV) suggested that *J. maritimus* would significantly promote Hg mobilization in sediments. Indeed, the concentration of Hg in the sediments was reduced when bioaccumulation was observed in *J. maritimus* and the growth rate was negatively correlated to bioaccumulation [21]. Conversely, bioaccumulation factors (BAFs) from sediments—defined as the ratio between the concentration in macrophyte roots and the initial concentration in sediments—were very similar for both species, highlighting our limited understanding of the processes.

The discrepancies between expected influence of pH and Eh and observed Hg accumulation are attributable to the co-influence of other determining factors affecting the mobility and speciation of Hg, such as the concentration of organic matter, Fe, Cl^-, and SO_4^{2-} [26]. The Eh can be significantly reduced by the

deposition of organic debris, suggesting a role of the plant metabolism and the surrounding environment in controlling this factor [22]. In the rhizosperic sediments of *P. australis*, organic matter content was lower at the contaminated site than at the reference site, suggesting an effect of Hg concentration in sediments on plant metabolism [9]. In both studies, organic matter content in sediments was the highest in autumn, when the highest accumulation was observed in *P. australis* but not in *J. maritimus* [9,21]. The exudation of organic ligands by roots of macrophytes is, conversely, expected to bind dissolved Hg and reduce the amount of bioavailable Hg [15]. Nevertheless, organic carbon released by plants into the rhizosphere also drives dissimilar sulfate reduction, influencing sulfur and Hg speciation [24]. Moreover, the presence of organic matter and sulfides (2–6 μM) can increase Hg bioavailability through formation of nanoparticles of Hg [27,28].

Overall, the scarce literature demonstrates that macrophyte roots significantly modify a number of factors in rhizospheric sediments, including pH, Eh, and organic matter content, which can in turn affect Hg mobility, as well as Hg availability and thus Hg fate. However, further research is necessary to have a more detailed understanding of the mechanisms behind the observations being made.

Effects on microbial communities and Hg methylation in the rhizosphere

Roots of macrophytes influence microbial community structure and activities, which can in turn affect Hg fate, notably the speciation and bioavailability of Hg to plants. Changes in microbial community composition have been reported in rhizospheric versus bulk sediments. Mercury-resistant bacteria were found associated to 24 macrophyte taxa isolated from lakes and biological supply houses across the United States [29]. Mercury concentrations in *Myriophyllum spicatum, Potamogeton crispus*, and *Spartina pectinata* collected in an Hg-contaminated lake were very low (0.06 ± 0.01 mg/kg dry wt), but the same macrophyte species lacking Hg-resistant bacteria collected in another lake showed greater toxicity symptoms and 15-fold higher Hg accumulation [29]. Bacteria were hypothesized to protect the macrophytes by reducing the amount of Hg entering into their cells [29]. In the same line, *Potamogeton pusillus* roots increased rates of Hg demethylation and inhibited Hg methylation in sediments of constructed wetlands [15].

Other studies, however, demonstrated that macrophyte microenvironments favor Hg accumulation. A clear link between bacterial activity in the rhizosphere and the increased accumulation of total Hg (THg; i.e., IHg + MeHg) was shown by comparing THg accumulation in wetland plants (*Scirpus robustus* and *Polypogon monspeliensis*) in antibiotic treated and untreated sediments [30]. Furthermore, high levels of THg and MeHg, as well as high methylation rates were associated with sediments colonized by macrophytes in salt marshes and wetlands, particularly in actively growing plants [17,31]. In salt marshes, MeHg in rhizospheric sediments of *Sarcocornia fruticosa, Halimione portulacoides*, and *Spartina maritima* reached 18% of the THg, which was 70-fold higher than the values found in bulk sediments, whereas a 2-fold difference was observed for MeHg [17]. In the same line, roots of *Elodea nuttallii*—a submerged and rooted plant found in temperate freshwater ecosystems—significantly changed the bacterial community structure in sediments and increased by 2-fold the MeHg proportion of the THg in porewater [11]. Briefly, 2 bacterial operational taxonomic units related to sulfate-reducing bacteria (SRB) became more abundant, whereas THg concentrations in porewater of rhizosphere sediments decreased 2-fold, and MeHg concentrations increased in porewater and in plants [11]. Because Hg methylation is thought to be linked to bioavailable Hg(II), data suggested that the presence of the roots favored methylation of Hg, maybe through SRB activity, and favored bioaccumulation of MeHg, despite the diminished THg concentration in porewater [11]. Similarly, the presence of *Elodea canadensis* increased MeHg production by 2-fold in sediments when compared with bulk sediments in microcosms [32]. Moreover, the MeHg percentage of the THg significantly increased along the part of an Hg-contaminated reservoir colonized by macrophytes [10]. Recent estimations of net MeHg budgets made in temperate lakes in France further suggested that aquatic rhizosphere was the principal location for MeHg production [16]. Bacteria and root-associated materials were also shown to drive high Hg methylation in the roots of floating macrophytes (e.g., *Eichhornia crassipes*) in the Amazon [20,33]. To our knowledge, no detailed study concerning this topic has been conducted in coastal areas, although a similar effect can be expected in the rhizosphere of macrophytes in these environments [34]. However, the overall available data do not clearly determine whether roots directly affect Hg distribution or solely affect the microbial community structure, which in turn alters Hg distribution.

Bacteria, especially SRB, are widely expected to be the main actor in Hg methylation, and the occurrence of these species in the rhizosphere explains the higher methylation rates and MeHg concentrations observed in vegetated areas of shallow water environments [11,16,35]. Increased sulfate-reducing activity was reported in the rhizosphere of marine and freshwater macrophytes with a predominance of SRB in rhizosphere compared with bulk sediments [36]. In wetland sediments, the exudation of labile organic carbon (e.g., acetate) by roots of plants resulted in enhanced microbial sulfate reduction activity in the rhizosphere, and consequently in increased rates of Hg(II)-methylation and MeHg concentrations [37]. Indeed, exudates and oxygen released by roots into the rhizosphere drive SRB activity in conjunction with organic carbon degradation through dissimilar sulfate reduction [24]. Consequently, SRB are often found in association with the roots of macrophytes. Their abundance and species depend on the macrophyte species and their specific exudates and on the sediment geochemistry. For example, differences in the composition and frequency of SRB subgroups in the rhizospheric sediments of C_3 and C_4 macrophytes have been observed [33]. The observed discrepancies about the influence of the rhizosphere on Hg methylation might be linked to conditions for microbial growth offered by macrophyte root systems and consequently to bacteria species favored by the local macrophyte communities. Not surprisingly, great variability of methylation rates was found among microorganisms isolated from different rhizospheric sediments at different sampling sites and from different macrophyte species [20]. Not all SRB are capable of Hg methylation, and other microorganisms, including iron-reducing bacteria or methanogens, may also be important for Hg methylation [37,38].

To summarize, available data support the hypothesis that some macrophyte species create a microenvironment more favorable for Hg methylation because of their direct and indirect effects on sediment (see *Effects on rhizospheric sediments*) and microbial communities. However, despite numerous studies on the subject, we are still unable to predict Hg methylation rates based on microbial community composition, because Hg methylation is influenced by a large number of interacting environmental variables. Moreover, whether roots provide only

growing support for microorganisms or also take an active part in Hg methylation remains unclear.

Accumulation of Hg in roots of macrophytes

Bioaccumulation of IHg and MeHg from sediments by macrophytes can affect the Hg concentration in sediments and also remobilize Hg from the sediments to the roots. Primary producers, such as macrophytes, are considered as a putative entry point of Hg into the food web. Therefore, their bioaccumulation and modifying factors have been studied. The bioaccumulation factor (BAF)—the ratio between the concentration in root of macrophytes and the initial concentration in sediment—is used to quantify and compare Hg bioaccumulation in macrophytes under different experimental settings and between different sites. Bioaccumulation factors from natural and artificial sediments to macrophyte roots range, in most cases, from 10^1 kg/kg to 10^{-2} kg/kg for THg, suggesting that THg contents in roots are generally lower than those found in surrounding sediments (Table 1). An exception was found in salt marshes in Portugal and Spain: concentrations in root biomass as compared with bulk sediment were 9-fold and 44-fold higher for THg and MeHg, respectively [17]. In coastal wetlands, the highest concentrations of THg and MeHg were also found in the roots of macrophytes, highlighting the importance of vegetation for Hg fate in wetlands [34]. Only 1 other study reported a BAF of 10^1 for MeHg [11].

Porewater is often considered as a pool of nutrients and trace elements available for roots, and therefore the Hg concentration in porewater might be a more relevant indicator of Hg fate than the Hg concentration found in sediments [11,39]. Only 1 study took into account porewater concentrations, reporting BAFs in the range of 10^4 L/kg and 10^3 L/kg range for MeHg and THg, respectively [11]. However, too few studies are available to be conclusive.

In general, the BAF approach is nevertheless rather limited, because it does not take into account the Hg speciation and different parameters affecting bioavailability to plants, the possible translocation or release from plants, or metals adsorbed onto the surface of plants and those internalized. Moreover in the few studies trying to distinguish adsorbed and internalized Hg, highly diverse washing protocols, in terms of reactants, concentrations, and time, are used [31,40–42]. Overall, the absence of a standardized method limits direct comparison between Hg bioaccumulation studies.

EFFECTS AT THE ROOTS/SHOOTS INTERFACE

Physical effects on sedimentation and water flow

Macrophyte biomass has direct impacts on hydrology and sediment dynamics [7,8]. High shoot density enhances the dissipation of current and wave energy up to 90%, reduces turbulence at the sediment/water interface up to 80%, and consequently reduces resuspension of sediments and cycling of elements [6,43–45]. The density of macrophyte beds can be high enough to reduce water flow and increase water residence time 10-fold and 18-fold, respectively [44,46]. A longer residence time in macrophyte stands may promote the accumulation of fine sediments, with their associated nutrients and pollutants, and could increase Hg transfer processes [44,46]. Indeed, finer grain sediments (<0.063 mm) showed higher Hg concentrations in Lake Geneva [47]. Rooted macrophytes are expected to promote sedimentation and protect against erosion and are consequently thought to stabilize shorelines and bottom sediments [45]. These effects vary with biomass, and the sedimentation process shows a strong seasonality between vegetation trapping and resuspension after plant decay [46]. Overall, the effects of macrophytes on sedimentation seem difficult to generalize and need to be explored locally to understand their impact on Hg fate.

Table 1. Accumulation and bioaccumulation factors (BAFs) of Hg in roots of macrophytes from natural and model sediments

Species	Exposure (μg/kg)[a]	Time	Hg[a] in roots (mg/kg dry wt)	BAF (kg/kg)	Reference
Natural sediment exposure					
Elodea nuttallii	7.5 (MeHg)		0.2	2.8×10^1	[11]
Egeria densa	19		0.2	9.3	[49]
Pistia stratiotes	19		0.2	1.1×10^1	[49]
Sagitaria montevidense	19		0.1	3.3	[49]
Caulerpa prolifera	300		0.1	3.6×10^{-1}	[48]
Cymodocea nodosa	300		0.1	3×10^{-1}	[48]
Posidonia oceanica	300		0.3	1.0	[48]
Ruppia cirrhosa	300		0.3	1.0	[48]
Zostera noltii	300		0.2	6×10^{-1}	[48]
Elodea nuttallii	3300		0.2	6.3×10^{-2}	[11]
Juncus maritimus	20 840		1.2	5.5×10^{-2}	[31]
Sarcocornia perennis	20 840		0.8	3.6×10^{-2}	[31]
Triglochin marimata	20 840		2.3	1.4×10^{-2}	[31]
Halimione portulacoides	20 840		2.1	9.9×10^{-2}	[31]
Hygrophila schulli	41 000		14	3.1×10^{-1}	[54]
Monochoria hastata	41 000		25	6.1×10^{-1}	[54]
Paspalum scrobiculatum	41 000		20	4.8×10^{-1}	[54]
Bacopa monniera monnieri	192 000		9	4.7×10^{-2}	[54]
Cyperus rotundus	192 000		141	0.7	[54]
Eichhornia crassipes	192 000		141	0.7	[58]
Paspalum scrobiculatum	192 000		200	1.0	[54]
Model sediment exposure					
Ipomoea aquatica	2.1 (MeHg)	10 d	0.003 (MeHg)	1.6	[56]
Ipomoea aquatica	9600	10 d	2.2	2.3×10^{-1}	[56]

[a] Total Hg except when mentioned.
MeHg = methylmercury.

Translocation of Hg between roots and shoots

Macrophytes are exposed to the water column and sediments and can accumulate Hg by both routes of exposure. Accumulated Hg can be further translocated from one organ to another, where it can be released to the environment or to the food web. In most macrophytes, THg concentrations found in roots are higher than those in shoots, with the exception of *Posidonia oceanica*, *Sagittaria montevidensis*, and *E. crassipes*, in which concentrations in roots were lower than in shoots (roots:shoots concentrations ratio = 0.66–0.95) [48,49]. On the opposite, most studies report a slightly greater difference, 1.2-fold to 5-fold of THg in roots than in shoots for a variety of species in laboratory and field studies (Table 2) [12,31,48–53]. Greater differences, between 5-fold and 30-fold, are also reported (Table 2) [31,54–57]. The largest differences were reported in *Ipomoea aquatica* with 48-fold to 72-fold and 1700-fold increases in concentration of roots versus shoots for THg and MeHg, respectively (Table 2) [56]. However, studies concerning *E. crassipes* report roots:shoots concentration ratios of 0.95, 1.6, 1.8, 8.0, and 9.8 [49,51,54,57,58], illustrating the difficulty in generalizing and comparing the different experimental settings and perhaps ecotypes.

Inorganic Hg and MeHg were accumulated by roots and shoots when the organ was directly exposed to Hg in the water column or sediment in *E. nuttallii*. Nevertheless, accumulation is not homogeneous between the different organs of plants. Mercury concentrations were further reported to be highest in roots, followed by leaves, and then stems and highest in the top, then the middle, then the bottom of shoots in *E. nuttallii* when exposed directly [12]. Transport of Hg from one organ to the other was reported in *E. nuttallii* and *Elodea densa*; however, basipetal transport (from shoots to roots) was predominant for IHg, whereas acropetal (from roots to shoots) transport was predominant for MeHg [11,12,26]. Basipetal transport of IHg was also reported in *Eriocaulon septangulare*, but no measurement of MeHg was performed [59]. Overall, the existing literature suggests that in shoots IHg is mainly accumulated from the water, whereas MeHg is accumulated

Table 2. Ratio of the Hg concentration between roots and shoots of macrophytes exposed to different media in the field and in the laboratory

Species	Exposure[a]	Time	Roots:shoots concentration[a]	Reference
Water column exposure				
Eichhornia crassipes	7000 ng/L ce	25 d	1.8	[51]
Lemna minor	7000 ng/L ce	25 d	1.6	[51]
Spirodela polyrhiza	7000 ng/L ce	25 d	1.9	[51]
Pistia stratiotes	10 000 ng/L ce	28 d	1.4	[52]
Azolla pinnata	10 000 ng/L ce	28 d	1.4	[52]
Eichornia spp.	3.9×10^6 ng/L ce		9.8	[57]
Model media $HgCl_2$ exposure				
Elodea nuttallii	0.2 μg/L	4 d	1.2	[12]
Ipomoea aquatica	33.9 μg/L	7 d	72	[60]
Ipomoea aquatica	54 μg/L	10 d	48	[56]
Vallisneria spiralis	5400 μg/L	7 d	4.5	[50]
Eichhornia crassipes	20 000 μg/L	9 d	1.8	[53]
Sediments exposure				
Cymodocea nodosa	0.025 μg/kg		1.1	[48]
Zostera noltii	0.025 μg/kg		1.8	[48]
Ruppia cirrhosa	0.025 μg/kg		5.0	[48]
Posidonia oceanica	0.1 μg/kg		0.7	[48]
Caulerpa prolifera	0.2 μg/kg		2.2	[48]
Elodea nuttallii	7.5 μg/kg (MeHg)		1.9 (MeHg)	[11]
Egeria densa	19 μg/kg		2.2	[49]
Sagittaria montevidensis	19 μg/kg		0.6	[49]
Pistia stratiotes	80 μg/kg		1.4	[49]
Salvina auriculata	80 μg/kg		1.4	[49]
Eichhornia crassipes	80 μg/kg		1.0	[49]
Elodea nuttallii	3300 μg/kg		3	[11]
Triglochin marimata	20 840 μg/kg		22	[31]
Juncus maritimus	20 840 μg/kg		28	[31]
Sarcocornia perennis	20 840 μg/kg		2.2	[31]
Halimione portulacoides	20 840 μg/kg		6	[31]
Bacopa monniera	41 000 μg/kg		7.5	[54]
Eichhornia crassipes	41 000 μg/kg		8	[54]
Hygrophila schulli	41 000 μg/kg		3.5	[54]
Ludwigia peploides	41 000 μg/kg		5.2	[54]
Marsilea spp.	41 000 μg/kg		7.8	[54]
Monochoria hastata	41 000 μg/kg		7.5	[54]
Paspalum scrobiculatum	41 000 μg/kg		7.4	[54]
Pistia stratiotes	41 000 μg/kg		1.5	[54]
Eichhornia crassipes	192 000 μg/kg		1.6	[58]
Cyperus rotundus L.	192 000 μg/kg		7.8	[54]
Paspalum scrobiculatum	192 000 μg/kg		6.7	[54]
Model sediments exposure				
Ipomoea aquatica	2.1 μg/kg (MeHg)	10 d	1 700 (MeHg)	[56]
Ipomoea aquatica	9600 μg/kg	10 d	27.4	[56]

[a] Total Hg except when mentioned.
ce = contaminated effluent; MeHg = methyl Hg.

from both water and sediments through roots to shoots translocation [11,18,26,59].

Temporal variations in Hg accumulation and translocation might explain some of the controversial data existing between different experimental settings. For example, the highest concentration in THg together with a significant increase in roots to shoots transfer in *P. australis* were found in the autumn in the Laranjo basin salt marsh (Ria de Aveiro, Portugal) [9]. In addition, Hg accumulated in roots was significantly remobilized by tides, although the mechanisms involved have not been characterized [19]. Data also suggested a higher accumulation of IHg and MeHg in juvenile and actively growing plant parts [49,56]. Growth stages, seasons, and tide cycles have not been considered in detail in most data reported to date but need to be taken more into account in future research. However, the precise mechanisms and factors controlling Hg translocation between roots and shoots still need to be determined.

Accumulation of Hg from sediments in shoots

Because Hg might be translocated from roots to shoots (see *Translocation of Hg between roots and shoots*), BAFs in shoots versus sediments have been calculated. Studies that consider only THg concentration in sediments and shoots generally result in low BAFs in shoots (10^{-1}–10^{0} kg/kg; Table 3), suggesting a low bioavailability or translocation of THg from sediments to shoots [32,59]. Nevertheless, in most cases no information is available to determine whether IHg or MeHg are predominantly accumulated in plants from the water column, from the sediments, or from porewater. When the Hg concentration in porewaters was considered as the Hg source in shoots, BAFs of 10^{3} and 10^{4} were reported for THg and MeHg, respectively (Table 3) [12]. In most cases, MeHg has been shown to result in higher BAFs than IHg in different organisms in the laboratory and in the field [26]. However, a higher BAF of IHg versus MeHg in roots of the submerged macrophyte *E. densa* was also reported [26]. These controversial results showed that the measurement of the THg in sediments alone is insufficient. A better understanding of the accumulation of Hg in shoots from sediments requires a systematic measurement of both THg and MeHg in porewater, the water column, and plants, with basic physiological information of the studied plant species and physicochemical characteristics of sediments and media being provided. Indeed, many studies do not provide a complete set of data, limiting the interpretation of their observations.

Nutrient availability seems, for example, to affect Hg accumulation [60]. Dilution of the nutrient solution resulted in higher concentrations of Hg in the leaves and stems of *I. aquatica* [60]. In contrast, Hg concentrations in roots were not influenced by the degree of nutrient dilution in the external medium [60]. In the same line, low phosphate concentrations increased Hg accumulation in shoots of *E. crassipes* [61]. Because biomass was not significantly affected in nutrient-rich

Table 3. Accumulation and bioaccumulation factors (BAFs) of Hg in shoots of macrophytes from natural sediments, porewater of natural sediments, and model sediments

Species	Exposure[a]	Time	Hg in shoots (mg/kg dw)[a]	BAF (kg/kg)[b]	Reference
Sediments					
Elodea nuttallii	7.5 μg/kg (MeHg)		0.1	9.7×10^{3}	[11]
Elodea spp.	15–882 μg/kg		0.1	2.6	[72]
Myriophylum spicatum	15–882 μg/kg		0.1	3.3	[72]
Nuphar variegatum	15–882 μg/kg		0.02	1.1	[72]
Egeria densa	19 μg/kg		0.1	4.3	[49]
Cymodocea nodosa	25 μg/kg		0.1	3.2	[48]
Ruppia cirrhosa	25 μg/kg		0.1	2.4	[48]
Zostera noltii	25 μg/kg		0.1	4	[48]
Vallisneria neotropicalis	72 μg/kg		0.02	2.6×10^{-1}	[73]
Eichhornia crassipes	80 μg/kg		0.1	6.6	[49]
Pistia stratiotes	80 μg/kg		0.2	8.1	[49]
Salvina auriculata	80 μg/kg		0.1	7.3	[49]
Posidonia oceanica	100 μg/kg		0.5	4.5	[48]
Caulerpa prolifera	160 μg/kg		0.1	3.1×10^{-1}	[48]
Eichhornia crassipes	160 μg/kg		0.5	2.8	[58]
Elodea nuttallii	3300 μg/kg		0.1	2.1×10^{-2}	[11]
Eichornia spp.	5300 μg/kg ce		103	1.9×10^{1}	[57]
Ceratophyllum demersum	5500 μg/kg		1.1	2.1×10^{-1}	[12]
Elodea nuttallii	5500 μg/kg		2.1	3.6×10^{-1}	[12]
Potamogeton nodosus	5500 μg/kg		0.1	2.7×10^{-2}	[12]
Potamogeton pectinatus	5500 μg/kg		2.2	4.0×10^{-1}	[12]
Eichhornia crassipes	41 000 μg/kg ce		2.0	4.8×10^{-2}	[54]
Ludwigia peploides	41 000 μg/kg ce		4.8	1.2×10^{-1}	[54]
Marsilea spp.	41 000 μg/kg ce		3.2	7.8×10^{-2}	[54]
Pistia stratiotes	41 000 μg/kg ce		13	3.2×10^{-1}	[54]
Porewater					
Elodea nuttallii	0.9 ng/L (MeHg)		0.1 (MeHg)	7.6×10^{4} L/kg	[11]
Elodea nuttallii	42 ng/L		0.1	1.6×10^{3} L/kg	[11]
Model sediments $HgCl_2$					
Elodea canadensis	50 μg/kg dry wt	29 d	0.5	9.6	[32]
Elodea canadensis	197 μg/kg dry wt	29 d	10.6	5.4×10^{1}	[32]
Eriocaulon septangulare	1358 μg/kg dry wt	31 d	0.4	2.9×10^{-1}	[59]
Eichhornia crassipes	1 970 000 μg/kg dry wt	68 d	286	1.0	[61]

[a] Total Hg except when mentioned.
[b] kg/kg except when mentioned.
ce = contaminated effluent; MeHg = methyl Hg.

media, the hypothesis of a biodilution effect on Hg accumulation could be eliminated [60]. Alternatively, an abundance of nutrients could influence the transport of Hg to the shoot, either as a result of competition with the essential metals or as the result of the presence of Hg species with a higher bioavailability, or a combination of both [56,60]. Conversely, available studies suggest that high metabolic rate and growth, which are not expected in limiting media, favor Hg accumulation [56,61]. Bioaccumulation of Hg is also influenced by pH, pCl (concentrations of Cl ions), light, temperature, and Hg speciation [26]. For example, in *E. densa*, an increase of Cl^- ions or a decrease in pH increased Hg accumulation as a result of their effects on Hg speciation [26]. Speciation is known to influence uptake; neutral species of Hg, such as $HgCl_2$ and CH_3HgCl, result in higher internalization than other species, and MeHg often showed a higher uptake rate than IHg (see *Uptake mechanisms of Hg in macrophytes*) [26,62]. Light increased the metabolism (photosynthesis and transpiration) of *Typha* spp and the transport of Hg to leaves and consequently volatilization of Hg(0) from leaves [62].

Based on available studies, macrophytes could be largely involved in the mobilization of MeHg from sediments to shoots and its subsequent transfer in the food web or the air (see *Release of Hg from macrophytes*).

EFFECTS OF SHOOTS

Physical effects on water/air interface

Macrophyte beds are involved in the reduction of Hg to elemental Hg(0), in particular under summer conditions, but precise mechanisms are still unclear [63]. Internal reduction by ascorbic acid has been proposed (see *Output of Hg from shoots*), but whether this is a widespread mechanism is not known [64,65]. Several mechanisms, both complementary and opposing, could also take part in Hg(0) production and distribution in the water column in the presence of macrophytes. The relative importance of the mechanisms involving macrophytes varies with plant species, season, plant biomass, and site characteristics. A year-long field dataset suggested a predominant photo-induced Hg(0) production, rather than a significant effect of macrophyte presence on Hg reduction in the St-Francois Bay (Canada) [63]. In the summer, however, macrophytes interfere with the evasion of Hg(0) to the atmosphere by modifying flow patterns, wind effect, and light penetration [63,66]. Dense vegetative mats of duckweeds limit emission through diminished vapor transport across the water/air interface, decreased photoreactions due to light attenuation, plant Hg accumulation, and oxidation of Hg(0) to Hg(II), resulting in reduced water to air fluxes of Hg(0) to 17% to 67% of open water (inversely correlated to percent cover) [66]. In *E. crassipes*, Hg(0) emission was also reduced in the presence of vegetation versus open water through a high accumulation of Hg in roots, thereby reducing the availability of Hg for volatilization [67]. Mercury retained in a lake as a result of hindered emission may increase the amount of Hg available for methylation and bioaccumulation. Field data indicate that the effects of macrophytes on Hg reduction are correlated to biomass and limited spatially to the close proximity of the plants [63,66].

Accumulation of Hg from the water column in shoots

Macrophytes can accumulate Hg in shoots directly from the water column. The accumulation of Hg in shoots of macrophytes has been reported in the laboratory using Hg spikes [12,32,50,53,55,56,60,68–71], and in the field [9,11,12,21,48,49,51,52,54,57,58,72,73]. Data concerning accumulation and calculated BAF, the ratio between the concentrations in shoots of macrophytes and the initial concentration in water or media, are summarized in Table 4. Studies concerning emerged plants were not included in the table because of low Hg accumulation usually observed in shoots. Regarding submerged species, 1 study suggested that monocots accumulate more in roots, whereas dicots translocate more to shoots; however, these observations were not supported by other studies as reflected by no significant difference in BAFs [31]. Ferns, such as *Salvinia* spp., also accumulate variable amounts of Hg, with BAFs ranging from 10^1 L/kg to 10^6 L/kg [49,52,54,70].

A broad range of exposure concentrations, from 0.1 ng/L to 20 mg/L, have been tested to examine Hg uptake by macrophytes. In many studies, tested concentrations were 10^3 to 10^6 higher than Hg concentrations found in water in the environment (pg/L to ng/L) [10]. Submerged and floating macrophytes in laboratory experiments, exposed to water spiked with IHg in the mg/L range, resulted in BAFs ranging from 10^{-1} L/kg to 10^2 L/kg (Table 4). Studies involving IHg spiked water in the μg/L range generally resulted in a BAF of 10^2 L/kg [12,71]. Exposure to lower concentrations of IHg in the ng/L range resulted in a BAF of 10^3 L/Kg in *E. nuttallii* [12]. The fact that BAFs increased with lower exposure concentrations suggests that high and low affinity transporters are involved in Hg uptake. A few studies were concerned with spiking water with MeHg in the ng/L range and reported BAFs reaching 10^4 L/kg, confirming the expected higher bioaccumulation of MeHg than IHg [12,69].

Uptake mechanisms of Hg in macrophytes

Accumulation of Hg by macrophytes needs to be understood in detail to gain better knowledge of the mechanisms controlling the fate and accumulation of Hg in macrophytes. The degree to which an organism accumulates a toxic metal depends on rates of internalization, distribution, metabolization, and elimination of the toxic metal. The absorption of Hg compounds through cell membranes seems essentially to be based on neutral chemical species crossing the membrane by passive or facilitated diffusion and Hg(II) and MeHg species passing via the anion, amino acid, and thiol transport systems [12,74,75]. However, uncertainties exist regarding the relative importance of these mechanisms, which may be to some extent species specific. Early studies concerning Hg uptake by artificial membranes proposed that the toxicity of $HgCl_2$ arises from its high permeability through lipid bilayer membranes [74]. More recent studies with model membranes demonstrated that neutral species of Hg, such as $HgCl_2$ and CH_3HgCl, diffuse through biological membranes, whereas other species such as $HgCl^+$, CH_3Hg^+, $HgCl_4^{2-}$adsorb at the membrane surface [26,76]. This is relevant because Hg bound to membranes and the cell wall is regarded as less likely to be transferred to the food web [77,78]. To our knowledge, however, only 1 study addressed Hg uptake mechanism in macrophytes [12].

In *E. nuttallii*, exposure at low temperatures or exposure of dead plants to IHg and MeHg significantly reduced their accumulation (Figure 2). Data suggested that Hg might be accumulated in *E. nuttallii* shoots by diffusion through the cell wall, but Hg internalization in the cell sap was linked to metabolism [12]. Moreover, a competition with Cu^+ (more than with Cu^{2+}) strongly reduced IHg accumulation in *E. nuttallii* (Figure 2) [79]. These data suggested that the Cu transporters COPT/CTRs could be the major routes for IHg assimilation; several studies concerning eukaryotic model organisms have shown the involvement of this transporter family in Cu^+

Table 4. Accumulation and bioaccumulation factors (BAFs) of Hg in shoots of macrophytes from natural or spiked water column

Species	Exposure[a]	Time	Hg[a] in Shoots (mg/kg dry wt)	BAF (L/kg)	Reference
Water column					
Egeria densa	0.1 ng/L		0.1	1.0×10^6	[49]
Eichhornia crassipes	0.1 ng/L		0.1	1.3×10^6	[49]
Pistia stratiotes	0.1 ng/L		0.2	1.9×10^6	[49]
Salvina auriculata	0.1 ng/L		0.1	1.4×10^6	[49]
Elodea nuttallii	0.17 ng/L (MeHg)		0.2 (MeHg)	4.4×10^5	[12]
Elodea nuttallii	0.9 ng/L (MeHg) pw		0.1 (MeHg)	$7.\ 6 \times 10^4$	[11]
Nuphar variegatum	3–19 ng/L		0.02.	7.0×10^3	[72]
Ceratophyllum demersum	8 ng/L		1.1	1.15×10^5	[12]
Elodea nuttallii	8 ng/L		2.1	2.3×10^5	[12]
Potamogeton nodosus	8 ng/L		0.	1.9×10^4	[12]
Potamogeton pectinatus	8 ng/L		2.2	2.4×10^5	[12]
Elodea nuttallii	42 ng/L pw		0.1	1.6×10^3	[11]
Eichhornia crassipes	4000 ng/L ce		2.0	5.0×10^2	[54]
Ludwigia peploides	4000 ng/L ce		4.8	1.2×10^3	[54]
Marsilea spp	4000 ng/L ce		3.2	8.0×10^2	[54]
Pistia stratiotes	4000 ng/L ce		13	3.3×10^3	[54]
Eichhornia crassipes	7000 ng/L ce	25 d	0.2	3.1×10^1	[51]
Lemna minor	7000 ng/L ce	25 d	0.2	2.7×10^1	[51]
Spirodela polyrrhiza	7000 ng/L ce	25 d	0.2	2.1×10^1	[51]
Azolla pinnata	10 000 ng/L ce	28 d	0.4	4.5×10^1	[52]
Pistia stratiotes	10 000 ng/L ce	28 d	0.4	4.2×10^1	[52]
Eichornia spp	3.9×10^6 ng/L ce		103	2.6×10^1	[57]
Model media $HgCl_2$					
Elodea nuttallii	0.02 μg/L	7 d	0.1	2.3×10^3	[12]
Elodea nuttallii	0.2 μg/L	7 d	1.1	5.7×10^3	[12]
Eriocaulon septangulare	0.5 μg/L	31 d	0.2	3.5×10^2	[59]
Elodea canadensis	0.8 μg/L	60 d	0.1	1.4×10^{-1}	[32]
Eriocaulon septangulare	1.8 μg/L	31 d	0.4	2.2×10^2	[59]
Elodea nuttallii	2 μg/L	7 d	1.9	9.6×10^2	[12]
Azolla caroliniana	1000 μg/L	12 d	70	7.0×10^1	[70]
Chara spp	1000 μg/L	5 d	0.3	0.3	[68]
Eichhornia spp	1000 μg/L	5 d	2.1	2.1	[68]
Hydrilla spp	1000 μg/L	5 d	0.5	0.5	[68]
Pistia stratiotes	1000 μg/L	10 d	92	9.2×10^1	[55]
Salvinia spp	1000 μg/L	5 d	0.7	0.7	[68]
Vallisneria spp	1000 μg/L	5 d	0.7	0.7	[68]
Vallisneria spiralis	5400 μg/L	7 d	68	1.3×10^1	[50]
Eichhornia crassipes	20 000 μg/L	9 d	2430	1.2×10^2	[53]
Model media CH_3HgCl					
Elodea nuttallii	3 ng/L	7 d	0.04	1.4×10^4	[12]
Egeria densa	30 ng/L	80 d	0.7 (fresh wt)	2.3×10^4	[69]
Elodea nuttallii	30 ng/L	7 d	0.8	2.6×10^4	[12]
Elodea nuttallii	300 ng/L	7 d	2.5	8.3×10^4	[12]

[a] Total Hg except when mentioned.
cw = contaminated effluent; MeHg = methylmercury; pw = porewater.

acquisition from the environment [80,81]. Transcriptomic data further revealed the down-regulation of the *EnCOPT1* gene by increasing concentrations of Hg(II) as well as Cu(II), supporting the hypothesis of Hg(II) uptake via high-affinity Cu transporters [79]. In summary, data suggest that high-affinity Cu transporters could be an important pathway for IHg assimilation in this macrophyte. In *E. nuttallii*, however, transport of MeHg appears to occur primarily through a different, yet to be identified mechanism, but it is also linked in part to metabolic activity [12,79]. In summary, the data currently available suggest that Hg uptake is more tightly regulated by biological mechanisms than previously thought. Nevertheless, to achieve a better understanding of influence of macophytes on Hg fate, a research priority should be placed on studying Hg uptake mechanisms in macrophytes, and notably on the possibility of a carrier-mediated transport pathway of MeHg.

Transformation of Hg in shoots

Demethylation, methylation, and volatilization of Hg occur in macrophytes, suggesting that the concentration found in organisms might not reflect the real Hg uptake from the environment [19,32,56,69]. In *E. densa* exposed to MeHg in the water column for 87 d, only 25% of the accumulated THg was in the form of MeHg, indicating a significant level of demethylation, which did not happen in the water [69]. On the contrary, in *I. aquatica*, an exposure to $HgCl_2$-spiked nutrient medium for a period of 4 d, followed by 4 d without any addition of external Hg, resulted in a 3-fold increase of MeHg concentrations in the shoots as compared with levels directly after the Hg exposure, suggesting methylation of Hg in *planta* [56]. Reduction of Hg(II) to Hg(0) in the leaves with transpiration of Hg(0) as a consequence was also observed [64,65]. Ionic Hg(II) is carried by the transpiration flow from roots to leaves and then reduced to Hg(0) in the apoplastic spaces of the spongy mesophyll by ascorbic acid [65]. Although the putative reduction with ascorbic acid has been described in barley, authors suggested that reduction of Hg(II) to Hg(0) occurs in macrophytes [62,82]. However, more species of macrophytes need to be studied to determine whether other macrophytes can methylate, demethylate, or reduce Hg and what mechanisms are involved.

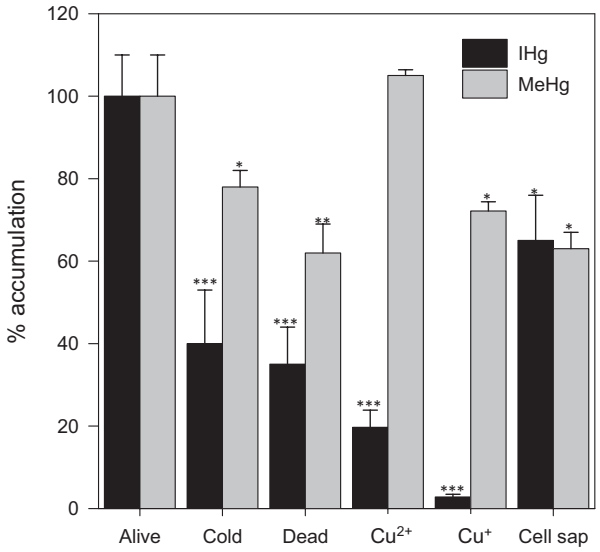

Figure 2. Accumulation of total mercury (THg) measured by AMA-254 in shoots of *Elodea nuttallii* exposed for 24 h to 200 ng/L $HgCl_2$ (inorganic mercury [IHg]) or 30 ng/L CH_3HgCl (methylmercury [MeHg]) is affected by temperature (4 °C), death (lyophilized shoots), as well as competition with 500 μg/L Cu^+. More than 60% of both Hg forms is internalized in shoots (cell sap; p values as compared with IHg or MeHg in alive plants: $^* < 0.05$, $^{**} < 0.01$, $^{***} < 0.005$; adapted from Regier et al. [12]).

Intracellular distribution and speciation of accumulated Hg

Distribution of Hg inside plants can give further hints on fate of Hg, because only cytosolic Hg is considered to be bioavailable to herbivores [77,78]. These aspects in macrophytes have unfortunately seldom been investigated until recently. In *E. nuttallii*, the distribution of Hg at the subcellular level was similar in shoots exposed for 24 h to 76 ng/L IHg and 23 ng/L MeHg; close to 65% of THg was internalized, most probably in the vacuole, whereas approximately 40% was bound to cell walls and 5% in membranes [12,83]. When exposed to MeHg, 30% of the THg in shoots was MeHg, but inside the cells 65% of THg was MeHg [12,83]. In plants exposed by roots only, 100% of THg was internalized in shoot cells in the form of MeHg [12]. Methylmercury is thus rapidly and preferentially accumulated intracellularly, whereas IHg is more accumulated in the cell wall [12]. After a longer exposure of 4 d, the proportion of THg changed to 65% in cell wall and 36% in cytosol, whereas membranes accounted for 3% of Hg, suggesting an important role of the cell wall in this plant in chronic exposure [83]. In the same line, field observations in the salt marsh plant *H. portulacoides* stressed the importance of cell wall immobilization of Hg [31]. Mercury could be retained in cell walls by means of extracellular carbohydrates, such as pectic sites or hystidyl groups forming very stable complexes. Mercury also could be associated with thiols or cysteines of cell wall proteins such as extensin, which contain several residues of cysteine [84].

Intracellular atoms found in close proximity to Hg were observed by X-ray absorption near edge spectroscopy (XANES) in *E. crassipes*. Mercury was in a form similar to MeHg-cysteine, Hg-dicysteine, and also Hg-methionine in *planta* [85]. Mercury-S binding, as β-HgS, comprised 0% to 43.5%, Hg-S binding in a form similar to Hg-cysteine represented 20.5% to 96.6%, and Hg-O coordination (including MeHg-acetate and Hg-diacetate) represented 0% to 36.0% of the chemical species in *Spartina* spp. [25]. In the same line, MeHg-cysteine was abundant in rice, but other possible Hg forms could not be identified by the analytical technique of trypsin hydrolysis followed by high-pressure liquid chromatography–inductive coupled plasma–mass spectrometry analysis [86]. The occurrence of MeHg, Hg-cysteine, and β-HgS in close proximity inside the roots suggested that Hg-cysteine was bound to cell wall proteins and further pointed toward precipitation of β-HgS with reduced sulfur concurrent with methylation [25]. However, factors controlling Hg methylation or precipitation in this environment await further elucidation. Overall, the studies demonstrated an important role of thiol and carboxyl groups of organic acids in controlling the intracellular fate of Hg. However, XANES data are obtained from samples exposed to very high Hg concentrations and therefore might not be fully representative of environmental conditions. Additionally, difficulty in differentiating between XANES spectra for species such as Hg-cysteine, Hg-glutathione, Hg-phytochelatins, and Hg bound to a sulfhydryl cysteine group of the biothiol or protein hinders their interpretation [84]. Mercury speciation in macrophyte tissues therefore may not be limited to Hg-cysteine and Hg-dicysteine. It is reasonable to expect that Hg also will have complexed with other thiol- containing biomolecules. However, data suggest that compartmentalization is involved in the greater biomagnification of MeHg versus IHg, because its higher intracellular accumulation would result in increased transfer through herbivores.

RELEASE OF Hg FROM MACROPHYTES

Output of Hg from shoots

Mercury is released during a plant's lifetime at senescence and decay. It can dissolve back to the water or be quickly integrated into the sediment with plant debris at the end of the growing season. For submerged species, a single study has shown THg distribution and accumulation over time in *E. canadensis* after a single spike of IHg in the water column [32]. During the first weeks, 50% of IHg was transferred from the water column to macrophytes, but after 1 mo IHg was increasingly present in sediments (90%), and MeHg appeared in sediments (3%) and also increased in the plants [32]. Overall, accumulation in plants affected Hg distribution in the ecosystem but was eventually released to other compartments.

Emergent and floating plants usually show significantly lower Hg concentrations than submerged plants found in the same sites [12,72]. This may be attributable to physiological differences affecting accumulation pathways, but also by excretion. Indeed, amongst emergent species, *Typha* spp. was shown to accumulate Hg in leaves from the atmosphere [18]. Conversely, a significant flux of Hg(0) from the leaves to the air was measured in vegetation of Portuguese salt marshes, suggesting that Hg was translocated from the sediments to the leaves through the roots and was released continuously to the air [19]. Wetland macrophytes are indeed important contributors to regional Hg(0) fluxes, and their ability to remobilize Hg from sediments is an important part of the biogeochemical cycle of Hg. Fluxes of Hg(0) range from 3 ng/m^2 h^1 in *Spartina patens* to 49 ng/m^2 h^1 in *Typha* spp. [62,82]. It was estimated that Hg(0) released in salt marsh areas accounted for up to 4.5% of world global Hg emissions [19]. Data suggest that Hg(0) was produced in the sediments and transported through lacunal space to the leaves [62,82] or was produced in the leaves after a reduction of Hg(II) to Hg(0), allowing transpiration of Hg to occur [64,65]. Ascorbic acid was proposed as principal reductant of Hg(II) in the apoplastic spaces of the spongy mesophyll where the bulk of ionic Hg(II) is carried by the transpiration flow [65]. Both

transpiration flow and regeneration and synthesis of ascorbic acid are stimulated by light. Such a synergism promoting the photoreduction of Hg(II) simultaneously is in line with the large differences observed between day and night emission of Hg(0) in the field [62,64]. More generally, both IHg and MeHg in macrophytes might be dispersed by drifting or hydrochory as well as through herbivory [87].

Trophic transfer

Mercury biomagnification, notably of MeHg in the food web, is one of the main hazardous consequences of Hg to mankind [3]. The MeHg bioaccumulation step from water to macrophytes represents the largest single increase for MeHg concentrations in aquatic ecosystems, reaching 10^4-fold and greater [10]. In shallow waters, macrophytes are often an important entry point of MeHg into the aquatic food web because they are the dominant primary producers in these ecosystems. Moreover, macrophytes can be exposed to both the water column and sediments, the major source of MeHg. For example in the Babeni reservoir in Romania, the average proportion of MeHg toward THg increased from approximately 2% to 13% in the surface water, to 28% to 56% in primary producers (macrophytes and plankton), to 71% in zooplankton and 94% to 97% in fish, whereas MeHg concentrations also increased (Figure 3) [10]. In this reservoir, *E. nuttallii* could retain up to 5.6 mg Hg/m^3, including 1.56 mg/m^3 MeHg based on the density found in the literature for these plants (350–2800 g dw/m^3) and the concentrations reported there [12].

The high biomass produced by macrophytes makes them significant for Hg fate in shallow water environments. Furthermore, several studies have shown that macrophytes are a food source themselves (i.e., alive and decaying tissues) in addition to serving as support to periphytic algae [3,10,73]. Thus, the factors controlling MeHg bioaccumulation from water and sediments to primary producers at the base of the food web are critical, yet they have been poorly investigated, in particular

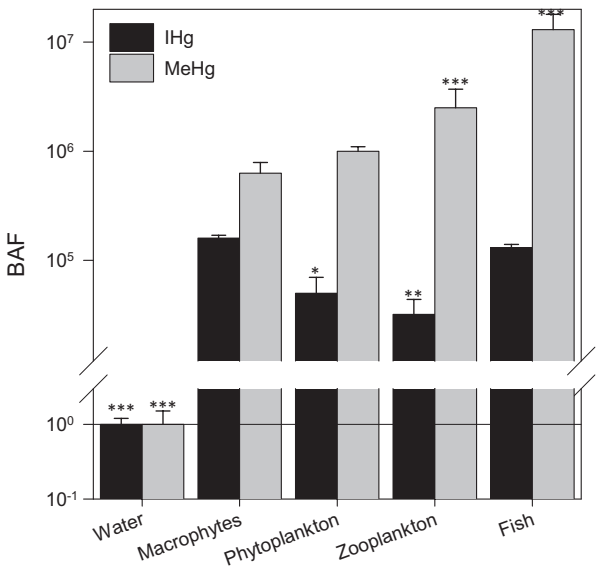

Figure 3. Biocaccumulation factors (BAFs) of inorganic Hg (IHg) and methylmercury (MeHg) measured in biota samples collected in August 2009 in the Babeni reservoir (Romania). The biggest bioconcentration step happens in primary producers, here submerged macrophytes and phytoplankton, whereas the highest MeHg concentrations are found in fish. Methylmercury is biomagnified in the food web (p values as compared with IHg or MeHg in macrophytes: $^* < 0.05$, $^{**} < 0.01$, $^{***} < 0.005$; adapted from Bravo et al. [10]).

regarding macrophytes. Experimental data showed that MeHg present in the biota is biomagnified from the portion of the prey that is assimilated by the predator (e.g., the cytoplasm), whereas other Hg species found in portions that are not assimilated by the consumers (e.g., membranes) are not biomagnified [77,78]. Data support the hypothesis of higher adsorption of IHg than MeHg and consequently more biomagnified MeHg versus IHg, although too few data really exist to generalize these findings to all organisms. Moreover, the precise mechanisms resulting in these differences of fate need to be further elucidated. However, the high amount of MeHg found in the cytosolic fraction in *E. nuttallii*, putatively available for biomagnification, together with studies that showed that Hg could be methylated in *I. aquatica*, suggest that aquatic plants could be a significant source of MeHg to the food web and therefore call for more investigation [56,88].

At a contaminated site in Romania, food web biomagnification and structure was studied [10]. Phytoplankton and macrophytes appeared at the base of 2 distinct food chains but resulted in similarly high accumulation and biomagnification of MeHg in fish [10]. In this reservoir, submerged macrophytes showed a higher accumulation of THg (up to 2 mg kg^{-1}) than other macrophytes and a similar MeHg accumulation (0.3 mg kg^{-1}) to plankton (0.4 mg kg^{-1}) and thus represent a significant entry point into the food web [10,12]. In the same line, other studies showed that emergent and submerged macrophytes such as *Scirpus fluviatilis* or *E. canadensis* made the greatest contributions to the diets of macroinvertebrate and were involved in bioaccumulation of MeHg in primary consumers in Canadian and Bolivian Lakes [3,89].

Trophic transfer from macrophytes to fish in the laboratory resulted in 3-fold higher accumulation of MeHg and IHg versus direct exposure in carp [69]. Exposure to MeHg lasting 2 mo resulted in 17% to 45% of the THg burden in *Lymnaea* and 4% to 8% in *Elodea* spp. [32]. Mercury intake by the leaves alone accounted for the MeHg amount measured in the muscle of the carp [69]. One fish consumed an average of 1.5 g *E. densa*/d, corresponding to 1030 ng MeHg and 2950 ng IHg [69]. In these 2 studies, the trophic transfer clearly predominated over direct exposure [32,69].

Macrophytes with high accumulation capacities therefore can represent a significant contamination source for herbivores and contribute to the transfer of MeHg into the food web. The transfer of MeHg to higher trophic levels could lead to cautionary levels for human consumption, in particular when elevated concentrations of THg and MeHg are found in macrophytes. Hence, macrophytes are valuable models for effective decision-making and environmental resource management in freshwater and estuarine systems. Clearly, further investigation into Hg biomagnification in shallow water ecosystems and the role of macrophytes in this process is needed.

CONCLUSION AND PERSPECTIVE

The overview of the existing literature demonstrated that macrophytes play important, but still overlooked, roles in Hg fate in aquatic systems. They affect the dispersion, speciation, and trophic transfer of Hg. Biomass production of macrophytes, notably of shoots, is significant, as is Hg accumulation. Furthermore, these plants play important roles in aquatic environments as sites for Hg methylation, as conveyors of Hg to the environment or the food web, and as biomonitors of environmental Hg concentrations. The current state of our knowledge on Hg uptake and biomagnification demonstrates the complexity and influence of a large number of biotic and abiotic

factors, including physicochemical characteristics of the medium and their variations, structural and functional properties of the macrophytes, Hg speciation, and contamination routes. Available research supports the hypothesis that some macrophytes create a microenvironment favorable for Hg methylation and that each macrophyte species might cope differently with Hg exposure, being able to alter its concentrations in the surrounding sediments, water, and air. This is of particular importance because it emphasizes the role of individual plant species and parameters of the surrounding media in the Hg retention in ecosystems. Nevertheless, a broad mechanistic understanding of the effect of macrophytes on Hg fate is also clearly lacking, notably in view of the potential risk linked with macrophyte communities found in contaminated sites.

Research priorities are to gain knowledge of the plant metabolism of macrophytes found in Hg contaminated sites to identify the relevant compartment of origin of the metal, as well as to understand bioaccumulation. Therefore, the development of a standardized leaching procedure would allow differentiating the adsorbed and absorbed metal fraction and more meaningful comparison of data in the literature. Methylmercury needs to be systematically measured in all compartments, including porewater in sediments and at the subcellular level in organisms when assessing Hg bioaccumulation and biomagnification. Eventually, more studies need to be conducted at environmental concentrations. Notably, the use of enrichment with Hg stable isotopes is recommended to help understand methylation and demethylation processes at environmental concentrations. Improving the existing basic knowledge on roots to shoots translocation is also necessary. Temporal information as well as the development stages need to be taken into account. Lastly, the uptake rate, internalization, and translocation mechanisms need to be characterized in more detail and for more species.

Acknowledgment—This work was supported by the SER (COST-FA0906 contract number C11.0117), and by the SNF (contract numbers 205321_138254 and IZERZO-142228). We are grateful to N. Graham for English improvement.

REFERENCES

1. Chambers PA, Lacoul P, Murphy KJ, Thomaz SM. 2008. Global diversity of aquatic macrophytes in freshwater. *Hydrobiologia* 595:9–26.
2. Noges T, Luup H, Feldmann T. 2010. Primary production of aquatic macrophytes and their epiphytes in two shallow lakes (Peipsi and Vrtsjarv) in Estonia. *Aquatic Ecology* 44:83–92.
3. Cremona F, Hamelin S, Planas D, Lucotte M. 2009. Sources of organic matter and methylmercury in littoral macroinvertebrates: A stable isotope approach. *Biogeochemistry* 94:81–94.
4. Cerba D, Mihaljevic Z, Vidakovic J. 2011. Colonisation trends, community and trophic structure of chironomid larvae (*Chironomidae: Diptera*) in a temporal phytophylous assemblage. *Fundamental and Applied Limnology* 179:203–214.
5. Gayet G, Guillemain M, Fritz H, Mesleard F, Begnis C, Costiou A, Body G, Curtet L, Broyer J. 2011. Do mute swan (*Cygnus olor*) grazing, swan residence and fishpond nutrient availability interactively control macrophyte communities? *Aquatic Botany* 95:110–116.
6. Wetzel RG. 2001. *Limnology: Lake and River Ecosystems*, 3rd ed, Academic Press, San Diego, CA, USA.
7. Bornette G, Puijalon S. 2011. Response of aquatic plants to abiotic factors: A review. *Aquatic Sciences* 73:1–14.
8. Desmet NJS, Van Belleghem S, Seuntjens P, Bouma TJ, Buis K, Meire P. 2011. Quantification of the impact of macrophytes on oxygen dynamics and nitrogen retention in a vegetated lowland river. *Phys Chem Earth* 36:479–489.
9. Anjum NA, Ahmad I, Válega M, Pacheco M, Figueira E, Duarte AC, Pereira E. 2012. Salt marsh macrophyte *Phragmites australis* strategies assessment for its dominance in mercury-contaminated coastal lagoon (Ria de Aveiro, Portugal). *Environ Sci Pollut Res* 19:2879–2888.
10. Bravo AG, Cosio C, Amouroux D, Zopfi J, Chevalley P-A, Spangenberg JE, Ungureanu V-G, Dominik J. 2014. Extremely elevated methyl mercury levels in water, sediment and organisms in a Romanian reservoir affected by release of mercury from a chlor-alkali plant. *Water Res* 49:391–405.
11. Regier N, Frey B, Converse B, Roden E, Grosse-Honebrick A, Bravo AG, Cosio C. 2012. *Elodea nuttallii* roots effect on bacterial communities and MMHg proportion in a Hg polluted sediment. *PLOS ONE* 7:e45565.
12. Regier N, Larras F, Bravo AG, Ungereanu VG, Cosio C. 2013. Hg bioaccumulation in the macrophyte *Elodea nuttallii* in the field and in microcosm: Hg in shoots accumulated from the water might involve Cu transporters. *Chemosphere* 90:595–602.
13. United Nations Environment Programme. 2013. *Global Mercury Assessment 2013: Sources, Emissions, Releases, and Environmental Transport*. Geneva, Switzerland.
14. Wu Y, Wang WX. 2011. Accumulation, subcellular distribution and toxicity of inorganic mercury and methylmercury in marine phytoplankton. *Environ Pollut* 159:3097–3105.
15. King JK, Harmon SM, Fu TT, Gladden JB. 2002. Mercury removal, methylmercury formation, and sulfate-reducing bacteria profiles in wetland mesocosms. *Chemosphere* 46:859–870.
16. Gentès S, Monperrus M, Legeay A, Maury-Brachet R, Davail S, André J-M, Guyoneaud R. 2013. Incidence of invasive macrophytes on methylmercury budget in temperate lakes: Central role of bacterial periphytic communities. *Environ Pollut* 172:116–123.
17. Canario J, Caetano M, Vale C, Cesario R. 2007. Evidence for elevated production of methylmercury in salt marshes. *Environ Sci Technol* 41:7376–7382.
18. Fay L, Gustin MS. 2007. Investigation of mercury accumulation in cattails growing in constructed wetland mesocosms. *Wetlands* 27:1056–1065.
19. Canario J, Vale C. 2012. Mercury in wetlands: A contribution to the definition of a global mercury policy. *Proceedings*, SETAC North America 33rd Annual Meeting, Long Beach, CA, USA, November 11–15, 2012, pp 105.
20. Correia RRS, Miranda MR, Guimaraes JRD. 2012. Mercury methylation and the microbial consortium in periphyton of tropical macrophytes: Effect of different inhibitors. *Environ Res* 112:86–891.
21. Figueira E, Freitas R, Pereira E, Duarte A. 2012. Mercury uptake and allocation in *Juncus maritimus:* Implications for phytoremediation and restoration of a mercury contaminated salt marsh. *J Environ Monit* 14:2181–2188.
22. Aldridge KT, Ganf GG. 2003. Modification of sediment redox potential by three contrasting macrophytes: Implications for phosphorus adsorption/desorption. *Mar Freshwater Res* 54:87–94.
23. Jing YD, He ZL, Yang XE. 2007. Effects of pH, organic acids, and competitive cations on mercury desorption in soils. *Chemosphere* 69:1662–1669.
24. Wu SB, Kuschk P, Wiessner A, Muller J, Saad RAB, Dong RJ. 2013. Sulphur transformations in constructed wetlands for wastewater treatment: A review. *Ecol Eng* 52:278–289.
25. Patty C, Barnett B, Mooney B, Kahn A, Levy S, Liu YJ, Pianetta P, Andrews JC. 2009. Using X-ray microscopy and Hg L-3 XANES to study Hg binding in the rhizosphere of *Spartina* cordgrass. *Environ Sci Technol* 43:7397–7402.
26. Boudou A, Delnomdedieu M, Georgescauld D, Ribeyre F, Saouter E. 1991. Fundamental roles of biological barriers in mercury accumulation and transfer in freshwater ecosystems (analysis at organism, organ, cell and molecular-levels). *Water Air Soil Pollut* 56:807–822.
27. Graham AM, Aiken GR, Gilmour CC. 2012. Dissolved organic matter enhances microbial mercury methylation under sulfidic conditions. *Environ Sci Technol* 46:2715–2723.
28. Gerbig CA, Kim CS, Stegemeier JP, Ryan JN, Aiken GR. 2011. Formation of nanocolloidal metacinnabar in mercury-DOM-sulfide systems. *Environ Sci Technol* 45:9180–9187.
29. Caslake LF, Harris SS, Williams C, Waters NM. 2006. Mercury-resistant bacteria associated with macrophytes from a polluted lake. *Water Air Soil Pollut* 174:93–105.
30. de Souza MP, Huang CPA, Chee N, Terry N. 1999. Rhizosphere bacteria enhance the accumulation of selenium and mercury in wetland plants. *Planta* 209:259–263.
31. Castro R, Pereira S, Lima A, Corticeiro S, Valega M, Pereira E, Duarte A, Figueira E. 2009. Accumulation, distribution and cellular partitioning of mercury in several halophytes of a contaminated salt marsh. *Chemosphere* 76:1348–1355.
32. Tessier E, Martin-Doimeadios RCR, Amouroux D, Morin A, Lehnhoff C, Thybaud E, Vindimian E, Donard OFX. 2007. Time course

transformations and fate of mercury in aquatic model ecosystems. *Water Air Soil Pollut* 183:265–281.
33. Acha D, Hintelmann H, Yee J. 2011. Importance of sulfate reducing bacteria in mercury methylation and demethylation in periphyton from Bolivian Amazon region. *Chemosphere* 82:911–916.
34. O'Driscoll NJ, Canario J, Crowell N, Webster T. 2011. Mercury speciation and distribution in coastal wetlands and tidal mudflats: Relationships with sulphur speciation and organic carbon. *Water Air Soil Pollut* 220:313–326.
35. Merritt KA, Amirbahman A. 2009. Mercury methylation dynamics in estuarine and coastal marine environments: A critical review. *Earth-Sci Rev* 96:54–66.
36. Vladar P, Rusznyak A, Marialigeti K, Borsodi AK. 2008. Diversity of sulfate-reducing bacteria inhabiting the rhizosphere of *Phragmites australis* in lake Velencei (Hungary) revealed by a combined cultivation-based and molecular approach. *Microb Ecol* 56:64–75.
37. Windham-Myers L, Marvin-Dipasquale M, Krabbenhoft DP, Agee JL, Cox MH, Heredia-Middleton P, Coates C, Kakouros E. 2009. Experimental removal of wetland emergent vegetation leads to decreased methylmercury production in surface sediment. *J Geophys Res-Biogeosciences.* DOI: 10.1029/2008JG000815.
38. Hamelin S, Amyot M, Barkay T, Wang YP, Planas D. 2011. Methanogens: Principal methylators of mercury in lake periphyton. *Environ Sci Technol* 45:7693–7700.
39. Roose T, Fowler AC, Darrah PR. 2001. A mathematical model of plant nutrient uptake. *J Math Biol* 42:347–360.
40. Lodenius A, Tulisalo E, Soltanpour-Gargari A. 2003. Exchange of mercury between atmosphere and vegetation under contaminated conditions. *Sci Total Environ* 304:169–174.
41. Schaefer JK, Morel FMM. 2009. High methylation rates of mercury bound to cysteine by *Geobacter sulfurreducens*. *Nature Geoscience* 2:123–126.
42. Le Faucheur S, Tremblay Y, Fortin C, Campbell PGC. 2011. Acidification increases mercury uptake by a freshwater alga, *Chlamydomonas reinhardtii*. *Environ Chem* 8:612–622.
43. Garcia E, Laroulandie J, Amyot M, Saint-Simon XR. 2003. Dissolved gaseous mercury distribution in a wetland: Influence of macrophyte beds. *J Phys IV* 107:509–512.
44. Vereecken H, Baetens J, Viaene P, Mostaert F, Meire P. 2006. Ecological management of aquatic plants: Effects in lowland streams. *Hydrobiologia* 570:205–210.
45. Le Hir P, Monbet Y, Orvain F. 2007. Sediment erodability in sediment transport modelling: Can we account for biota effects? *Continental Shelf Research* 27:1116–1142.
46. Kleeberg A, Kohler J, Sukhodolova T, Sukhodolov A. 2010. Effects of aquatic macrophytes on organic matter deposition, resuspension and phosphorus entrainment in a lowland river. *Freshwater Biol* 55:326–345.
47. Bravo AG, Bouchet S, Amouroux D, Pote J, Dominik J. 2011. Distribution of mercury and organic matter in particle-size classes in sediments contaminated by a waste water treatment plant: Vidy Bay, Lake Geneva, Switzerland. *J Environ Monit* 13:974–982.
48. Sanchiz C, Garcia-Carrascosa AM, Pastor A. 2000. Heavy metal contents in soft-bottom marine macrophytes and sediments along the Mediterranean coast of Spain. *Marine Ecology-Pubblicazioni Della Stazione Zoologica Di Napoli I* 21:1–16.
49. Molisani MM, Rocha R, Machado W, Barreto RC, Lacerda LD. 2006. Mercury contents in aquatic macrophytes from two reservoirs in the Paraiba do Sul: Guandu river system, SE Brazil. *Brazilian Journal of Biology* 66:101–107.
50. Gupta M, Chandra P. 1998. Bioaccumulation and toxicity of mercury in rooted-submerged macrophyte *Vallisneria spiralis*. *Environ Pollut* 103:327–332.
51. Mishra VK, Upadhyay AR, Pathak V, Tripathi BD. 2008. Phytoremediation of mercury and arsenic from tropical open cast coal mine effluent through naturally occurring aquatic macrophytes. *Water Air Soil Pollut* 192:303–314.
52. Mishra VK, Tripathi BD, Kim KH. 2009. Removal and accumulation of mercury by aquatic macrophytes from an open cast coal mine effluent. *J Hazard Mat* 172:749–754.
53. Giri AK, Patel RK. 2011. Toxicity and bioaccumulation potential of Cr (VI) and Hg (II) on differential concentration by *Eichhornia crassipes* in hydroponic culture. *Water Sci Technol* 63:899–907.
54. Lenka M, Panda KK, Panda BB. 1992. Monitoring and assessment of mercury pollution in the vicinity of a chloralkali plant. 4. Bioconcentration of mercury in *in situ* aquatic and terrestrial plants at Ganjam, India. *Arch Environ Contam Toxicol* 22:195–202.
55. Qian JH, Zayed A, Zhu YL, Yu M, Terry N. 1999. Phytoaccumulation of trace elements by wetland plants: III. Uptake and accumulation of ten trace elements by twelve plant species. *J Environ Qual* 28:1448–1455.
56. Gothberg A, Greger M. 2006. Formation of methyl mercury in an aquatic macrophyte. *Chemosphere* 65:2096–2105.
57. Rai PK. 2008. Mercury pollution from a chloralkali source in a tropical Lake and its biomagnification in aquatic biota: Link between chemical pollution, biomarkers, and Human health concern. *Hum Ecol Risk Assess* 14:1318–1329.
58. Marrugo-Negrete J, Benitez LN, Olivero-Verbel J, Lans E, Gutierrez FV. 2010. Spatial and seasonal mercury distribution in the Ayapel Marsh, Mojana region, Colombia. *Int J Environ Health Res* 20:451–459.
59. Coquery M, Welbourn PM. 1994. Mercury uptake from contaminated water and sediment by the rooted and submerged aquatic macrophyte *Eriocaulon septangulare*. *Arch Environ Contam Toxicol* 26:335–341.
60. Gothberg A, Greger M, Holm K, Bengtsson BE. 2004. Influence of nutrient levels on uptake and effects of mercury, cadmium, and lead in water spinach. *J Environ Qual* 33:1247–1255.
61. Chattopadhyay S, Fimmen RL, Yates BJ, Lal V, Randall P. 2012. Phytoremediation of mercury- and methyl mercury-contaminated sediments by water hyancinth (*Eichhornia crassipes*). *International Journal of Phytoremediation* 14:142–161.
62. Lindberg SE, Dong WJ, Chanton J, Qualls RG, Meyers T. 2005. A mechanism for bimodal emission of gaseous mercury from aquatic macrophytes. *Atmospheric Environment* 39:1289–1301.
63. Garcia E, Laroulandie J, Saint-Simon XR, Amyot M. 2006. Temporal and spatial distribution and production of dissolved gaseous mercury in the Bay St. Francois wetland, in the St. Lawrence River, Quebec, Canada. *Geochimica Et Cosmochimica Acta* 70:2665–2678.
64. Siegel SM, Siegel BZ, Barghigiani C, Aratani K, Penny P, Penny D. 1987. A contribution to the environmental biology of mercury accumulation in plants. *Water Air Soil Pollut* 33:65–72.
65. Battke F, Ernst D, Halbach S. 2005. Ascorbate promotes emission of mercury vapour from plants. *Plant Cell Environ* 28:1487–1495.
66. Wollenberg JL, Peters SC. 2009. Diminished mercury emission from waters with duckweed cover. *J Geophys Res-Biogeosciences.* DOI: 10.1029/2008JG000770.
67. Silva Correia RR, Martins de Oliveira DC, Guimaraes JRD. 2012. Total mercury distribution and volatilization in microcosms with and without the aquatic macrophyte *Eichhornia crassipes*. *Aquatic Geochemistry* 18:421–432.
68. Shrivastava S, Rao KS. 2000. Observations on the experimental assessment of optimal exposure time for mercury detoxification by an integrated aquatic macrophyte base system. *Bull Environ Contam Toxicol* 65:91–97.
69. Simon O, Boudou A. 2001. Direct and trophic contamination of the herbivorous carp *Ctenophatyngodon idella* by inorganic mercury and methylmercury. *Ecotoxicol Environ Safety* 50:48–59.
70. Bennicelli R, Stepniewska Z, Banach A, Szajnocha K, Ostrowski J. 2004. The ability of *Azolla caroliniana* to remove heavy metals (Hg(II), Cr(III), Cr(VI)) from municipal waste water. *Chemosphere* 55:141–146.
71. Kamal M, Ghaly AE, Mahmoud N, Cote R. 2004. Phytoaccumulation of heavy metals by aquatic plants. *Environ Int* 29:1029–1039.
72. Thompson-Roberts ES, Pick FR, Hall GEM. 1999. Total Hg in water, sediment, and four species of aquatic macrophytes in the St. Lawrence River, near Cornwall, Ontario. *J Great Lakes Res* 25:294–304.
73. Lafabrie C, Major KM, Major CS, Cebrian J. 2011. Arsenic and mercury bioaccumulation in the aquatic plant, *Vallisneria neotropicalis*. *Chemosphere* 82:1393–1400.
74. Gutknecht J. 1981. Inorganic mercury (Hg^{2+}) transport through lipid bilayer-membranes. *J Membr Biol* 61:61–66.
75. Schaefer JK, Rocks SS, Zheng W, Liang LY, Gu BH, Morel FMM. 2011. Active transport, substrate specificity, and methylation of Hg(II) in anaerobic bacteria. *Proc Natl Acad Sci U S A* 108:8714–8719.
76. Morel FMM, Kraepiel AML, Amyot M. 1998. The chemical cycle and bioaccumulation of mercury. *Annu Rev Ecol Syst* 29:543–566.
77. Mason RP, Reinfelder JR, Morel FMM. 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environ Sci Technol* 30:1835–1845.
78. Lawson NM, Mason RP. 1998. Accumulation of mercury in estuarine food chains. *Biogeochemistry* 40:235–247.
79. Regier N, Cosio C. 2012. Hg bioaccumulation in shoots of the macrophyte *Elodea nuttallii* happens through copper transporters. *Proceedings*, SETAC North America 33rd Annual Meeting, Long Beach, CA, USA, November 11–15, 2012, pp 46.
80. Hassett R, Kosman DJ. 1995. Evidence for Cu(II) reduction as a component of copper uptake by *Saccharomyces cerevisae*. *J Biol Chem* 270:128–134.

81. Page MD, Kropat J, Hamel PP, Merchant SS. 2009. Two *Chlamydomonas* CTR copper transporters with a novel Cys-Met motif are localized to the plasma membrane and function in copper assimilation. *Plant Cell* 21:928–943.
82. Lee X, Benoit G, Hu XZ. 2000. Total gaseous mercury concentration and flux over a coastal saltmarsh vegetation in Connecticut, USA. *Atmos Environ* 34:4205–4213.
83. Larras F, Regier N, Planchon S, Poté J, Renaut J, Cosio C. 2013. Physiological and proteomic changes suggest an important role of cell walls in the high tolerance to metals of *Elodea nuttallii*. *J Hazard Mat* 263:575–583.
84. Carrasco-Gil S, Alvarez-Fernandez A, Sobrino-Plata J, Millan R, Carpena-Ruiz RO, Leduc DL, Andrews JC, Abadia J, Hernandez LE. 2011. Complexation of Hg with phytochelatins is important for plant Hg tolerance. *Plant Cell Environ* 34:778–791.
85. Rajan M, Darrow J, Hua M, Barnett B, Mendoza M, Greenfield BK, Andrews JC. 2008. Hg L(3) XANES study of mercury methylation in shredded *Eichhornia crassipes*. *Environ Sci Technol* 42:5568–5573.
86. Li L, Wang FY, Meng B, Lemes M, Feng XB, Jiang GB. 2010. Speciation of methylmercury in rice grown from a mercury mining area. *Environ Pollut* 158:3103–3107.
87. Manny BA, Nichols SJ, Schloesser DW. 1991. Heavy-metal in aquatic macrophyte in a large river. *Hydrobiologia* 219:333–344.
88. Greger M, Dabrowska B. 2010. Influence of nutrient level on methylmercury content in water spinach. *Environ Toxicol Chem* 29:1735–1739.
89. Molina CI, Gibon FM, Duprey JL, Dominguez E, Guimaraes JRD, Roulet M. 2010. Transfer of mercury and methylmercury along macroinvertebrate food chains in a floodplain lake of the Beni River, Bolivian Amazonia. *Sci Total Environ* 408:3382–3391.