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Hg L_3 XANES Study of Mercury Methylation in Shredded *Eichhornia crassipes*

MICHAEL RAJAN,[†] JEANNINE DARROW,[†] MICHAEL HUA,[†] BRANDY BARNETT,[†] MIGUEL MENDOZA,[†] BEN K. GREENFIELD,[‡] AND JOY C. ANDREWS*[,†]

Department of Chemistry and Biochemistry, California State University East Bay, 25800 Carlos Bee Boulevard, Hayward, California 94542, and San Francisco Estuary Institute, 7770 Pardee Lane, Oakland, California 94621

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Eichhornia crassipes (water hyacinth) is a non-native plant found in abundance in the Sacramento-San Joaquin River Delta (hereafter called Delta). This species has become a problem, clogging waterways and wetlands. Water hyacinth are also known to accumulate mercury. Recent attempts to curb its proliferation have included shredding with specialized boats. The purpose of this research is to better understand the ability of water hyacinth to phytoremediate mercury and to determine the effect of shredding and anoxic conditions on mercury speciation in plant tissue. In the field assessment, total mercury levels in sediment from the Dow Wetlands in the Delta were found to be 0.273 ± 0.070 ppm Hg, and levels in hyacinth roots and shoots from this site were 1.17 ± 0.08 ppm and 1.03 ± 0.52 ppm, respectively, indicating bioaccumulation of mercury. Plant samples collected at this site were also grown in nutrient solution with 1 ppm $HgCl_2$ under (1) aerobic conditions, (2) anaerobic conditions, and (3) with shredded plant material only. The greatest accumulation was found in the roots of whole plants. Plants grown in these conditions were also analyzed at Stanford Synchrotron Radiation Laboratory using Hg L_3 X-ray Absorption Near Edge Spectroscopy (XANES), a method to examine speciation that is element-specific and noninvasive. Least-squares fitting of the XANES data to methylated and inorganic mercury(II) model compounds revealed that in plants grown live and aerobically, 5 ± 3% of the mercury was in the form of methylmercury, in a form similar to methylmercury cysteine. This percentage increased to 16 ± 4% in live plants grown anaerobically and to 22 ± 6% in shredded anaerobic plants. We conclude that shredding of the hyacinth plants and, in fact, subjection of plants to anaerobic conditions (e.g., as in normal decay, or in crowded growth conditions) increases mercury methylation. Mechanical removal of the entire plant is significantly more expensive than shredding, but it may be necessary to avoid increased biomagnification of mercury in infested areas.

* Corresponding author phone: 650-926-4285; fax: 650-926-4100; e-mail: jandrews@slac.stanford.edu. Corresponding author address: Stanford Synchrotron Radiation Laboratory (SSRL), 2575 Sand Hill Road, SLAC MS 69, Menlo Park, CA 94025.

[†] California State University East Bay.

[‡] San Francisco Estuary Institute.

Introduction

From the onset of the California Gold Rush in 1849 until the 1970s, mercury was mined for its use in extracting gold from ores, amalgamation, and distillation processes (*1*–*3*). It has been reported that approximately 3600 tons of Hg^0 were mined near the California coast and transported to the Sierra-Nevada mountains (*4*). A major pollution problem was created when the dispersal of this mercury into the surrounding soils and sediments, along with the mercury tailings from abandoned mines, eventually led to mercury surface water runoff into nearby tributaries and watersheds (*5, 6*). As a result of this pollution, high mercury levels in the Sacramento-San Joaquin River Delta (hereafter, Delta) were observed, posing risks to both wildlife and humans (*7*). Once in the environment, interconversion between inorganic and organic mercury takes place (*8, 9*). Conversion of inorganic mercury to the more dangerous (*10*) and bioaccumulative methylmercury is facilitated primarily by sulfate reducing bacteria (SRB), especially in aquatic systems (*9, 11*). Several factors contribute to the degree to which mercury becomes methylated. These factors include but are not limited to the following: sulfate levels (*12*), dissolved organic matter (DOM) (*13*), oxidation/reduction environment (*14*), pH, temperature, and the bioavailability of mercury to SRB (*12*).

Methylmercury is biomagnified up the food chain resulting in an increased concentration of up to ten million times greater in fish than in water (*15*). Once mercury is methylated, its bioavailability is greatly enhanced, and it is more easily stored in body tissue, thereby increasing the half-life from 6 days to 80 days (*10*). The impact of methylmercury ingestion on the human nervous system includes numbness in the extremities (fingers, nose, and lips), impaired vision, adverse changes in gait, speech, hearing, and ultimately death (*10, 16*).

A prevailing factor that contributes to the solubility, presence, and speciation of mercury is the amount of DOM. DOM is an assorted mixture of carbohydrates, carboxylic acids, amino acids, and hydrocarbons which comprise 20% of its makeup and humic substances which account for 80% (*17*). DOM has a high affinity for binding trace metals (*13*), including a strong bonding between mercury and DOM-associated reduced sulfur (*14*). The preferred affinity of mercury to sulfur is supported by their Lewis characteristics. Mercury(II) acts as a "soft" Lewis acid which combines easily with the "soft" Lewis base character of sulfur (*18*). However, total sulfur only makes up 1% of DOM, with only 0.2% in the reduced form (*14*). Mercury methylation and subsequent biomagnification has been found to be supported by increased mass of DOM (*19*). On the other hand, increased levels of DOM can conversely reduce bioaccumulation of methylmercury because DOM competes for the binding of Hg(II) with sulfate reducing bacteria thereby hindering the rate of mercury conversion to the methylated form (*20*).

Also prevalent in the Delta, Sacramento, and San Joaquin rivers is the non-native aquatic plant species, water hyacinth (*Eichhornia crassipes*). Originally from South America, water hyacinth is an invasive plant that grows in dense mats, obstructing waterways and hindering fish migration (*21*). The plant's known ability to accumulate heavy metals, including mercury, has resulted in its use in phytoremediation (*22, 23*). In one study when hyacinth were exposed to 0.5 and 2.0 ppm Hg, only trace levels were left in the water after the hyacinth were removed (*23*). Mercury is mainly biomethylated by anaerobic sulfidogenic bacteria found at the rooting zone (*24*), although some abiotic methylation processes can occur (*19*). In the rhizosphere, mercury is converted by SRB

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from less toxic forms to methylmercury which can be biomagnified up the food chain, resulting in toxic levels in higher organisms (*25*).

Because of its adverse effects on the ecosystem, efforts to curb water hyacinth growth have been made over time including chemical and mechanical methods (*26, 27*). Experimental pilot studies have been conducted to evaluate the use of mechanical shredding boats to control the growth of water hyacinth (*27*). Shredding large mats of water hyacinth can result in an anoxic environment, especially when shredded plants remain trapped in the area (*27*). Previous studies indicate that the proliferation of anaerobic bacteria increases the amount of methylmercury production (*28, 29*). Our goal is to elucidate the potential ramifications of shredding water hyacinth, particularly the effect on methylmercury presence, by assessing the degree of mercury uptake and speciation in live and shredded water hyacinth. This includes investigation of the binding and speciation changes of mercury in these plants using X-Ray Absorption Near Edge Spectroscopy (XANES). Hg L_3 XANES fingerprinting has been used to elucidate mercury speciation in swordfish and orange roughy (*30*) and in largemouth bass from Guadalupe (*31*).

The purposes of this study were as follows: 1) to determine total mercury levels in sediment and water hyacinth at the Dow Wetlands, a site where shredding took place; 2) to assess the degree of mercury uptake from shredded and live water hyacinth in a growth chamber study; and 3) to investigate the binding of mercury in hyacinth and speciation changes due to shredding, using Hg L_3 XANES.

Experimental Methods

Field Study. The study area included a tidal wetland complex along the south shore of the San Joaquin River called the Dow Wetlands (38° N 01′11″ 121° W 49′51″) due south of Sherman Island. Sample locations were in areas heavily infested with *E. crassipes* (*26*). In April of 2004 after shredding, water hyacinth and sediment samples were collected from two docks at the Dow Wetlands. Other environmental indicators relevant to our findings such as dissolved oxygen (DO), temperature, pH, and conductivity were also measured (*27*). Sediment samples were collected within 0.1 m of the surface, and plant samples were collected at various locations within the hyacinth bed. Handled with plastic gloves, plants were rinsed with distilled water and placed in resealable plastic bags. Sediment and plant samples were transported on ice and refrigerated until digestion (within 10 days). Sediment and field plant samples (approximately 1 g for sediment and 1−3 g for plants) were digested with 30% trace metal grade (TMG) HNO_3 at 95 °C for 10−15 min, cooled and refluxed with 5 mL of additional TMG acid, treated with 30% H_2O_2 until effervescence subsided, and filtered with TMG acid-rinsed Whatman #42 filter paper.

Chamber Study. Water hyacinth plant samples were collected from the Dow Wetlands and grown in a Percival Scientific Environmental Chamber. The temperature was set to 20 °C during 16 h of light and 16 °C during 8 h of darkness, and the humidity level range was set to 15−20% throughout the experiment. Water hyacinth were grown in 1-L acid-washed beakers containing one-quarter strength Hoagland's plant nutrient solution. Plant samples were divided into three groups: shredded, live, and mixed (live and shredded plants growing together) Atmospheric air was piped into the aerobic samples, while the anaerobic samples were not aerated. Shredded plants were not aerated, because we did not expect this to be a relevant environmental condition. Sample size was four plants of each unique type. Once the plants were acclimated for seven days, 1 ppm Hg as $HgCl_2$ was added to all 25 experimental samples excluding controls.

Hg Analysis. Root and shoot samples were harvested after 1 week, acid digested as for field samples above, and analyzed along with field samples (sediments, hyacinth roots, and hyacinth shoots) for total mercury using a Perkin-Elmer Analyst 300 atomic absorption spectrophotometer (AAS) in the cold vapor mode with 1.1% stannous chloride reduction. Blanks, duplicates (1 per 10 samples), spikes (1 per 10 samples), and NIST sediment standards were included for quality control. Sediment spike recoveries were 94%−111%. Plant shoot samples had spike yields of 77% ± 19%, and analysis of samples with spike yield <85% was repeated with the method of standard additions. NIST standard recovery was 107% for sediment, but the available NIST plant samples were significantly lower in concentration (0.2−0.3 ppm) than our field samples and too close to the method detection limit to be useful. Analysis of calibration standards ($R^2 > 0.998$) and samples were done in triplicate, with <1% RSD within replicates.

XAS Study. Plant samples from the chamber study were rinsed with distilled water, ground in liquid nitrogen, and placed in aluminum spacers bound with kapton tape. Aqueous saturated mercury(II) model compounds which included $HgCl_2$, mercuric acetate, Hg(II)glutamic acid, Hg(II)methionine, Hg(II)cysteine, Hg(II)dicysteine, HgS (wet in a slurry), methylmercury chloride, methylmercury glutamic acid, methylmercury methionine, and methylmercury cysteine were prepared by placing 1−2 drops in an aluminum spacer, bound by kapton tape. Mercury L_3 edge X-ray absorption spectra were collected at the Stanford Synchrotron Radiation Laboratory (SSRL) using beam line 10−2, a wiggler end-station with Si-220 crystals, a double-crystal monochromator, and an upstream focusing mirror. Sample temperature was maintained at 10 K using an Oxford Instruments CF 1204 liquid helium flow cryostat. Hg L_3 spectra for the hyacinth plants were collected by monitoring the Hg $L_{\alpha 1}$ fluorescence (9989 eV) using a 30-element Germanium detector. Data analysis was carried out according to standard methods (*32*) using EXAFSPAK (*33*). After calibration with $HgCl_2$ (first edge inflection 12284.4 eV) and adding the spectra, the pre-edge background was removed by subtracting a linear function from the entire spectrum. The ratio of the first edge jump to the EXAFS region was essentially the same for all of the spectra of these Hg(II) compounds; thus the data were normalized so that the maximum of each edge spectrum was set equal to unity. All spectra were smoothed using a third order polynomial with 2.5 eV smoothing, before derivatives were taken.

Mercury L_3 X-ray Absorption Near Edge Structure (XANES) spectra generally have two large main peaks in the first derivative, due to transitions from the core level binding energy of the L_3 electron, to unoccupied orbitals mainly 6s and 6p in character. Peak spacing and relative heights can provide significant clues to speciation. From the position of the first edge inflection (first maximum of the first derivative, E_1), the oxidation state can be determined. The spacing between these two main peaks (ΔE) can be used to determine the level of ionic or covalent bonding (*22, 34*), although factors besides this may be involved (*35*).

First and second derivative spectra of hyacinth spectra were compared with aqueous model compound spectra using the least-squares fitting program DATFIT, which is part of the EXAFPAK suite of programs (*33*). Fitting to the first or second derivative is more accurate for Hg samples because there is more variation in shape (*36*). Initially the data for the plant samples were fit to all 11 model compounds, including inorganic and methylated compounds. In successive fits the components comprising the lowest percentage (≪1%) were removed, and the remaining compounds were then refit until a reasonable fit was achieved, resulting in the roots and shoots each fitting to four main compounds. A second approach

TABLE 1. Total Hg (mg/kg dry weight) in Roots and Shoots of *E. crassipes* exposed 1 Week to 1 ppm Hg

aerobic conditions	[Hg] shoots	[Hg] roots
shredded control plants (no Hg)	0.10 (±0.01)	0.12 (±0.03)
live plants with Hg	36.5 (±32.4)	62.1 (±33.4)
live and shredded with Hg	6.6 (±3.8)	18.7 (±5.6)
amerebic conditions	**[Hg] shoots**	**[Hg] roots**
shredded plants with Hg	0.42 (±2.44)	4.38 (±2.20)
live plants with Hg	13.4 (±4.8)	67.3 (±29.5)
live and shredded with Hg	9.9 (±1.5)	20.6 (±3.3)

was employed to corroborate the findings. In this approach, each sample was sequentially fit to each model compound (1-shell fit) to find the best fit. It was then fit to this compound, plus each of the others (a 2-shell fit) that best explained the remaining variation, and was followed by 3- and 4-shells fits until the percentage of each model type in the unknown samples was determined. Both techniques yielded the same results. Data from two sets of roots from each treatment were averaged for the final fit values. Only one set of shoots data was fit because these samples were less concentrated, and thus it took significantly longer to collect suitable XANES data.

Results and Discussion

Field Study. Sediment from the Dow Wetlands in Pittsburgh, California contained 0.273 ppm mercury (SD = 0.070; N = 10), which is between the San Francisco Estuary ambient sediment concentration of 0.25 ppm for coarse sediment and 0.43 ppm for fine sediment (*37*). Average total mercury (dry weight) concentrations in the hyacinth roots and shoots collected at the Dow Wetlands after shredding were comparable at 1.17 ppm (SD = 0.08; N = 3) and 1.03 ppm (SD = 0.075; N = 3), respectively. The relatively elevated concentrations, in comparison to sediments, indicate that the roots and the shoots of the water hyacinth are bioaccumulating mercury. DO readings at the water surface from the Dow Wetlands ranged from 3.3 mg/L−4.8 mg/L prior to shredding and increased to 4.7 mg/L−5.5 mg/L post shredding (*27*). This increase in DO was a result of tidal flow in the San Francisco Bay-Delta, restored in the previously crowded hyacinth beds. The preshredding water flow from wind and tidal mixing was reduced due to the dense plant vegetation and this prevented introduction of oxygen into the water. Once the hyacinth were chopped into smaller pieces, they were able to freely float away with the Delta currents and allow the water to become turbulent and aerated. This is in contrast with a nearby slough which is land locked, in which shredding of the water hyacinth resulted in a decreased DO content (*27*).

Chamber Study. Table 1 shows the levels of total mercury in the roots and shoots of water hyacinth that were grown for 1 week in Hoagland's plant solution plus 1 ppm Hg (as $HgCl_2$). Under both aerobic and anaerobic conditions, hyacinth root samples had significantly more mercury than hyacinth shoots. Live plants under both aerobic and anaerobic conditions yielded the highest uptake of mercury, resulting in 62.1 ± 33.4 ppm and 67.3 ± 29.5 ppm in roots grown under aerobic and anaerobic conditions, respectively, and 36.5 ± 32.4 ppm and 13.4 ± 4.8 ppm in shoots of aerobic and anaerobically grown plants. The intricate structure of the root system provides greater surface area for the passive uptake of mercury (*22*) and is also the site for SRB. In earlier studies (*22*) it was shown that initially roots do take up more mercury, whereas mercury may accumulate for longer periods in the shoots. Shredded plants plus mercury (which were in all cases anaerobic) had the least uptake in roots (4.38 ± 2.20 ppm), and in shoots, at 0.42 ± 2.44 ppm, indicating that metabolic processes of live plants contribute to Hg accumulation. Control plants grown in Hoagland's only for 2 weeks have lower total Hg than field samples, due to leaching of Hg from these plants.

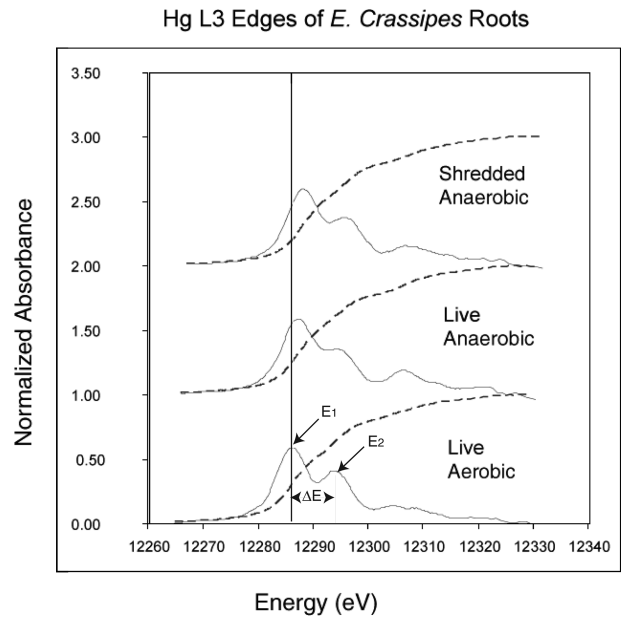

FIGURE 1. Hg L_3 edge spectra (dotted lines) and first derivatives (solid lines) of water hyacinth grown in various conditions (live aerobic, live anaerobic, and shredded anaerobic). The vertical line is provided as a reference to compare first edge inflection energies. The first and second inflections (E_1 and E_2) and their separation ΔE are indicated on the bottom spectrum.

XAS Study. Figure 1 shows Hg L_3 spectra (dotted lines) and first derivatives (solid lines) of hyacinth roots exposed to $HgCl_2$ in aerobic and anaerobic conditions (live plants) and shredded anaerobic plants. (Spectra are offset for clarity.) The first derivatives of the data (with 3.0 eV smoothing) all show two distinct transitions, a trend demonstrated in earlier Hg L_3 edge studies (*22, 34*).

Table 2 indicates the first edge and second edge inflection points E_1 and E_2 (i.e., the maxima in the first derivative spectra), and the distance between them ΔE, for the hyacinth samples and model compounds. The E_1 values range from 12284.4 eV−12286.2 eV in the inorganic model compounds and 12286.0 eV−12286.4 eV in methylated model compounds; all are in the range for Hg(II) compounds (*22, 34*). All of these model compounds were aqueous, for better comparison with hydrated plant tissue. (The presence of water ligands can affect XANES.) The generally higher E_1 in methylated compounds indicates a shift to a slightly more electron-poor form as Hg becomes methylated, indicating that the methyl group holds a significant portion of the electron density, even when compared to ligands containing chloride, oxygen, and sulfur. (Edge energies increase when electron density decreases around the absorbing atom because electrons become more tightly bound.) For mercury and methylmercury cysteine the above trend is very small, within the edge energy error of 0.2 eV, with E_1 of 12286.2 vs 12286.4; probably because the Hg−S bond is already significantly covalent and electron-withdrawing in the inorganic compound. Because cysteine has a sulfhydryl group on the end of the amino acid side chain it has a high potential to form a covalent bond between the sulfur and the mercury atoms. On the other

TABLE 2. Hg L_3 Edge Inflections of Model Compounds and Hyacinth

model compound	E_1 (eV)	E_2 (eV)	ΔE (eV)
$HgCl_2$	12284.6	12292.6	8.0
Hg(II) acetate	12286.3	12295.7	9.4
Hg(II) glutamic acid	12285.3	12299.9	8.6
Hg(II) methionine	12284.8	12293.4	8.6
Hg(II) cysteine	12286.2	12293.9	7.7
Hg(II) dicysteine	12285.8	12293.1	7.3
HgS wet	12284.4	12298.3	13.8
methylmercury chloride	12286.0	12295.5	9.5
methylmercury glutamic acid	12286.3	12295.1	8.8
methylmercury methionine	12286.2	12296.0	9.8
methylmercury cysteine	12286.4	12294.1	7.7
samples	**E_1 (eV)**	**E_2 (eV)**	**ΔE (eV)**
roots aerobic live 1	12286.2	12293.9	7.8
roots aerobic live 2	12285.9	12294.0	8.1
roots anaerobic live 1	12286.3	12293.6	7.3
roots anaerobic live 2	12286.3	12293.6	7.3
roots anaerobic shredded 1	12286.6	12293.7	7.1
roots anaerobic shredded 2	12286.4	12293.5	7.1
shoots aerobic live	12286.0	12293.0	7.0
shoots anaerobic live	12286.1	12293.0	7.0
shoots anaerobic shredded	12286.0	12293.6	7.6
dow sediment	12286.2	12294.1	7.9

hand, methionine is flanked with a carbon atom on both sides of the sulfur atom and is less likely to cleave those covalent bonds and form a new one with mercury, resulting in a more ionic linkage (wider ΔE) and a smaller electron withdrawal from mercury in mercury(II) methionine (E_1 = 12284.8) compared with mercury(II) cysteine.

In the hyacinth root samples, E_1 increased from 12286.1 eV (in live hyacinth root samples grown aerobically) to 12286.3 eV (in live hyacinth root samples grown anaerobically) to 12286.5 eV (in shredded hyacinth root samples grown anaerobically), indicating the presence of more electron-withdrawing ligands in the anaerobic plants. Compared with the ΔE from spectra of roots grown aerobically (average 8.0 eV), the spectra of roots grown anaerobically have a narrower ΔE (7.3 eV) which indicates that the speciation is changing from ionic to covalent, and this is changed further in shredded roots indicated by an even narrower ΔE value of 7.1 eV (Table 2). This leads us to hypothesize that as the mercury encounters more anaerobic conditions, its binding becomes more covalent in character.

There is no appreciable change in E_1 in the hyacinth shoot samples—aerobically and anaerobically grown shoot samples and shredded shoots had similar values, close to the E_1 values for live hyacinth roots grown aerobically. In hyacinth shoots, ΔE is consistent in the live aerobic and anaerobic plants, with $E_1 \approx 12286$ and $\Delta E = 7.0$ eV (Table 2). However, the speciation became more ionic in the shredded plants, with $\Delta E = 7.6$ eV. This suggests that the dead plants are probably forming a more ionic association between Hg and organic acids in the plants, as has been found previously in studies of Cr and Pb binding to alfalfa biomass (*38, 39*). This is verified later by least-squares fitting (Table 4), which indicate 7.6% Hg-carboxyl binding in the shredded plants.

Least-Squares Fitting (Fingerprinting) to Model Data. The trends in E_1, E_2, and ΔE as well as the overall shape of the first and second derivatives indicating intensities of transitions to unoccupied molecular orbitals (*22*) can be used to speciate mercury (*36*). Fingerprinting (least-squares fitting using DATFIT, a subroutine of EXASPAK (*33*)), indicated that all of the hyacinth root samples (Table 3) fit to a combination of mercuric acetate (representing Hg—O binding to organic acids in the plant), Hg Cl-cysteine and Hg dicysteine (Cys-Hg-Cys) (both representative of Hg binding to sulfhydryl groups in proteins or phytochelatins), and methylmercury cysteine. The more stable Hg—S bonds to cysteine (79—80% of the mercury present, summing Hg cysteine and Hg dicysteine) were consistently observed in roots of plants grown under all three conditions.

Methylmercury Content. In roots of plants grown live and aerobically, 5.0 ± 3.0% (1 × SD) of the mercury was in methylmercury form, in a form similar to methylmercury cysteine. This percentage increased to 15.7 ± 4.0% in live plants grown anaerobically and to 22.0 ± 6.4% in shredded plants grown anaerobically. The major conversion of mercury to the methylated form is accompanied by a loss of percentage of mercury(II) acetate, a model compound for Hg-carboxyl bonding to organic acids in the plant. Reduction from 16.0% Hg-carboxyl bonding in live aerobic plants to 6.7% and 9.5% in live and shredded hyacinth roots grown anaerobically, respectively, accounts for the speciation changes from a more ionic to a more covalent form, methylmercury, thus explaining the decrease in ΔE values. (Note that the total percentages do not add exactly to 100% because the numbers provided are averages from the fitting of two separate sets of hyacinth root samples).

The results from fingerprinting of the hyacinth shoots revealed two of the same compounds as the roots data: Hg(II) dicysteine and methylmercury cysteine (Table 4). This work confirms that live hyacinth shoots have more covalent binding than roots, as concluded earlier (*22*), but not due to a simple difference e.g. Hg—S in shoots vs Hg—O binding in roots, as earlier hypothesized. In contrast with root samples, mercury cysteine was replaced by (ionic) mercury methionine in shoots, and no Hg carboxyl binding was found in the live

TABLE 3. Percent Composition of Mercury Species in Hyacinth Roots as determined by Least Squares Fitting of Hg L_3 Edge Spectra (Second Derivatives) To Model Data

growth conditions	Hg(II) acetate	Hg(II) cysteine	Hg dicysteine	methylHg cysteine
live, aerobic	16.0 ± 2.8	11.2 ± 3.6	69.0 ± 3.3	5.0 ± 3.0
live, anaerobic	6.7 ± 3.8	10.5 ± 4.8	69.4 ± 4.4	15.7 ± 4.0
shredded, anaerobic	9.5 ± 4.9	9.9 ± 7.3	68.6 ± 7.2	22.0 ± 6.4

TABLE 4. Percent Composition of Mercury Species in Hyacinth Shoots as determined by Least Squares Fitting of Hg L_3 Edge Spectra (Second Derivatives) To Model Data

growth conditions	Hg(II) acetate	Hg methionine	Hg dicysteine	methylHg cysteine
live, aerobic	not detected	19.6 ± 1.8	58.7 ± 5.9	26.2 ± 2.3
live, anaerobic	not detected	24.6 ± 2.8	53.5 ± 3.9	32.6 ± 3.6
shredded, anaerobic	7.6 ± 1.3	not detected	76.9 ± 1.7	10.1 ± 2.3

shoots. In shoots of live plants, 26% to 33% of the mercury was in methylmercury form, in a form similar to methylmercury cysteine. Overall, these differences can account for the greater covalent Hg binding in shoots vs roots. Speciation in dead shoots however is significantly different from live, with 10% methylmercury in shredded plants grown anaerobically, and also 7.6% carboxyl binding, consistent with Hg-organic acid binding in the dead plants. Methylmercury content in shoots of shredded plants is significantly lower than in live, indicating that methylmercury production in the rhizosphere must be transported to shoots by metabolic processes in live plants.

These results and a companion study (*27*) suggest that growth and decay of water hyacinth may play a significant effect on total and methylmercury levels in wetland ecosystems and that shredding of hyacinth can increase this effect. Numerous studies have shown that water hyacinths are able to dramatically sequester mercury (*22, 23, 40*). There is often a considerably higher concentration of methylmercury in roots of water hyacinth than in benthic sediment and water, therefore, suggesting the rooting zone may be an important mercury methylation site (*25, 41, 42*). This was confirmed in a study (*42*) in which addition of sodium molybdate inhibition of SRB resulted in a significant decrease in mercury methylation. The SRB in the hyacinth root zone are likely associated with periphyton, or biofilms secreted by plants, that provide organic matter for their growth (*42*). Because the water hyacinth support diverse biota (*21*), methylmercury produced by SRB in the root zone may be regarded as highly accessible by other organisms that feed on the living plant or its detritus (*43*) and may also be transported into surrounding waters (*44*).

Although live water hyacinth takes up more total mercury than shredded, the presence of shredded water hyacinth and associated anaerobic conditions (which can be found in field conditions with dense hyacinth populations (*27*)) increases the degree of mercury methylation. This is consistent with the work of Guimarães et al. (*44*). In the field overcrowding in hyacinth mats can lead to lower dissolved oxygen (DO) content in the water (*27, 45*) and increased levels of organic matter (DOM), which could both lead to increased methylation. This methylmercury is apparently transported to shoots and could be consumed by water birds that feed on water hyacinth.

Due to its ability to biomagnify up the food chain, an increase in methylmercury leads to greater toxic loads of mercury in sport fish (*3*), resulting in greater risk to humans. This, combined with our results demonstrating increased methylation in shredded and anoxic plants, suggests that alternative methods such as mechanical removal are more appropriate to control the growth of water hyacinths and potentially to bioremediate mercury in Delta waterways. This might contribute to phytoremediation of mercury from the water system because the water hyacinth is a fast growing hyperaccumulator of heavy metals, sometimes accumulating more than five times the concentration of other species (*23*).

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