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Potential sources of methylmercury in tree foliage

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ABSTRACT

Litterfall is a major source of mercury (Hg) and toxic methylmercury (MeHg) to forest soils and influences exposures of wildlife in terrestrial and aquatic ecosystems. However, the origin of MeHg associated with tree foliage is largely unknown. We tested the hypothesis that leaf MeHg is influenced by root uptake and thereby related to MeHg levels in soils. Concentrations of MeHg and total Hg in deciduous and coniferous foliage were unrelated to those in soil at 30 urban and rural forested locations in southwest Ohio. In contrast, tree genera and trunk diameter were significant variables influencing Hg in leaves. The fraction of total Hg as MeHg averaged 0.4% and did not differ among tree genera. Given that uptake of atmospheric Hg^0 appears to be the dominant source of total Hg in foliage, we infer that MeHg is formed by in vivo transformation of Hg in proportion to the amount accumulated.

1. Introduction

Litterfall is a major source of mercury (Hg) and toxic methylmercury (MeHg) to forest soils (Munthe et al., 1995; St. Louis et al., 2001; Rea et al., 2002; Graydon et al., 2008) and influences exposures of wildlife in terrestrial (Rimmer et al., 2005) and aquatic ecosystems (Tsui et al., 2008). Inorganic forms of Hg associated with leaves can be derived from both wet and dry atmospheric deposition (Guentzel et al., 1998; St. Louis et al., 2001) as well as stomatal uptake of Hg^0 – the dominant pathway of accumulation (Browne and Fang, 1978; Mosbaek et al., 1988; Rea et al., 2001, 2002; Ericksen et al., 2003; Graydon et al., 2008; Bushey et al., 2008). A less significant source to foliage is from xylem sap (Beauford et al., 1977; Bishop et al., 1998; Schwesig and Krebs, 2003; Ericksen et al., 2003), which can translocate Hg species from soil via roots. In litterfall, Hg derived from the atmosphere is considered a new input to forest soils, whereas that from soil is considered to be recycled within the forest ecosystem (St. Louis et al., 2001).

Importantly, the source of MeHg in tree leaves and litterfall is largely unknown. Live and senescing leaves contain MeHg (St. Louis et al., 2001; Schwesig and Matzner, 2001; Schwesig and Krebs, 2003; Ericksen et al., 2003). Potential sources of MeHg in foliage include translocation from roots (Bishop et al., 1998; Schwesig and Krebs, 2003), wet atmospheric deposition, uptake and demethylation of gaseous dimethylmercury (DMHg), and, potentially, production in/on the leaves from chemical and biological methylation of inorganic Hg species. Both Hg^0 and Hg(II) are substrates for formation of MeHg. Hg^0 can be transformed to MeHg by reaction with a methyl carbonium ion, and Hg(II) can be methylated by donors of methyl carbanions (Bertilsson and Neujahr, 1971). These types of Hg methylating agents are associated commonly with plant foliage, including, for example, acetate, coniferol, and para-hydroxybenzaldehyde (Falter, 1999).

Here, we investigate the source of MeHg in foliage of deciduous and coniferous trees in southwest Ohio. Based on the work of others (Bishop et al., 1998; Schwesig and Krebs, 2003), we posited that uptake from soil is an important source of MeHg in tree leaves. This hypothesis was examined by comparing MeHg levels in tree foliage to those in associated A-horizon soils among multiple tree genera and locations. We found that MeHg concentrations in foliage were unrelated to those in soil, but proportional to total Hg levels in leaves among nine genera of trees. We interpret these results to suggest that MeHg in tree foliage is derived from in vivo methylation of Hg accumulated from the atmosphere, which implies that MeHg in litterfall is a new source and not recycled from soil.

2. Methods

2.1. Sampling leaves and soil

Tree leaves and soil were sampled from 30 locations in southwest Ohio ($n = 132$ trees; Fig. 1). The study area (1100 km^2) is in the Till Plains region of the Central Lowland Province of Ohio, with the geology comprised mainly of glacial moraine deposits and Ordovician-Silurian age calcareous shale and limestone (Schiefer, 2002). There are no large Hg-emitting facilities in this area, and it appears to have

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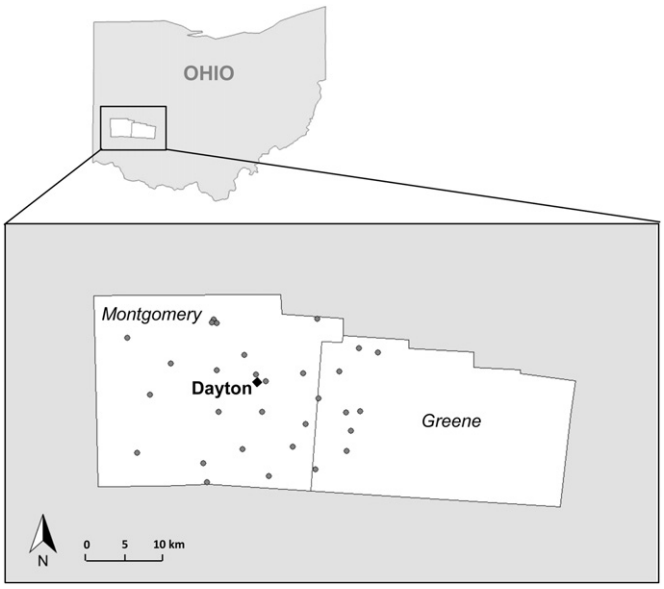

Fig. 1. Foliage and soil sampling locations in Montgomery and Greene Counties, Ohio, USA.

spatially homogeneous atmospheric fluxes of Hg(II) (Naik and Hammerschmidt, 2011) and, by extension, atmospheric levels of Hg^0 and fluxes of MeHg. Sampling locations were mostly in forested public parks (urban and rural) around the Dayton metropolitan region that were selected to have inferred differences of soil total Hg and MeHg concentrations, based on the spatial variability of other heavy metals (Ritter and Rinefierd, 1983). Nine tree genera were targeted for sampling at each site. These included maple (*Acer* sp., $n = 40$), oak (*Quercus* sp., $n = 28$), elm (*Ulmus* sp., $n = 12$), sweetgum (*Liquidambar* sp., $n = 7$), poplar (*Liriodendron* sp., $n = 7$), buckeye (*Aesculus* sp., $n = 9$), mulberry (*Morus* sp., $n = 10$), pine (*Pinus* sp., $n = 10$), and spruce (*Picea* sp., $n = 9$). Each genus was not represented at all sampling locations and, at some sites, multiple species of the same genus were sampled.

From each tree, between five and 10 live leaves, or about 50 spruce needles, were sampled from a single branch 1–2 m above ground. Leaves were sampled with gloved hands and transferred to food-grade plastic zip bags. Trunk diameter was measured at breast height as a proxy for tree size and age. About 100 cm^3 of soil was sampled within 1 m of the trunk of each tree with a stainless steel trawl by removing the upper 1–2 cm of loose debris and transferring soil from 2 to 6 cm depth (i.e., A-horizon) to an acid-cleaned jar (Fitzgerald et al., 2005). Triplicate samples of soil were collected beneath about 10% of sampled trees to evaluate variability of soil Hg. Soil and foliage were sampled between July 7–27, 2009. Leaves also were sampled in October 2009 from a subset of 14 maples to examine seasonal variation of Hg speciation. Soil and leaves were stored frozen (−20 °C) until freeze drying, homogenization, and Hg analysis.

2.2. Determination of Hg

MeHg and total Hg in foliage were determined after digestion with dilute acid (Hammerschmidt and Fitzgerald, 2006a). Leaves were freeze dried, pulverized and homogenized inside plastic zip bags, and 0.1–0.2 g subsamples were digested with 7.0 mL of 4.57 M HNO_3 for 12 h in a covered water bath at 60 °C. MeHg in leaf digestates was quantified by 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 total Hg after oxidation with BrCl (Bloom and Crecelius, 1983) for 12 h. NH_2OH (12% wt:vol) was added to oxidized digestates prior to reduction with $SnCl_2$. Total Hg was quantified by dual-Au amalgamation CVAFS (Fitzgerald and Gill, 1979; Bloom and Fitzgerald, 1988).

MeHg was extracted from soil by aqueous distillation (Horvat et al., 1993). Subsamples of freeze-dried soil (0.3–0.5 g, not size fractionated) were weighed accurately into 60-mL Teflon vials and slurried with 30 mL of reagent-grade water (resistivity, ≥18 MΩ-cm), 0.2 mL of 20% (wt:vol) KCl, and 0.4 mL each of 9 M H_2SO_4 and 1 M $CuSO_4$. MeHg was distilled from soils at 150 °C and analyzed by gas-chromatographic CVAFS.

Total Hg was extracted from soil with concentrated HNO_3 and HCl (Fitzgerald et al., 2005). Freeze-dried subsamples of bulk soil (0.1–0.2 g) were weighed accurately into 50-mL Teflon bombs to which were added 3 mL of HNO_3 and 2 mL of HCl. Bombs were sealed hermetically and irradiated intermittently for 5 min in a 1000-W microwave oven. Digestates were diluted with 25 mL of reagent-grade water and oxidized with 1 mL of BrCl solution for 12 h prior to addition of 0.5 mL of NH_2OH solution. Total Hg in soil digestates was measured, after $SnCl_2$ reduction, by dual-Au amalgamation CVAFS.

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

2.3. 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. Masses of soil and leaf samples were measured (±0.001 g) with a balance calibrated with ASTM Class 1 reference weights before each use. Measurements of total Hg in soil and foliage were calibrated versus known amounts of Hg^0 and verified by comparison to measurements of an aqueous Hg^{2+} standard traceable to the U.S. National Institute of Standards and Technology (NIST). Mean recovery of aqueous Hg^{2+} versus Hg^0 was 101% ($n = 95$) during analyses of total Hg. Sample MeHg was determined after calibration with a solution of CH_3HgCl that was standardized versus Hg^0 and a NIST-traceable Hg^{2+} solution. Standard calibration curves for total Hg and MeHg were prepared at the start of each analytical batch and verified with internal standards after analysis of every 10–14 samples.

Accuracy of Hg determinations was assessed by analysis of (1) certified reference materials (MESS-3 soil and TORT-2 lobster hepatopancreas) from the National Research Council of Canada, (2) procedural replicates, (3) recoveries of known additions, and (4) procedural blanks. Measured total Hg in MESS-3 averaged (±SD, $n = 10$) 88 ± 9 ng/g, within the certified range of 82–100 ng/g. Measured concentrations in TORT-2 averaged 163 ± 14 ng/g for MeHg ($n = 23$; certified range = 139–165 ng/g) and 252 ± 21 ng/g for total Hg ($n = 6$; certified range = 210–330 ng/g). Reproducibility between procedural duplicates during total Hg analysis averaged 5.4% relative difference for soils ($n = 29$), and 7.9% relative difference for leaves ($n = 27$). Precision among procedural replicates during MeHg analyses averaged 8.4% relative standard deviation for soils ($n = 60$), and 29% relative difference for leaves ($n = 16$). Greater imprecision of MeHg determinations in foliage compared to soils can be attributed to leaves having very low concentrations (most <0.1 ng/g dry weight). Indeed, the mean precision of replicate analyses of the same digestate was 28% relative difference ($n = 11$) and comparable to the average precision between separate digestates of the same parent sample (i.e., 29% relative difference). Recoveries of known MeHg additions from soils and leaves averaged 103% (range = 81–137%; $n = 51$). Estimated detection limits (sample dry-weight basis) were about 0.01 ng/g for MeHg and total Hg in a 0.1-g sample of foliage; 2 ng/g for total Hg in a 0.1-g sample of soil; 0.01 ng/g for MeHg in a 1-g sample of soil. Precision of organic content determinations in soil averaged 1.4% relative standard deviation ($n = 24$).

2.4. Statistical analyses

Relationships between different paired-variables (i.e., MeHg in leaves and soil, total Hg in leaves and soil, % LOI, and trunk diameter) were examined by linear regression analyses. For MeHg, total Hg, and MeHg:total Hg ratios measured in foliage and soil, differences among tree genera were examined with one-way analyses of variance (one-way ANOVA). Tukey *post hoc* tests were used in cases of significant ANOVA differences to determine which tree genera differed. Total Hg and MeHg concentrations in maple leaves sampled from the same trees in July and October were compared by paired *t*-test. When necessary, data were square root-transformed before statistical analysis to meet the assumptions of homoscedasticity and normality. Significance for all statistical analyses was accepted at $p < 0.05$. All statistical analyses were performed with R software (http://www.r-project.org/).

3. Results and discussion

Hg in foliage varied among individual trees and genera. Among individual trees, MeHg in leaves differed by a factor of 25× (range, 0.01–0.25 ng/g dry weight) and total Hg by 11× (range, 3.56–38.7 ng/g). Total Hg in foliage also varied significantly among tree genera, with spruce needles having the lowest levels and buckeye the greatest (one-way ANOVA, $p < 0.001$; Tukey *post hoc* tests in Table 1). In contrast, foliar MeHg concentrations did not differ significantly among tree genera (one-way ANOVA, $p > 0.05$; Table 1), which may be due, in part, to the relatively greater degree of MeHg variability among trees within each genus. As noted, some of the variability of foliar MeHg levels can be attributed to analytical imprecision at such low concentrations.

Foliar Hg concentrations in southwest Ohio are less than those measured in the same genera of trees at other temperate locations