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Distribution and Uptake Dynamics of Mercury in Leaves of Common Deciduous Tree Species in Minnesota, U.S.A.

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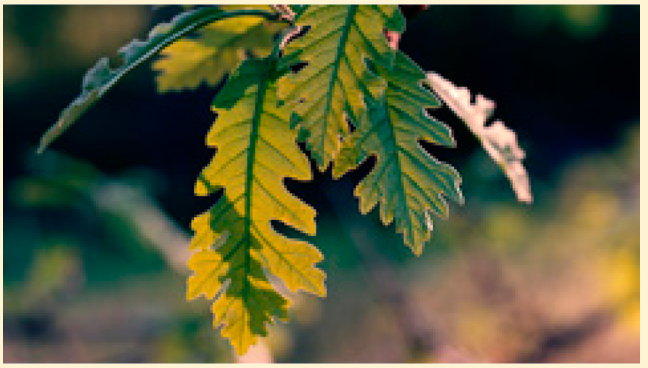
Supporting Information

ABSTRACT: A sequential extraction technique for compartmentalizing mercury (Hg) in leaves was developed based on a water extraction of Hg from the leaf surface followed by a solvent extraction of the cuticle. The bulk of leaf Hg was found in the tissue compartment (90–96%) with lesser amounts in the surface and cuticle compartments. Total leaf concentrations of Hg varied among species and was most closely correlated with the number of stomates per sample, supporting the hypothesis that stomatal uptake of atmospheric Hg (most likely Hg^0) is a potential uptake pathway. Mercury concentrations in leaves were monitored from emergence to senescence and showed a strong positive correlation with leaf age. Leaves accumulated Hg throughout the growing season; the highest uptake rates coincided with periods of high photosynthetic activity. Concentrations of Hg in leaf tissue increased steadily throughout the season, but no such trends were observed for surficial or cuticular accumulation. Factors affecting the variability of Hg in leaves were analyzed to improve protocols for the potential use of leaves as passive monitors of atmospheric Hg. Results show that total leaf Hg concentrations are affected by leaf age and leaf placement in the crown.



INTRODUCTION

Mercury (Hg) is a significant global pollutant due to its biogeochemical properties and its toxicity.[1,2] Anthropogenic activities related to industrialism are the main source for increased emissions of Hg to the atmosphere,[3,4] and to its subsequent availability for methylation in aquatic environments. In the atmosphere, gaseous elemental mercury (Hg^0) is the dominant form of Hg,[5,6] typically constituting >95% of atmospheric Hg. Lesser amounts of reactive gaseous mercury (RGM) and particle-bound mercury (Hg_p)[7] are also present, though little is known about their forms, speciation, or abundance.

Leaves have long been recognized as a sink for atmospheric Hg,[8−10] and the contribution of soil solution to leaf Hg has been deemed marginal.[11−15,8] Because vegetation covers nearly 80% of terrestrial surfaces with leaf area index up to 20 times the ground surface area,[16] foliage can play a significant role in the capture and cycling of many atmospheric pollutants.[17] For example, forested watersheds have been found to capture dry deposition of Hg more efficiently than open fields.[18−21] Leaves, therefore, represent an intermediate repository of atmospheric Hg,[9,22] and play a significant role in the global biogeochemical cycle and movement of Hg between the atmosphere and the lithosphere and thus aquatic environments.

The majority of leaf Hg is hypothesized to be associated with dry deposition of Hg^0 via stomatal routes[14,23,24] and non-stomatal routes[25−27] and, although numerous studies have pointed to the significant role of leaves in the uptake of atmospheric Hg, little information is available about the fate of Hg in the leaf after uptake. The location of mercury within the leaf, whether it is adsorbed to the surface of leaves or more tightly bound in the tissues (epidermis, mesophyll, and vascular tissues), will affect its fate and potential to persist and accumulate and can also indicate what forms are taken up.

Solvent extraction of the cuticle has been successfully applied to study the distribution and uptake dynamics of many airborne persistent organic pollutants (POPs) in leaves.[28−31] Analysis of the cuticular extract and of the remaining leaf tissues can provide an understanding of uptake pathways and the subsequent fate and behavior of those compounds. For example, low molecular weight polycyclic aromatic hydrocarbons (PAHs) have been found to diffuse through both the cuticle and the stomates, and accumulate in the cuticle and tissue of the leaf. Higher molecular weight and particle-associated PAHs, on the other hand, stay on the surface of leaves.[31,32] Unfortunately, such pertinent information is lacking for Hg.

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Table 1. Subcomponents of the Study

substudies	species	sampling time	location (SI)	no.
mercury distribution in leaves	American elm (*Ulmus americana* L.)	June 22, 2005	St Paul campus, University of Minnesota (SI).	9
	ginkgo (*Ginkgo biloba* L.)	August 31, 2005		
	sugar maple (*Acer saccharum* L.)	October 5, 2005		
	tamarack (*Larix larcinia* (Du Roi) K. Koch)			
intraseasonal uptake of mercury	elm	growing season 2005: tamarack and elm	St Paul campus, University of Minnesota.	3
	ginkgo			
	horse chestnut (*Aesculus hippocastanum* L.)	growing season 2004: ginkgo, horse chestnut, red oak and sugar maple		
	red oak (*Quercus rubra* L.)			
	sugar maple			
	tamarack			
effect of leaf placement in the crown on leaf Hg concentration	red maple (*Acer rubrum* L.)	August 11, 2005	City of Minneapolis	6

Despite agreement among scientists on the significant role of leaves in the uptake and cycling of atmospheric Hg,[7,18−20,33−38] results on interspecies variation are also unsettled. Some researchers found significant differences among broadleaf species;[39−41] however, Obrist et al.[37] reported no significant differences among 17 different tree species from 14 forest sites in the United States. Differences exist between deciduous and coniferous trees in Hg deposition fluxes. For instance, Kolka et al.[18] demonstrated that conifers are more efficient scavengers of Hg than broadleaf species. However, according to Demers et al.,[36] litterfall fluxes of Hg to the soil are greater in deciduous forests while throughfall fluxes are greatest in coniferous forests. Likewise, the results of research on the rate of Hg uptake in leaves are also inconsistent. For example, Poissant et al.[42] and Bushey et al.[39] reported a season long increase in leaf Hg concentrations, with leaf Hg reaching maximum concentrations at the end of the season. Ericksen et al.[14] however, reported that leaf Hg contents leveled off after 2−3 months of growth in a controlled environment.

The overarching objective of this study is to improve our understanding of the uptake of mercury by leaves. More specifically, we seek to determine the partitioning of Hg in the surface, cuticle, and tissue compartments of leaves/needles. We also investigate the intraseasonal dynamics of leaf Hg uptake and we address the variability in leaf uptake of Hg in the context of the potential use of leaves as passive monitors of atmospheric Hg.

MATERIALS AND METHODS

Leaf Sampling and Processing. For all parts of this study, we collected fresh, fully developed, undamaged leaves from several branches at heights over 2 m using the clean hands/dirty hands technique.[43] A composite sample was taken for each species from two adjacent trees, double bagged, and transported to the lab for analysis unless otherwise stated. All samples were collected within the Twin Cities metropolitan area (Figure S1 of the Supporting Information, SI), and therefore were assumed to have similar atmospheric exposure profiles. Species included in various parts of this study are provided in Table 1.

We determined the dry weight/fresh weight (dw/fw) ratio in a subsample of each composite sample following drying at 60 °C for 24 h. We measured the one-sided leaf surface area of the broadleaf species using a leaf area meter (LICOR LI-3050). Specific leaf area was determined as the ratio of the one-sided leaf surface area to leaf dry weight. For tamarack, the total surface area was calculated from geometric measurements.[44] Stomatal densities of the broadleaf species were measured from epidermal impressions.[45] Stomata on tamarack needles were counted directly without the aid of an impression.[46,47]

In the intraseasonal Hg uptake study, we monitored total Hg concentrations in leaves of six deciduous species (Table 1) over the full growing season from emergence to senescence to determine Hg uptake dynamics and to evaluate differences among species. Additionally, we monitored total Hg in the surface, cuticle, and tissues of tamarack needles throughout the 2005 season to investigate the contribution of each compartment to total Hg uptake. Composite samples of leaves were collected between May and October, at 1−4 week intervals depending on weather conditions. The first set of leaves was sampled at emergence and the last one during senescence, but while leaves were still on the tree. American elm was sampled an additional time (May 4, 2005).

Cuticle Separation and Sequential Extraction Technique. We developed a technique for extraction of the cuticle from leaf tissues using dichloromethane (CH_2Cl_2), similar to methods used for cuticle extraction in leaf uptake of POPs.[28,30,48] An optimal extraction time was determined for each species because differences in leaf cuticle thickness and composition can affect extraction times.[29,48] A subsample of 2 to 4 g of fresh leaves was rinsed with distilled deionized water (DDI), and then placed in a 125 mL Teflon bottle with 100 mL of dichloromethane. The leaves were gently agitated on a lab shaker and solution extracts were taken at 2 h intervals. The appropriate duration was defined as the longest extraction time that did not extract chlorophyll (a or b) from the leaves.

We used a UV−visible spectrophotometer to detect the presence of chlorophyll[49] in the extract. The presence of peaks at 413 and 666 nm for chlorophyll a and 454 and 650 nm for chlorophyll b,[50] respectively, in the dichloromethane extract was an indication of chlorophyll leaking out of the leaf and was therefore considered an overexposure. Additionally, we used Fourier transform infrared (FT-IR) spectroscopy to determine that the extract contained the cuticular material of the leaf.[51] The presence of carbonyl groups (1710−1740 cm^{-1}) in the extract,[52] which are known components of wax esters and fatty acids of the cuticle,[53] was used to verify extraction of the cuticle.

We used the following procedure to extract and to measure the amount of Hg associated with the surface, cuticle, and tissue compartments of leaves. We defined surface Hg as all forms of

Table 2. Optimal Cuticle Extraction Time in Dichloromethane and Hg Distribution in Leaf Compartments[a]

species	first observance of chlorophyll					Hg distribution in leaves		
	16 h	18 h	20 h	22 h	24 h	surface Hg (%)	cuticle Hg (%)	tissue Hg (%)
sugar maple	−	+	+	+	+	1.6 ± 0.3	2.5 ± 1.0	95.9 ± 1.1
American elm	−	−	+	+	+	2.2 ± 0.3	4.3 ± 2.5	93.5 ± 2.6
ginkgo	−	−	−	−	+	2.1 ± 0.5	3.1 ± 1.4	94.8 ± 1.8
tamarack	−	−	−	−	+	4.0 ± 2.2	6.0 ± 2.4	90.0 ± 3.1

[a]A "+" sign means a detection of chlorophyll in the extract by UV-visible adsorption. Hg distribution is reported in percent of total quantity of Hg in the leaf. The uncertainty represents the 95% C.I; n = 9.

Hg removable by a gentle shaking in water; cuticle Hg as all forms of Hg associated with the cuticle and recoverable in the dichloromethane extraction; and tissue Hg as all forms of Hg associated with the tissues of the leaf (epidermis, mesophyll, and vascular tissues) that are not removable by water and solvent extraction.

Surface Hg. A 2 to 4 g sample of fresh leaves was weighed, placed in a 125 mL Teflon bottle with 100 mL DDI water, and allowed to gently shake for 2 h in a horizontal lab shaker. The rinsate was then analyzed for Hg.

Cuticle Hg. Following extraction of surface Hg, 100 mL of dichloromethane was added to the sample, and it was placed back on the shaker for the appropriate time to remove the cuticle as determined above. Once cuticle removal was complete, 20 mL of the dichloromethane extract was placed in a 60 mL PFA Teflon impinger vessel and covered with 20 mL of nanopure water. A stream of N_2 gas was gently bubbled through the cuticle extract which was heated to 45 °C to evaporate the dichloromethane through the water. Mercury present in the dichloromethane extract was transferred into the aqueous phase. Once the dichloromethane had evaporated completely, the mercury in the aqueous phase was digested with BrCl at 70 °C and then analyzed for total Hg.

Tissue Hg. Leaf samples used in the previous treatment, now minus their cuticle, were transferred into a Teflon digestion bomb and then digested overnight with 40 mL of concentrated (15.8 M) nitric acid (HNO_3) at a temperature of 70 °C followed by Hg analysis.

Evaluation of the sequential extraction technique. To evaluate the overall efficiency and completeness of the extraction technique we compared the total Hg content of two subsamples of leaves for each species. The first subsample was immediately digested with HNO_3 without undergoing sequential extraction and the second subsample underwent the sequential extraction technique. Recovery was determined by comparing the sum of the Hg content of the three individual steps of the sequentially extracted leaves to the Hg content of the whole leaf digest. No significant difference was observed between the results of analyses of whole, untreated leaves, and the Hg contents of the sum of analyses of the three leaf compartments, indicating that the sequential extraction technique did not introduce contamination into the analyses nor lead to losses of Hg from the extractions (p = 0.68, 0.14, 0.13, and 0.12, respectively for tamarack, elm, sugar maple, and ginkgo).

Samples were analyzed for Hg in a clean room laboratory in Nater's Hg biogeochemistry lab at the University of Minnesota using cold vapor atomic fluorescence spectroscopy (CVAFS) by the double gold amalgamation method.[54] Statistical analyses were conducted using the R software[55] and the uncertainty represents the 95% confidence interval (C.I.). Student-t test was used to compare between two means, and Pearson correlation coefficients used to investigate possible correlation between parameters. Analysis of variance (ANOVA) was conducted to test for the significant difference between means. When this difference was significant, Tukey's honest significant difference (HSD) was used to find homogeneous groups. Single linear regression was used to fit prediction models for foliage Hg uptake. The slopes were compared using confidence intervals.

RESULTS AND DISCUSSION

Distribution of Mercury in Leaf Compartments. *Cuticle Removal.* The optimal extraction times for cuticle removal, considered the longest agitation period that did not extract chlorophyll, are summarized in Table 2. These extraction times are not to be taken as standards since they will vary, among other factors, with leaf age and cuticle thickness.[29,48,56] Other researchers reported different extraction times for studies that focused on the uptake of organic pollutants by plants. According to Bakker et al.,[48] 30 s were sufficient for the extraction of the cuticle of lettuce (*Latuca sativa* L.) and 15 min were necessary for Torpedo grass (*Panicum repens* L.) However, longer extraction times were reported for pine needles,[57] reaching up to 48 h.

Distribution of Hg in Leaves. Although Hg occurred in all leaf compartments, the majority was associated with leaf tissues (Table 2) remaining after cuticle extraction. Additionally, the contribution of each compartment to total leaf content Hg was consistent over time for the three compartments (ANOVA time effect was not significant for the three compartments; p > 0.05).

Surface Hg. Surface Hg constituted between 1.6% and 4% of the total leaf Hg for all four species. Tamarack had the highest mass-based concentration of surface Hg (2.66 ± 1.5 ng g^{-1}), while gingko had the lowest (0.34 ± 0.1 ng g^{-1}; Table S1 of the SI). Similar results were obtained for Hg concentrations normalized by leaf surface area. Surface Hg is likely related to both Hg_p that is removable by water (as in throughfall Hg), and RGM that is soluble in water, as has been speculated in previous studies.[20,58] Hg^0 is less likely to be involved in surface Hg since it is less soluble in water. Our findings are comparable to leaf behavior with regard to PAH uptake, where particulate and nonvolatile forms of these air pollutants were associated exclusively with the surface of leaves.[31] Overall, leaf surface Hg constituted only a small fraction of total leaf Hg; however, these samples were collected on a university campus and therefore may not be representative of all environments. We speculate that this fraction would increase if leaves were sampled near a point source of particulate or reactive Hg as has been demonstrated in analysis of throughfall for Hg following forest fires.[58] In comparison to other studies, Rea et al.[20] reported a concentration 3 times higher (75 ng m^{-2}) on the surface of

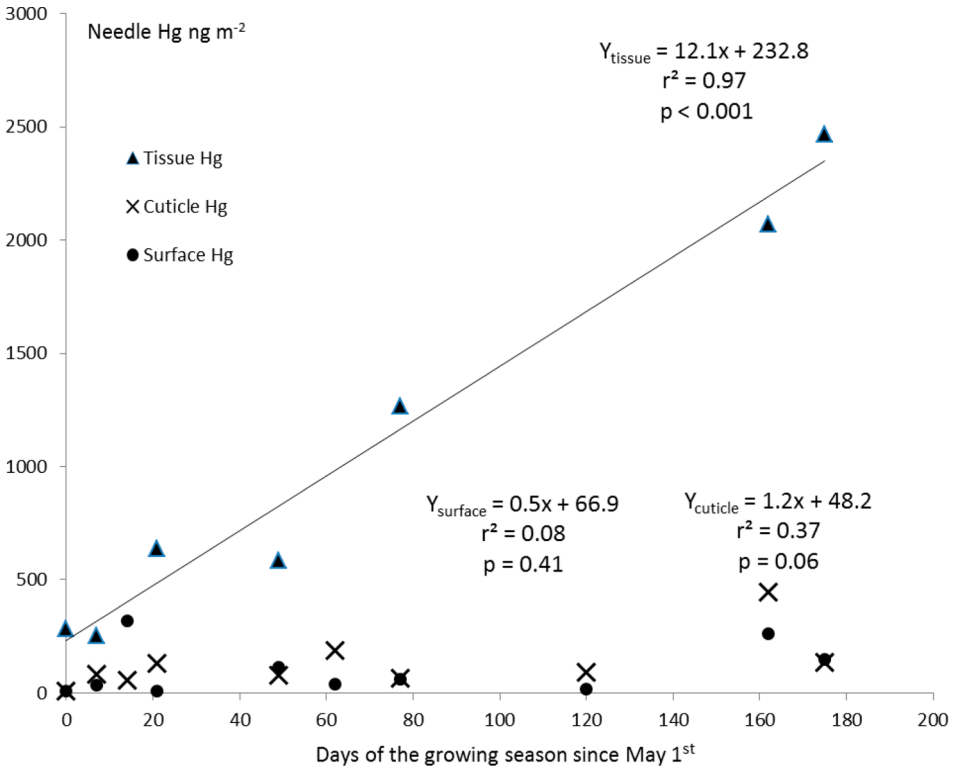

Figure 1. Intraseasonal trend in surface, cuticle, and tissue Hg concentrations of tamarack needles.

maple leaves in the eastern United States compared to levels found in this study (23 ng m^{-2}).

Cuticle Hg. Cuticular Hg constituted about 4% of the total leaf Hg. American elm (1.5 ± 0.8 ng g^{-1}) had significantly higher concentrations of Hg in the cuticle extract than the other species (Table S1 of the SI). Stamenkovic and Gustin[26] monitored leaf–atmosphere Hg fluxes in a controlled environment and observed mercury deposition into leaves during darkness and elevated CO_2 concentrations. Consequently, they suggested that a nonstomatal pathway plays a significant role in leaf Hg uptake. We speculate that the cuticle may be involved in nonstomatal pathway(s), similar to the role attributed to the cuticle in the uptake of polycyclic aromatic hydrocarbons (PAHs). Kuhn et al.[31] showed that nonvolatile PAHs are adsorbed at the cuticle, while volatile PAHs diffuse across it and reach the inner tissues of the leaf. The cuticle has both polar and nonpolar routes[59] and can potentially permit the passage of both Hg^0 (nonpolar) and Hg^{2+} (polar). Ionic forms of Hg could reach the inside of the leaf the same way herbicides and foliar fertilizers do, following polar routes to gain access to the tissues of the leaf.[60,61]

Tissue Hg. Leaf tissue was the dominant reservoir of Hg for all four species (Table 2). In terms of mass-based concentrations (ng g^{-1}), tamarack (36.8 ± 9.6 ng g^{-1}) displayed the highest concentrations, while ginkgo had the lowest (Table S1 of the SI). The proportion of leaf tissue Hg was consistent over the three sampling times, suggesting that Hg is held inside the leaf and most likely incorporated in leaf tissues. A similar conclusion was reached by Lodenius et al.[62] where they subjected leaves to high temperature over a period of four weeks and did not notice any loss of leaf Hg concluding that leaf uptake of Hg is irreversible.

Temporal Change of Hg Concentrations in the Surface, Cuticle, And Tissue of Leaves. We monitored Hg concentrations in these three compartments in tamarack from emergence to senescence to determine temporal trends in uptake (Figure 1). Tamarack was chosen because it had the highest Hg concentrations among the species.

Surface Hg. Surface Hg concentration (ranging from 9.03 to 263 ng Hg m^{-2}) varied throughout the growing season and constituted 1 to 14% of total leaf Hg with no clear trend of accumulation (Figure 1). Surface Hg showed periods of apparent accumulation followed by depletion, suggesting removal of surface Hg by precipitation.[19] Researchers[18,58] have observed that throughfall concentrations were higher and more variable for total Hg than open-air precipitation, indicating washoff of surficial Hg from leaf surfaces. Surface Hg could also be removed by biotic and abiotic reduction processes[63] causing re-emission of Hg to the atmosphere. Alternatively, surface Hg may migrate into the cuticle, effectively removing it from the leaf surface compartment. A combination of these scenarios is likely the cause of the fluctuation in surface Hg concentration during the growing season.

Cuticle Hg. Cuticular Hg behaved similarly to surface Hg and showed periods of accumulation followed by depletion (Figure 1). Cuticle Hg concentrations varied from 9 to 446 ng m^{-2} and contributed 3 to 24% of total needle Hg. No significant accumulation occurred over the growing season (p = 0.35), indicating that, at least for tamarack, the cuticle is not a storage site for Hg as it is for many airborne POPs.[31,64] Given the low concentrations of Hg encountered in the cuticle, it is likely that the stomatal route is more dominant in atmospheric gaseous Hg uptake while the cuticle is involved in ionic Hg uptake. These observations support recent speculation that the cuticle is involved in Hg uptake.[26,27]

Tissue Hg. Tissue Hg increased significantly ($p < 0.001$) and continuously between emergence and senescence of needles

(Figure 1), constituting 75% to 94% of total Hg in needles and reaching a mean concentration of 2420 ng m^{-2} at a rate of 14.4 ng m^{-2} day^{-1}. Although the rate of Hg uptake varied over the season, tissue Hg concentrations increased continuously throughout the growing season, suggesting that Hg is irreversibly incorporated in the tissue of needles and is unavailable for release back to the atmosphere. The majority of atmospheric Hg is in the form of Hg^0 and several laboratory studies[14,65] have shown that leaves can take up Hg^0 from the atmosphere. Although the mechanism is not known, it is apparent that Hg^0 taken up by leaves must be oxidized to Hg^{2+} inside the leaf for it to be irreversibly incorporated into leaf tissues. Hg^{2+} has a high affinity for thiols,[66,67] which are essential components of the two amino acids cysteine and methionine, and tripeptide glutathione, all of which are common constituents of plant cells and tissues.[68] This high affinity was confirmed in a recent study where the majority of Hg in *Brassica juncea* leaf tissues was found bound to sulfur.[69] The high affinity of Hg^{2+} for thiols would render Hg incorporation irreversible. The continuous increase in leaf tissue Hg shows that atmospheric Hg and needle Hg never reached an equilibrium, and therefore the use of partitioning coefficients that assume this equilibrium is not appropriate.

Influence of Surface Area, Dry Weight and Stomates on Leaf Mercury. Simple linear regression analysis of leaf Hg content based on surface area and dry weight for sugar maple, ginkgo, elm, and tamarack suggests the importance of both parameters in Hg uptake. Leaf total Hg content (THg) for these four species combined was significantly and positively correlated with the dry weight of the leaves ($y = 31.4x - 0.12$; where x is sample weight in g, and y is total Hg in ng, $r = 0.76$, $n = 12$, $p < 0.05$). Likewise, the correlation between leaf THg and surface area was also significant and positive ($y = 0.16x + 2.63$; where x is the surface area in cm^2, and y is total Hg in ng, $r = 0.76$, $n = 12$, $p < 0.05$). However, when fresh weight was used instead of dry weight, the correlation was insignificant ($r = 0.30$, $p = 0.28$). These results suggest that leaf accumulation of atmospheric Hg relies on both the surface area (stomates), and the biomass of the leaf (storage). Therefore, reporting leaf Hg concentration both in ng g^{-1} and in ng m^{-2} would provide complementary information that would be useful for species comparison despite the strong correlation between dry weight and surface area ($r = 0.91$). In POP uptake studies, Simonich and Hites[70] recommended normalizing leaf concentrations of atmospheric pollutants by surface area. Moeckel et al.[71] reached the same conclusion while studying plant uptake of polychlorinated biphenyls (PCBs).

Regression analyses showed that the number of stomates per sample (Table S2 of the SI) was significantly and positively correlated with Hg content of the leaf interior ($y = 3.96 \times 10^{-6}x + 10.6$; where x is the total number of stomates in the leaf sample, and y is the total Hg in ng, $n = 12$, $r = 0.81$, $p < 0.001$). This correlation was better than that provided by Hg/surface area or Hg/mass, suggesting a potential role of the stomates in Hg uptake, in agreement with conclusions reached by Ericksen et al.[14] and Choi et al.[24]

Mercury Uptake over the Growing Season. *Leaf Hg Concentrations.* All species accumulated Hg ($p < 0.001$) throughout the duration of the growing season (Table 3; Figure S2 of the SI) and peak concentrations were reached at the end of the growing season. While it would be better to compare data from the same year, we found no significant interannual difference in Hg concentrations between 2004 and 2005

Table 3. Leaf Hg Concentration, Seasonal and Intra-Seasonal Uptake Rates

species	emerging leaves hg		senescing leaves hg		regression of the averageseasonal uptake rate	adjusted R^2	95% C.I of the slope	intraseason uptake rates ng m^{-2} day^{-1}		
	ng g^{-1}	ng m^{-2}	ng g^{-1}	ng m^{-2}				early season	midseason	late season
gingko	1.73 ± 0.6	168 ± 37.8	[a]19.1 ± 2.8[A]	1420 ± 180[A]	[b]$y = 7.37x + 99$	0.92	5.60−8.44	[c]2.3Aa	10.5Ba	13.6B
horse chestnut	1.51 ± 0.8	60 ± 29.3	29.9 ± 3.4[B]	1200 ± 134[A]	$y = 7.50\ x + 11.16$	0.91	5.86−9.10	7.4ab	10.0a	7.3
red oak	2.10 ± 0.7	94 ± 29.4	25.2 ± 5.5[B]	1140 ± 250[A]	$y = 4.71x + 100$	0.82	3.23−6.33	1.9Aa	5.4Ba	12.0B
sugar maple	2.70 ± 2.1	114 ± 77.1	30.6 ± 4.8[BC]	1460 ± 123[A]	$y = 7.72\ x + 78.12$	0.97	5.39−10.58	7.2ab	10.3a	8.5
American elm	3.00 ± 1.7	134 ± 27.2	41.0 ± 6.8[CD]	1520 ± 175[A]	$y = 8.78\ x + 190.49$	0.95	4.43−13.13	13.4Bb	5.4Aa	10.1A
tamarack	2.85 ± 1.3	338 ± 50.5	43.1 ± 1.7[D]	2980 ± 116[B]	$y = 16.09\ x + 265.19$	0.93	12.57−19.61	9.8Aab	40.4Bb	6.6A

[a]For Hg concentrations, means with different letters in the same column are statistically different ($p < 0.05$) and the uncertainty represents the 95% C.I.; $n = 3$. [b]For the regression, y is mercury concentration in ng m^{-2} and x is leaf age in days; $n = 3$ ($p < 0.05$). [c]For the intraseason uptake rates, means with different upper case letters in the same row are significantly different. For comparison between species, means with different lower case letters in the same column are significantly different ($p < 0.05$); $n = 3$.

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collections of senescing elm leaves (p = 0.11) and tamarack needles (p = 0.27).

Hg concentrations measured in this study are roughly comparable to those reported in other studies.[39−41] Leaf Hg concentrations among the broadleaf species showed no significant interspecies difference when end of season leaf Hg concentrations were normalized by surface area (Table 3), a result also observed by Siwik et al.[40] When the species were compared on a mass basis, however, significant differences were observed among species. Tamarack attained higher area-based Hg concentrations (p < 0.001) than any of the broadleaf species. Conifers generally have greater surface roughness and more leaf hairs than broadleaf species, and a structure that slows air flow.[72,73] Moreover, tamarack needles have stomata in both the abaxial and adaxial surfaces, unlike the broadleaf species, potentially allowing the needles to intercept larger amounts of atmospheric Hg than broadleaf leaves. Additionally, the cuticle of coniferous needles is known to have more lipids than that of broad-leafed leaves,[74,75] which could be a factor in Hg uptake and/or accumulation given the lipophilicity of Hg^0. The higher scavenging ability of Hg by conifers was also observed in watershed studies that evaluated throughfall Hg in deciduous and coniferous species.[18,19,36,76]

Seasonal Uptake Rates and Regression Analyses. Sugar maple, gingko, red oak and horse chestnut showed similar mean seasonal uptake rates (Table 3), whereas tamarack had a much steeper slope of 16.09 ng Hg m^{-2} leaf area day^{-1}, not surprising given that tamarack needles had the highest Hg concentrations at the end of the season.

Among the broadleaf species, red oak had the lowest Hg uptake rate of 4.71 ng m^{-2} day^{-1} corroborating results of recent studies by Siwik et al.[40] and Juillerat et al.[41] where red oak also had the lowest uptake rate among different deciduous species. However, Siwik et al.,[40] who studied leaf uptake of Hg in Ontario, Canada, reported a steeper slope for red oak (17 ng m^{-2} day^{-1}) than we observed, possibly due to higher Hg exposure and/or different environmental conditions. Higher uptake rates were also calculated by Bushey et al.,[39] who found that sugar maple from the Huntington Wildlife Forest in NY accumulated a daily average of 14.40 ng m^{-2} in the 2005 growing season, and Poissant et al.[42] who reported an uptake rate of 13.20 ng m^{-2} day^{-1} for maple leaves in a Canadian forest. Leaf Hg concentrations correlated significantly and positively with leaf age for all species. Similar results were observed in laboratory studies[14] and field studies.[20,35,42] Leaf uptake of Hg continued throughout the growing season, and leaf Hg concentration did not level off after two months, contrary to results of a laboratory study by Ericksen et al.[14] In the Ericksen et al.[14] EcoCELLs experiment, however, Hg exposure was higher than that in natural settings, and leaves reached Hg concentrations nearly five times what we observed before they leveled off.

Intraseasonal Changes in Hg Uptake Rates in Leaves. To gain better insight into the seasonal variation in the Hg uptake rate, we divided the season into three periods of two months each; early season (May−June), midseason (July−August) and late season (September−October), and compared Hg uptake rates between these periods (Table 3). For most species, the uptake rate changed during the season. The bulk of Hg uptake occurred during the midgrowing season when leaves reached maturity and photosynthetic activity was at its peak,[77,78] as also observed by Obrist[79] who related the seasonal decline in global atmospheric Hg concentrations to the annual oscillations of atmospheric CO_2 concentrations and the seasonal cycles of photosynthetic activity in the Northern Hemisphere.

The rate of leaf/needle Hg uptake leveled off or decreased toward the end of the growing season for most species. Similar late declines in leaf Hg uptake rates were also observed in controlled environment studies[14] and in natural settings.[42] This decrease in Hg uptake rate appears to be related to a decrease in photosynthetic activity at the end of summer experienced by deciduous leaves,[80] especially since leaf Hg uptake has been related to the stomatal route.[23,81,82] The decline in Hg uptake later in the season could also be explained by leaves/needles reaching a saturation point with regard to Hg assimilation. However, the latter scenario is unlikely since much higher leaf Hg concentrations have been reported for the same species in other environments.[41,83] In controlled environments, however, and under higher Hg exposures, saturation generally occurs within 2−3 months of leaf emergence.[14]

Gingko and red oak displayed higher late season uptake rates (13.55 and 11.95 ng m^{-2} day^{-1}, respectively) than the other species (Table 3). Siwik et al.[40] also reported a higher late season uptake rate (>20 ng m^{-2} day^{-1}) for red oak, potentially related to the prolonged photosynthetic activity of red oak, which does not decline until late in the season.[80,84]

Even when Hg uptake rates declined, leaf Hg concentrations continued to increase until senescence. If a compensation point for Hg uptake by leaves exists,[85] then it did not seem to cause a loss of leaf Hg, again supporting the findings of Lodenius et al.[62] that Hg uptake is irreversible. It is possible that leaf Hg emissions observed in other studies[86] are a result of leaf surface Hg (Hg^{2+}) being reduced to Hg^0 by biotic and/or abiotic mechanisms[63] and emitted back to the atmosphere. Additionally, the continuous increase in Hg concentrations until leaf senescence indicates that leaf Hg is not translocated to the stem and other tree storage sites before leaf abscission unlike nitrogen,[87] starch[88] and some microelements.

Potential Use of Leaves As a Passive Monitor for Atmospheric Hg Concentrations. Deciduous leaves have long been used as a passive monitoring tool for many airborne POPs[89,90] and more recently for atmospheric Hg[91−93] because trees are generally present in the landscape and because it is less expensive and more convenient to measure these chemicals in leaves than it is to actively monitor them in the atmosphere. However, use of leaves as a proxy for atmospheric Hg measurements requires a comprehensive understanding of the dynamics of uptake and its variability in leaves so that the uncertainty can be assessed and reduced. Leaf height[39,41] and leaf placement on the branch with regard to apex leaves are known to affect leaf Hg content.[40] Here we examine factors such as leaf age and leaf placement with regard to the crown of the tree.

Leaf Age. Leaf age is an important factor that affects leaf Hg content. Our study showed a strong and significant positive correlation between age and Hg content of leaves for all species investigated (Table 3). Therefore, for the purpose of accurate estimation of leaf Hg, particular attention is needed when sampling deciduous tree species with indeterminate growth, that leaf out throughout the summer such as species of *Populus*,[94,95] *Alnus*,[95] *Ulmus*,[96] and *Salix*.[95] This holds also for heterophyllous species with repeated flushing such as *Butela*,[97] *Quercus*,[98] and other species that can produce leaves throughout the growing season such as ginkgo.[99] Furthermore, many deciduous tree species produce sucker leaves on sucker branches. These branches can leaf out later in the growing

season.[100] Analysis of sucker leaves may complicate comparisons among sites because they may be considerably younger than canopy leaves and thus may have significantly lower Hg concentrations. An analysis of basswood (*Tilia Americana* L.) sucker leaves (Figure S3 of the SI) showed that they had less than half the Hg concentration of canopy leaves (Table S3 of the SI). Therefore, comparison of the concentration of Hg in leaves from different sites should focus on leaves of similar age. Inadvertent incorporation of leaves of different ages into a leaf monitoring study could lead to inconsistent results.

Leaf Placement. The position of leaves with respect to a tree's crown affects their exposure to many environmental parameters (e.g., solar radiation intensity[101]) and may also affect Hg uptake. Leaves positioned near the outside of the crown usually have a smaller specific leaf area (SLA), a measure of leaf area per unit mass typically expressed in $cm^2\ g^{-1}$, than those positioned nearer the interior of the crown.[101]

Outside crown leaves of red maple were collected from the exterior of the eastern side of the crown at a height of 2 m, while the inside crown leaves were collected near the tree trunk at the same height and direction. Outside and inside crown leaves had similar Hg concentrations on a per mass basis (54 ± 6.7 ng g^{-1} and 52 ± 5.5 ng g^{-1}, respectively). However, when leaf Hg concentrations were reported on a per area basis, outside crown leaves contained 77% more ($p = 0.01$) Hg than inside crown leaves (3600 ± 412 ng m^{-2} and 2030 ± 142 ng m^{-2}, respectively). The lower SLA ($p = 0.0001$) of outside crown leaves (149 ± 1.8 $cm^2\ g^{-1}$ vs 261 ± 31.1 $cm^2\ g^{-1}$ for the inside crown leaves) indicates that they are thicker and have more biomass per unit of surface, which may potentially store more Hg. Outside crown leaves typically have thicker palisade mesophyll layers and highly vacuolated cells.[101] Outside crown leaves also have higher stomatal conductance,[102,103] which could result in higher Hg uptake on a per unit area basis. To minimize variability, samples should be collected from the same position within the crown.

CONCLUSIONS

Leaf tissue was found to be the primary storage site for Hg in leaves, and its behavior indicates that gaseous Hg is the main form of uptake. Tissue Hg therefore may be a good proxy for regional and global Hg exposure, while surface Hg is more important near Hg point sources.

The potential for monitoring atmospheric Hg concentrations by using leaves as a passive receptor is gaining increasing attention (i.e., litterfall network) because it is cheaper to seasonally measure Hg in foliage/litterfall compared to continuously measuring Hg in air, and because litterfall Hg has a longer time fingerprint. The variability of such observations can be decreased and the comparability among sites increased by modifying sampling protocols to account for leaf age (older leaves have higher Hg concentrations), position of the leaves within the crown of the tree (inner and outer crown leaves typically have different specific leaf areas and outer crown leaves commonly have more Hg associated with the leaf surface), and proximity to potential sources of particulate or aerosol Hg, which can significantly increase leaf surface Hg. Long-term monitoring programs for leaf Hg in proximity to atmospheric Hg monitoring may be needed to better understand its behavior and to permit a meaningful interpretation of interannual comparisons. Lastly, a better understanding of leaf uptake of Hg will improve our estimation of Hg associated with leaves and thus the contribution of dry deposition to the global Hg cycle.

ASSOCIATED CONTENT

Supporting Information

Detailed experimental methods, study site, and supporting data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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