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Mercury Speciation in Temperate Tree Foliage

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Cycling of mercury (Hg) and monomethylmercury (MMHg) in forest ecosystems can affect exposures of terrestrial and aquatic wildlife within the watershed. Litterfall has been posited to be a major source of MMHg and total Hg to the forest floor; however, the origin of MMHg associated with tree foliage is largely unknown. I tested the hypothesis that leaf MMHg would be controlled by root uptake and thereby proportional to levels in soil. Fresh leaves and associated soil samples were sampled from nine tree species (deciduous and coniferous) at 30 locations spanning a 1145 km² area in southwest Ohio, a region presumed to have relatively homogeneous atmospheric deposition of Hg and MMHg. Concentrations of Hg species in tree leaves were unrelated to those in soil. In contrast, tree genera and trunk diameter were dominant variables influencing Hg levels in tree foliage. The fraction of total Hg as MMHg was relatively constant among all genera and averaged 0.4%. Results of this study suggest that uptake of gaseous Hg⁰ from the atmosphere is the dominant source of total Hg in foliage and that MMHg is formed by in vivo transformation of Hg(II) in proportion to the concentration accumulated. Via litterfall, it appears that processes associated with tree leaves are a major source of total Hg and MMHg to the forest floor.
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I. Introduction

Sources and Speciation of Mercury

Mercury (Hg) is derived from a variety of natural and anthropogenic sources and is distributed throughout earth’s environments and ecosystems. Coal-fired power plants are the leading source of Hg to the atmosphere (Lawson and Mason, 2001; Munthe et al., 2007) and comprise about 60% of Hg emissions globally (Pacyna and Pacyna, 2006). The influence of anthropogenic sources, namely fossil fuel combustion, on the global Hg cycle is evident from sedimentary archives that show atmospheric Hg deposition has increased about 3-fold worldwide since the advent of the Industrial Revolution (Fitzgerald et al., 1998). Natural sources, such as volcanoes and wild fires, also emit Hg to the atmosphere (Pirrone et al., 2010). The forms of Hg that are emitted from both anthropogenic and natural sources are gaseous elemental Hg (Hg\(^0\)), which constitutes 95–99% of the Hg in the atmosphere (Iverfeld, 1991; Lindqvist et al., 1991; Zhang et al., 1995; Ericksen et al., 2003; Millhollen et al., 2006; Graydon et al., 2008), and divalent inorganic Hg (Hg\(^2+\); Lindqvist et al., 1991). While Hg\(^0\) is a gas, Hg\(^2+\) is present in the atmosphere in dissolved aqueous, particulate, and gaseous phases (Lindqvist et al., 1991; Lindberg et al., 2007).

Methylated Hg compounds also exist in the atmosphere. Gaseous dimethylmercury (DMHg) is hypothesized to exist in the atmosphere, although concentrations are often lower than detection limits (Bloom and Watras, 1989). Monomethylmercury (MMHg) is observed frequently in wet deposition at levels of about
1–10% of total Hg (HgT; Hammerschmidt et al., 2006) and is believed to result from abiotic and biologically mediated methylation of inorganic Hg species. MMHg is the most toxic form of Hg in the environment, mainly because of its ability to bioaccumulate in organisms and biomagnify in food webs (Hultberg et al., 1995; Schwesign and Matzner, 2001; Hammerschmidt and Fitzgerald, 2006b; Fitzgerald et al., 2007; Munthe et al., 2007; Swain et al., 2007; Tsui et al., 2008).

**Mercury Toxicity**

MMHg can have modest to severe toxicological effects across a wide array of animal species, ranging from small aquatic organisms and fish to piscivorous wildlife and humans (Mergler et al., 2007; Scheuhammer et al., 2007). Toxic effects in fish include suppressed gonadal development and reduced production of eggs and hormones (Scheuhammer et al., 2007). MMHg also can pass from mother to embryo (Hammerschmidt et al., 1999), which may be attributed to its affinity for thiols in amino acids (Mergler et al. 2007), and potentially affect the health of offspring (Alvarez et al., 2006). Because MMHg increases in concentration with trophic level (i.e., biomagnification), large piscivorous wildlife are most susceptible to mercury poisoning. Symptoms of toxicity in birds include suppressed immune systems (Spalding et al., 1994), undevoloped embryos, and death (Scheuhammer et al., 2007). Mammals, such as minks and otters, can experience effects such as blindness, seizures, abnormal startle reflexes, and neurotoxicity leading to lethargy ataxia, limb paralysis, and death (Wolfe et al., 1998; Strom, 2008).
Toxic effects of Hg exposure to humans are known more extensively than those for other animal species. Since the Industrial Revolution, the first major outbreak of human Hg poisoning occurred in Minamata, Japan, in the 1950’s (WHO, 1976) and was caused by consumption of fish contaminated with MMHg; however, Hg poisoning also can occur via inhalation of Hg\(^0\) vapors, as observed in mine workers and “mad hatters” (Lindqvist et al., 1991; Levin, 2007). The term “mad hatters” was coined in the late 1830’s to describe men working in the felt hat industry (Levin, 2007). Hg was used in a process known as “carroting,” which used mercury nitrate to expedite the process of separating fur from the pelt (Levin, 2007). The workers exhibited symptoms such as drooling, twitching, hair loss, and the inability to think and talk coherently (Levin, 2007). Effects of MMHg exposure are similar and also include ataxia, constricted vision, tremors, and death (Mergler et al., 2007). Fetuses of women exposed to MMHg consuming fish during pregnancy are more susceptible to illness. Brain damaging symptoms, such as those seen with cerebral palsy, can lead to physical and mental deficiencies in the growing fetuses (WHO, 1976; Lindqvist et al., 1991; Mergler et al., 2007).

**MMHg in the Environment**

Most MMHg in the environment is thought to result from methylation reactions involving organic and inorganic complexes of Hg\(^{2+}\) (i.e., Hg(II)) that are mediated by sulfate-reducing bacteria (Compeau and Bartha, 1984; Gilmour et al., 1992), although iron-reducing bacteria also have been implicated (Kerin et al., 2006; Fleming et al.,
2006). The mechanism by which these anaerobic bacteria produce MMHg is understood poorly, but is known to be influenced by both activity of the microorganisms (King et al., 1999) and availability of Hg(II) for uptake and methylation (Hammerschmidt and Fitzgerald, 2004). Active MMHg production has been observed to occur in, for example, wetlands (Langer et al., 2001), forest soils (St. Louis et al., 2001; Hall and St. Louis, 2004), freshwater and marine sediments (King et al., 1999; Hammerschmidt et al., 2006; Hammerschmidt and Fitzgerald, 2004, 2006, Hammerschmidt and Fitzgerald, 2008), the water column of marine systems (Monperrus et al., 2007, Lamborg et al., 2008), and is hypothesized to occur in the atmosphere (Hammerschmidt et al., 2006).

*Hg in Foliage*

Studies near the Canadian Experimental Lakes Area (St. Louis et al., 2001) and Adirondacks (Demers et al., 2007) suggest that litterfall from the forest canopy is a major source of Hg to the forest floor. St. Louis and coworkers (2001) found that fluxes of MMHg in throughfall + litterfall to the boreal forest floor were about 2-fold greater than fluxes from wet atmospheric deposition alone. Moreover, Demers and others (2007) also found that fluxes of total Hg to the soil via litterfall were greater in deciduous forests, and throughfall fluxes were more important in coniferous forests. Results of these studies indicate that processes associated with terrestrial vegetation may affect loadings of Hg and MMHg to soils and receiving waters in some locations (Siegel et al., 1984; Gustin et al., 2000; Ericksen et al., 2003).
Sources of Hg(II) adsorbed to leaf surfaces include mainly wet (i.e., precipitation) and dry atmospheric fluxes. Dry deposition comprises Hg(II) associated with atmospheric particles (dust, aerosols), deposition of reactive gaseous mercury (gaseous Hg$_{2}^{2+}$, RGM; Lindberg et al., 2007), and may include Hg(II) resulting from oxidation of Hg$^{0}$ passing over leaf surfaces, which produce and emit chemical oxidants. The relative significance of dry versus wet atmospheric deposition of Hg(II) is unknown for vegetated surfaces (St. Louis et al., 2001), but mass-balance investigations suggest that dry depositional fluxes can range from 10–100% of wet deposition (Guentzel et al., 1998; St. Louis et al., 2001; Lamborg et al., 2002). Dry deposition of Hg can be affected by plant species, age and size of the leaves, and amount of moisture on the leaves (Rea et al., 2000). Hg species that are either wet or dry deposited to leaves are most likely rinsed off in throughfall, meaning that Hg in foliage most likely comes from soil or stomatal uptake (St. Louis et al., 2001). Stomatal uptake of Hg$^{0}$ is an important pathway of accumulation by leaves (Browne and Fang, 1978; Lindberg et al., 1979, Mosbaek et al., 1988; Bishop et al., 1998; Rea et al., 2001; St. Louis et al., 2001; Schwesig and Krebs, 2003; Hall and St. Louis, 2004; Millhollen et al., 2006; Graydon et al., 2008). Hg$^{0}$ can be oxidized to Hg(II) inside the substomatal spaces of the leaves, similar to oxidation reactions that may occur on leaf surfaces. A presumably less significant source of inorganic Hg to leaves is from xylem sap, which transports Hg from the soil, via the roots, to the leaves in the canopy (Beauford, 1977; Bishop et al., 1998; Millhollen et al., 2006; Schwesig and Krebs, 2003). The source of MMHg in foliage, however, is largely unknown.

Both Hg$^{0}$ and Hg(II) are potential substrates for formation of MMHg by either chemical or biological processes. Hg$^{0}$ can be transformed to MMHg by reaction with a
methyl carbonium ion donor, and Hg(II) can be methylated by reaction with a donor of methyl carbanion (Bertilsson and Neujahr, 1971). These types of methylating agents are associated commonly with plants. Vitamin B\textsubscript{12}, for example, is a prolific methylator of Hg(II) by transfer of methyl carbanion (Bertilsson and Neujahr, 1971). Acetic acid, coniferol, and para-hydroxybenzaldehyde also can methylate Hg(II) to MMHg (Falter, 1999). Coniferol is a chemical compound that gives coniferous trees their distinctive odor, while para-hydroxybenzaldehyde can be found in nitriolosides or vitamin B\textsubscript{17}, a common component of cereal millet plants. Acetic acid is a compound that is common on and within plants (Hopkins and Hüner, 2009) and may be the principal methylating agent for Hg(II) in the atmosphere (Hammerschmidt et al., 2006).

MMHg produced inside or on leaf surfaces can be transported to the soil by two pathways. One route is by throughfall, a process where precipitation rinses adsorbed MMHg from leaf surfaces (Rea et al 2000; Rea et al., 2001). However, throughfall appears to be a minor flux of MMHg to the forest floor relative to other sources (Hojdova et al., 2007; St. Louis et al, 2001). Litterfall is another pathway of MMHg introduction to underlying soils (St. Louis et al, 2001; Schwesig and Matzner, 2001; Schwesig and Krebs, 2003). Senescing leaves contain MMHg both internally and adsorbed to the surface, and litterfall is the major flux of MMHg to the forest floor (St. Louis et al., 2001). Both throughfall and litterfall are fluxes of MMHg to underlying soils and can influence loadings to nearby streams (Tsui et al., 2008). MMHg in soil also may be available for uptake into terrestrial food webs (Gnamuš et al., 2000).
II. Hypotheses

Relatively little is known about whether MMHg associated with leaves may result from in-vivo transformation of inorganic Hg species either on the leaf surface or within the leaf structure. The purpose of this study is to examine the hypothesis that MMHg in tree leaves is related to the mercury content of underlying soil. This hypothesis was tested by examining MMHg in leaves and soils associated with several tree genera in southwestern Ohio. This region is ideal because wet atmospheric Hg deposition is presumed to be relatively homogenous over the area (MDN, 2009), although soil Hg is expected to vary 10x based on variations of other heavy metal (Ritter and Rinefierd, 1983). In a comparison of three locations, Fleck and others (1999) found concentrations of total Hg in needles of red pines were unrelated to soil Hg and were greatest near an urban center. In contrast, Schweg and Krebs (2003) suggest transport of MMHg from the roots to canopy of trees as a major source. Results from the current investigation will reveal whether MMHg concentrations in leaves is related to that in soil, promoting the idea of soil-to-leaf mercury transport.

III. Methods

Sampling

Tree leaves and soil were sampled from 30 locations in southwestern Ohio ($n = 132$ trees; Figure 1). These locations were selected to span a geographic range around the Dayton metropolitan region and, presumably, differences in soil total Hg and MMHg
based on variation of other heavy metals (Ritter and Rineferd, 1983). Several tree genera were selected for sampling at each site. These include maple (genus *Acer*, *n* = 40), oak (genus *Quercus*, *n* = 28), elm (genus *Ulmus*, *n* = 12), sweetgum (genus *Liquidambar*, *n* = 7), poplar (genus *Liriodendron*, *n* = 7), buckeye (genus *Aesculus*, *n* = 9), mulberry (genus *Morus*, *n* = 10), pine (genus *Pinus*, *n* = 10), and spruce (genus *Picea*, *n* = 9). Each tree was considered individually because availability of tree genera varied among locations. For each tree, between five and 10 live leaves or about 50 spruce needles were sampled from a single branch and about 100 cm$^3$ of soil was collected from under the tree canopy within 1 m of the trunk. Leaves were sampled with gloved hands and transferred to plastic zip bags, and trunk diameter was measured at breast height. Soil was sampled with a stainless steel trawl by removing the upper 1–2 cm of loose debris and transferring soil from 2–6 cm depth (i.e., A Horizon) to an acid-cleaned specimen jar. Triplicate samples of soil were collected at multiple locations beneath about 15% of sampled trees to evaluate the degree of soil Hg variability. Soil and leaves were sampled in July 7$^{th}$ – 27$^{th}$, 2009. Leaves also were sampled in October 2009 from a subset of these trees to examine seasonal variation of Hg speciation. Soil and leaves were stored frozen (-20°C) until freeze drying, homogenization, and analysis.

**Determination of Hg**

Methods for measuring MMHg and total Hg in tree leaves were based on those of Hammerschmidt and Fitzgerald (2006c). Leaves were freeze dried, pulverized and homogenized inside plastic bags, and 0.1–0.2 g aliquots were digested with 7.0 mL of
4.57 M HNO$_3$ for 12 h in a covered water bath at 60 °C. MMHg in leaf digestates was measured with flow-injection gas chromatographic cold vapor atomic fluorescence spectrometry (CVAFS; Tseng et al., 2004) after aqueous phase ethylation (Bloom, 1989). The same digestates also were used for determination of HgT after oxidation with BrCl for 12 h. NH$_2$OH (12% wt:vol) was added to oxidized digestates prior to reduction with SnCl$_2$. HgT was determined by dual-Au amalgamation CVAFS (Fitzgerald and Gill, 1979; Bloom and Fitzgerald, 1988).

MMHg was distilled from soil (Horvat et al. 1993). Dried soil (~ 0.3–0.5 g) were weighed accurately into a 60-mL Teflon vials and slurried with 30 mL of reagent-grade water ( > 18 MΩ-cm), 0.2 mL of 20% KCl, and 0.4 mL each of 9 M H$_2$SO$_4$ and 1 M CuSO$_4$. MMHg was distilled from a hot block at ~150 °C. Soil distillates were analyzed by gas chromatographic CVAFS.

HgT in soil was determined with methods described by Fitzgerald et al. (2005). Freeze-dried aliquots (0.1–0.2 g) were weighed accurately into 50-mL Teflon bombs to which was added 5 mL of a 3:2 mixture of HNO$_3$/HCl. The bombs were sealed hermetically and heated for 5 min in a microwave. Digested samples were diluted with 25 mL of reagent-grade water and oxidized with 1 mL of BrCl for 12 h prior to addition of 0.5 mL NH$_2$OH solution. HgT in soil digestates was measured, after SnCl$_2$ reduction, by dual-Au amalgamation CVAFS (Fitzgerald and Gill, 1979; Bloom and Fitzgerald, 1988).

Organic content of soils was determined as loss-on-ignition (LOI; Heiri et al. 2001). Lyophilized soil samples (5–10g) were ignited at 550 °C for 1 h, with the mass
difference inferred to be the organic content.

Quality Assurance

Trace-metal clean techniques were used for sample collection, preparation, and analysis (Gill and Fitzgerald, 1985). All equipment was cleaned rigorously with acid and rinsed with reagent-grade water. Soil and leaves (± 0.001g) were measured using a balance calibrated with ASTM-Class 1 certified reference masses. Standard calibration curves for HgT and MMHg were made at the start of each analytical batch and internal standards were analyzed every 10–14 samples. Measurements of HgT in soil and leaves were calibrated versus known quantities of Hg⁰ and verified by comparison to measurements of an aqueous Hg²⁺ standard traceable to the U.S. National Institute of Standards and Technology (NIST). Average recovery of aqueous Hg²⁺ versus Hg⁰ was 101% (n = 95) during analysis of HgT. Sample MMHg was determined after calibration with a solution of CH₃HgCl that was standardized versus Hg⁰ and NIST-traceable Hg²⁺ solution.

Accuracy of Hg determinations was assessed by analysis of (1) certified reference materials (MESS-3 soil and TORT-2 lobster hepatopancreas, National Research Council of Canada), (2) procedural replicates, (3) recoveries of known additions, and (4) procedural blanks. HgT in MESS-3 soil averaged (± SD) 88 ± 9 ng/g, within the certified range of 82–100 ng/g. Measured concentrations of Hg in TORT-2 averaged (± 1 SD) 163 ± 14 ng/g for MMHg (n = 23; certified range, 139–165 ng/g) and 252 ± 21 ng/g for HgT.
Reproducibility of procedural replicates during HgT analysis averaged 5.4 relative percent difference (RPD; \( n = 29 \)) for soils, and 7.9 RPD \( (n = 27) \) for leaves. Precision of procedural replicates during MMHg analyses averaged 8.4 relative standard deviation (RSD; \( n = 60 \)) for soils, and 29 RPD \( (n = 16) \) for leaves. Relatively greater uncertainty of MMHg determinations in leaves can be attributed to their having very low concentrations (most <0.1 ng/g dry weight). Indeed, the average precision of replicate analyses of the same digestate was 28 RPD \( (n = 11) \) and comparable to the average procedural precision. Recoveries of known MMHg additions averaged 103\% (range, 81–137\%; \( n = 51 \)).

Precision of organic content determination in soil averaged 1.4\% RSD \( (n = 24) \). Estimated detection limits were as follows (dry-weight basis): 0.01 ng/g for MMHg and HgT in a 0.1-g aliquot of leaves; 2 ng/g for HgT in a 0.1-g sample of soil; 0.01 ng/g for MMHg in a 1-g aliquot of soil.

**Statistical analysis**

Results were analyzed with R statistical to examine if correlations existed between multiple factors: location, tree species, HgT in leaves, HgT in soil, MMHg in leaves, MMHg in soil, % LOI, and trunk diameter. One way analysis of variance tests were used to determine if there were tree species effects on MMHg and HgT in leaves when comparing one pair of tree species at a time. A univariate test of significance was first applied to the entire population. For MMHg and HgT in leaves, if the \( p \)-value was less than 0.05 respectively, then Tukey HSD (Honestly Significant Different)
test were applied. No statistically significant differences in tree species were observed if values for either test were greater than 0.05. The Tukey HSD test identified how significantly different values of MMHg and HgT in leaves were for each pair of tree species. One-way ANOVA also was applied to test for significant difference in percent MMHg in leaves and soil of all tree species.

IV. Results and Discussion

Hg in leaves varied among individual trees and tree genus groups. Tree species within a genus were grouped together (e.g. sugar, red, and silver maples were grouped together as maple trees for this study) because there were no statistical differences (\(p > 0.05\)) in leaf Hg concentrations among species. Among individual trees, MMHg in leaves ranged by a factor of 25× (range, 0.010–0.247 ng/g dry weight) and total Hg differed by 11× (range, 3.56–38.7 ng/g dry weight). Total Hg in leaves also differed significantly among tree genus, with spruce needles having the lowest levels and buckeye the greatest (Table 1; Appendix Figure 1a). Inter-genus differences of leaf MMHg (Table 1; Appendix Figure 1b) were not as pronounced as total Hg, which was due, in part, to the greater relative degree of variability among trees in each genus. Some of this variability can be attributed to analytical uncertainty at such low concentrations. Although mean levels of total Hg and MMHg in leaves differed 3–4× among tree genera, the fraction of total Hg as MMHg was relatively consistent (Figure 2).

Leaf Hg concentrations in southwest Ohio are less than those in similar species at other temperate locations in North America (Table 2). In general, HgT in Ohio tree
leaves was 2–3× less than those from Minnesota and the Experimental Lakes Area in Ontario, Canada. Ohio spruce, pine, and maple had about 10-fold less MMHg than comparable trees at the Experimental Lakes Area. Variations of foliar Hg levels among locations may reflect differences in either atmospheric or soil Hg conditions. However, the average fraction of total Hg as MMHg in Ohio tree leaves (mean, 0.4%; range, 0.04–2.3) is within the range of that for trees at the Experimental Lakes Area in Ontario (0.1–2%; St. Louis et al., 2001; Graydon et al., 2008). The small fraction of MMHg as total Hg is comparable to that observed in other primary producers, including river periphyton (1–12% MMHg; Bell and Scudder, 2007) and marine phytoplankton (3–10% MMHg; Fitzgerald et al., 2007). However, the fraction of total Hg as MMHg in leaves was much less than that in soil.

Total Hg and MMHg in soil varied widely among sampling locations. Total Hg ranged 7.0–373 ng/g dry weight and MMHg from 0.04 to 7.69 ng/g. Neither total Hg ($r = -0.02$, $p$-value = 0.5; Appendix Figure 2a) nor MMHg ($r = 0.05$, $p$-value = 0.9; Appendix Figure 2b) in soil was related to the organic content of the substrate. This was surprising because organic matter is often strong control on the distribution of both total Hg and MMHg in aquatic ecosystems (Hammerschmidt et al., 2004, 2008; Hammerschmidt and Fitzgerald, 2006b). In contrast, MMHg in soil appeared to be influenced by the concentration of total Hg (Figure 3). The average fraction of total Hg as MMHg in soils was 1.4% (range, 0.10–5.9 %MMHg). This ratio is similar to that other soil (Revis et al., 1990) and aquatic sediments throughout North America (Sullivan and Mason, 1998; Conaway et al., 2003).
MMHg in tree leaves was unrelated to concentrations of either MMHg or total Hg in soil from the A-horizon under the canopy of each (Figure 4). Similarly, total Hg in leaves was unrelated to total Hg in soil ($r = -0.05$, $p$-value = 0.4; Appendix Figure 3). These results are supported by the observation that, while total Hg and MMHg in leaves varied among tree genera (Table 1), there was no significant difference in the mean soil concentration of either MMHg (ANOVA $p$-value = 0.31; Appendix Figure 4) or total Hg (ANOVA $p$-value = 0.96; Appendix Figure 5) under the canopies of each tree genus.

Soil Hg speciation results are presumed to be representative of those throughout the A-horizon under each tree because total Hg varied by 7.2% RSD (range 2.7–20% RSD) and MMHg by 14% RSD (range, 4.3–29% RSD) among triplicate samples collected at from beneath each of 14 different trees. The absence of a relationship between Hg species in leaves and soil implies that root uptake from soil is not a major pathway of Hg accumulation in leaves. Prior studies have suggested that uptake from soil is not a dominant source of total Hg in leaves based on either observational correlation studies (Fleck et al., 1999), and as conducted here, or experimentally with added Hg isotopes (Schwesig and Krebs, 2003). Siwik and others (2010) also show root uptake is not the dominant source of total Hg to leaves by examining trends in wood, bark, soil, and leaf Hg. This is the first study to examine MMHg in leaves and soil, and just as with total Hg, there is no connection.

Hg speciation in maple leaves varied between summer and fall (Figure 5). Senescing leaves sampled in October had significantly greater levels of total Hg than those sampled from the same tree in July (paired t-test, $p$-value < 0.0001). Total Hg
increased, on average, about 42% in concentration over this three-month period. In contrast to total Hg, MMHg did not increase or decrease consistently from July to October among all sites (paired $t$-test, $p$-value = 0.36). Differences in the foliar behavior of total Hg and MMHg suggest that either total Hg and MMHg may have different dominant sources to the leaves or that there is a decoupling in the biogeochemistry of the two Hg species as the leaves senesce.

Mean, tree-genus specific concentrations of total Hg and MMHg in leaves were related inversely with average trunk diameter among the nine tree genera examined (Figure 6). This relationship implies that either larger trees or larger tree genera have lower levels of Hg in their leaves. A similar correlation was observed among individual oak trees sampled on the Wright State University campus (Figure 7), where total Hg and MMHg soil varied by 7.1% RSD and 40% RSD ($n = 9$), respectively. Such relationships might be expected if there were a finite source of either MMHg or total Hg to the trees, as a larger tree will have more leaves to dilute the contaminant concentration. However, I have found that soil does not appear to be the major source of either MMHg or total Hg in leaves (Figure 4). This suggests that the source of Hg in the leaves is the atmosphere.

Ionic forms of Hg can be wet and dry deposited to tree leaves and gaseous elemental Hg can be accumulated through stomata. Prior studies (Browne and Fang, 1978; Lindberg et al., 1979, Mosbaek et al., 1988; Bishop et al., 1998; Rea et al., 2001; St. Louis et al., 2001; Schwegess and Krebs, 2003; Hall and St. Louis, 2004; Millhollen et al., 2006; Graydon et al., 2008) have indicated that stomatal uptake of elemental Hg is a major source of Hg in foliage. In contrast, wet and dry deposition of Hg to leaf surfaces
does not appear to be a significant input, based on the similarity of wet deposition and throughfall fluxes measured in both temperate (St. Louis et al., 2001; Graydon et al., 2008) and subtropical locations (Guentzel et al., 1998). That is, if leaves were a sink for atmospherically deposited Hg, then throughfall fluxes should be less than wet deposition.

I found that total Hg in leaves was related inversely to trunk diameter (Figures 6 and 7). If uptake of gaseous elemental Hg were the major source to leaves, then these relationships would suggest that gas exchange of Hg\(^0\) decreases with tree size or some other variable correlated with trunk diameter. This is supported by inverse relationships between stomatal conductance and tree height (Schäfer et al., 2000) and stomatal diameter and tree age (Franich et al., 1977). Thus, larger trees have lower stomatal conductance and correspondingly reduced uptake of Hg\(^0\), resulting in relatively lower levels of Hg\(_T\) in the leaves. However, the source of MMHg in the leaves is less clear.

If the atmosphere were a direct source of MMHg to tree leaves, then this could occur either by wet deposition or accumulation of gaseous DMHg, analogous to Hg\(^0\). Just as with total Hg, atmospheric deposition does not appear to be a principal source (St. Louis et al., 2001; Graydon et al., 2008). Additionally, it is unknown if DMHg exists in the atmosphere, let alone at levels sufficient to support its accumulation and monodemethylation to MMHg in leaves. A key constraint on the source of MMHg in foliage is that it appears to be proportional to the source of Hg\(_T\) (Figure 2). Comparable correspondences between MMHg and Hg\(_T\) concentrations or loadings have been observed in multiple environments, including the atmosphere (Hammerschmidt et al., 2006), marine sediments (Hammerschmidt and Fitzgerald, 2004, 2006b; Fitzgerald et al.,
2007) and biota (Hammerschmidt and Fitzgerald, 2006a) and have been interpreted to suggest that the production of MMHg is limited by HgT availability. Hence, a plausible source of MMHg in foliage is that it is produced within the leaves from either Hg$^0$ or Hg(II) resulting from the oxidation of atmospheric Hg$^0$ inside the leaves.

The latter hypothesis is supported by the lack of relationship observed between MMHg and HgT in leaves between July and October. MMHg was proportional to HgT in leaves sampled in July (Figure 2); however, and while HgT continued to increase in concentration until senescence, MMHg was unchanged statistically (Figure 5). If atmospheric deposition or uptake of DMHg were a leading source of MMHg, then one would expect MMHg levels to increase with time, as they do for HgT, which is presumed to result from uptake of Hg$^0$.

Results of this research imply that HgT and MMHg in tree leaves result ultimately from uptake of Hg$^0$ in the atmosphere and transformation within the foliage. The mechanism by which MMHg is produced in leaves is unknown, but prior studies have suggested that chemicals associated with foliage have a potential for methylation of either Hg(II) or Hg$^0$, including, for example, vitamin B$_{12}$, coniferol, acetate, and para-hydroxybenzaldehyde (Bertilsson and Neujahr, 1971; Falter, 1999; Hammerschmidt et al., 2006). The source of Hg$^0$ entering the tree leaves may be from either soil emissions beneath the canopy, which would comprise a recycling of Hg between foliage and soil via litterfall and emission, or distant sources. If the Hg$^0$ were from distant sources, the litterfall flux of HgT and MMHg would represent a new source of Hg species to the forest floor and ecosystem.
V. Literature Cited


Table 1. Summary characteristics (±1 SE) of trees examined for Hg speciation in leaves

<table>
<thead>
<tr>
<th>Tree genus</th>
<th>n</th>
<th>Trunk diameter (cm)</th>
<th>Total Hg</th>
<th>MMHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spruce</td>
<td>9</td>
<td>122 ± 18</td>
<td>9.4 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.025 ± 0.004</td>
</tr>
<tr>
<td>Pine</td>
<td>10</td>
<td>113 ± 9</td>
<td>13 ± 1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.029 ± 0.005</td>
</tr>
<tr>
<td>Sweetgum</td>
<td>7</td>
<td>111 ± 23</td>
<td>13 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.043 ± 0.009</td>
</tr>
<tr>
<td>Oak</td>
<td>28</td>
<td>91 ± 17</td>
<td>13 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.071 ± 0.008</td>
</tr>
<tr>
<td>Poplar</td>
<td>7</td>
<td>112 ± 24</td>
<td>16 ± 1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.049 ± 0.015</td>
</tr>
<tr>
<td>Mulberry</td>
<td>10</td>
<td>125 ± 30</td>
<td>16 ± 1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.086 ± 0.023</td>
</tr>
<tr>
<td>Elm</td>
<td>12</td>
<td>45 ± 6</td>
<td>20 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.095 ± 0.027</td>
</tr>
<tr>
<td>Maple</td>
<td>40</td>
<td>69 ± 9</td>
<td>20 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.071 ± 0.007</td>
</tr>
<tr>
<td>Buckeye</td>
<td>9</td>
<td>42 ± 11</td>
<td>30 ± 2.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.090 ± 0.024</td>
</tr>
</tbody>
</table>

*One-way ANOVA was used to determine whether MMHg or HgT in leaves was different among tree species. For a given tree species, different symbols are significantly different (Tukey HSD post hoc test P< 0.001 for HgT). No statistical differences in MMHg were observed for between tree genera.
Table 2. Comparison of foliar Hg concentrations in Ohio with those at other North American locations.

<table>
<thead>
<tr>
<th>Tree genus</th>
<th>Total Hg (ng/g dry weight)</th>
<th>MMHg (ng/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>MN&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Spruce</td>
<td>9.4 ± 2.0</td>
<td>--</td>
</tr>
<tr>
<td>Pine</td>
<td>13 ± 1.0</td>
<td>7–30</td>
</tr>
<tr>
<td>Maple</td>
<td>20 ± 0.9</td>
<td>--</td>
</tr>
<tr>
<td>Oak</td>
<td>13 ± 0.5</td>
<td>--</td>
</tr>
</tbody>
</table>

<sup>a</sup> This study  
<sup>b</sup> Fleck et al., 1999  
<sup>c</sup> Tsui et al., 2008  
<sup>d</sup> St. Louis et al., 2001  
<sup>e</sup> Zhang et al., 1995  
<sup>f</sup> Graydon et al., 2008  
<sup>g</sup> Siwik et al., 2010
Figure 1. Locations in Montgomery and Greene Counties, Ohio, where soil and leaves were sampled for analysis of total mercury and monomethylmercury.
Figure 2. Relation between mean concentrations of MMHg and HgT in leaves among nine tree species. Error bars are ± 1 SE.
Figure 3. Correlation between MMHg and HgT in soil sampled from under the canopies of trees in this study. Sample circled in red was not included in linear regression analysis.
Figure 4. MMHg in tree leaves versus MMHg and total Hg (HgT) in soil under the canopy of each tree.
Figure 5. Seasonal variation of MMHg and total Hg (HgT) in maple leaves examined by repeat sampling.
Figure 6. Relation between mean concentrations of MMHg and total Hg (HgT) in leaves and average trunk diameter among nine tree genera. Error bars are ± SE.
Figure 7. MMHg in oak leaves versus trunk diameter of individual oak trees sampled from within a 100-m range at on the campus of Wright State University.
Appendix Figure 1. Inter-genus differences of MMHg and total Hg (HgT) in tree leaves.
Appendix Figure 2. MMHg and HgT versus organic content of soil sampled from under the canopies of trees in this study.
Appendix Figure 3. Relationship between total Hg (HgT) in tree leaves and soil under the canopy of each tree.
Appendix Figure 4. Variation of MMHg in soil under the canopies of nine tree genera examined in this study.
Appendix Figure 5. Variations of HgT in soil under the canopies of nine tree genera examined in this study.