ASSESSING THE SOURCE OF MERCURY IN FOLIAR TISSUE OF QUAKING ASPEN

TOBY F. FRESCHOLTZ,† MAE S. GUSTIN,*† DAVID E. SCHORRAN,‡ and GEORGE C.J. FERNANDEZ§
†Department of Environmental and Resource Sciences, University of Nevada–Reno, ERS Department, MS 370, Reno, Nevada 89557, USA
‡Desert Research Institute, 2215 Raggio Parkway, Reno, Nevada 89512, USA
§Department of Applied Economics and Statistics, University of Nevada–Reno, Reno, Nevada 89557, USA

(Received 9 July 2002; Accepted 15 January 2003)

Abstract—Foliar accumulation of mercury has been demonstrated to occur as plants leaf out, yet the primary source of this mercury is not known. Using closed-system growth chambers, uptake of mercury by quaking aspen (Populus tremuloides) foliage was measured over time as a function of soil mercury concentrations (0.01, 6.2, and 25.6 μg/g) and atmospheric mercury exposure concentrations (1.4, 14.9, and 68.5 ng/m³). Foliar mercury concentrations increased as a function of time for all exposures. Twice during the experiment, leaf washes were analyzed for mercury to assess surface deposition, and little mercury was removed (0.02–0.04 ng/m²), suggesting that direct deposition to the leaf surface was not significant during this experiment. At the end of the four-month experiment, whole-plant mercury concentrations were determined. It was found that whereas mercury in the atmosphere primarily influenced foliar uptake, root concentrations were related to the soil mercury concentration. The implication of this study is that litterfall may serve as a pathway for new, atmospherically derived mercury to be deposited to forest soils. This has significant implications for watershed management of ecosystems where mercury is of concern.

Keywords—Mercury Quaking aspen Foliar uptake Bioaccumulation

INTRODUCTION

Mercury (Hg) is designated as a global pollutant because of the presence of volatile phases that are transported across regional and global boundaries. Emission to the atmosphere occurs from both natural and anthropogenic sources and primarily involves the elemental form (Hg⁰) [1]. Recent work has contributed significantly to our understanding of emission and deposition of Hg to and from terrestrial systems [2–5], although much remains unknown about its biogeochemical cycling [1].

Studies of single plants have suggested that plants accumulate Hg via root uptake [2,6–8] and emit Hg from foliar surfaces [4,9–11]. However, to our knowledge, studies have not been conducted that differentiate between root uptake versus atmospheric deposition as the primary source of Hg in foliar tissue. Earlier field studies also did not demonstrate whether the Hg in foliage was new Hg being input into the ecosystem [12,13].

Mercury that is assimilated both in and on leaves may be a pathway for atmospheric Hg to be transferred to forest soils [12,14]. In a study of atmospheric deposition in the Lake Champlain watershed (North America), Rea et al. [14] found significant Hg contributions to forest soils via throughfall and litterfall, with the contribution of litterfall being the greatest.

Plants can transfer Hg from the soil to the atmosphere [9,10]. Hanson et al. [9] suggested that plant uptake and emission were dependent on the atmospheric Hg⁰ concentration. They demonstrated that at air concentrations between 0.5 to 1.5 ng/m³, emission from the foliage of hardwood species occurred. At concentrations between 50 and 68.5 ng/m³, deposition to foliage occurred. However, at concentrations between 9 and 20 ng/m³, little net exchange of Hg⁰ between the atmosphere and foliage was observed. Hanson et al. deemed this to be the compensation point.

The goals of this study were to quantify the significance of root versus atmospheric uptake as the primary source of Hg in vegetation and to assess Hg accumulation in foliage as a function of time as well as air and soil exposure concentrations using a deciduous plant species. A deciduous species was chosen because the annual litterfall of such species provides a distinct source of Hg input to the forest floor.

MATERIALS AND METHODS

Quaking aspen (Populus tremuloides) were purchased commercially as one-year-old, unbranched tree shoots, or whips. Ninety trees were used in the experiment, 30 of which were potted in one of three soil Hg concentrations (low, 0.01 ± 0.001 μg/g; intermediate, 6.2 ± 0.9 μg/g; and high, 25.6 ± 2.1 μg/g). The soil consisted of a subsoil derived from decomposed granite, which was purchased from a local company. To achieve the intermediate- and high-Hg concentrations, the soil was amended with contaminated mill tailings from the Bessels Mill site (500 μg/g) from the Carson River Superfund Site (NV, USA), where elemental Hg was amended to ore for removal of gold and silver [15]. The soils were digested in aqua regia, and digestates were analyzed for total Hg by atomic absorption spectrometry using a Varian® Spectra AA 220 (Varian, Walnut Creek, CA, USA) [16].

Plant exposures were conducted within six environmentally controlled, closed-system growth chambers, or ecopods. These were located in the Frits Went Laboratory at the Desert Research Institute (Reno, NV, USA). Each ecopod had dimensions of 1.14 × 0.99 × 1.48 m and was enclosed on all sides by glass walls, with one being a glass door. The pods were located within a naturally lit greenhouse. Air temperatures in the pods were maintained at 26.1 ± 0.3°C during daytime hours and 18.1 ± 0.2°C during nighttime hours. The temperature set...
Eighteen of the pots (six pots per soil concentration) were equipped with time-domain reflectometry rods to measure the soil moisture content. Because all soil weights were similar, it was assumed that the soil moistures of these 18 pots were representative of the soil moisture of all 90 trees. At the initiation of the experiment, each pot was watered by weight to approximately 14% moisture; this was done to ensure that the plants would have sufficient moisture to avoid water stress. This concentration of moisture also reduced the risk of over-watering the plant pots, which had no drainage. During the experiment, soil moisture was measured three times per week. When soil moisture content reached 11% or less, the trees were watered to adjust the soil moisture to 14%. Plants were watered with tap water (Hg concentration, 3.26 ± 0.06 ng/L; n = 6).

The headspace air above the soil in the plant pots was isolated from the atmosphere in the ecopods by the application of airtight lids. The lids, consisting of two Plexiglas® plates with a hole in the center for the tree stem, were fitted on pot tops and sealed with silicone sealant. A polyethylene foam collar around the tree stem plugged the hole in the center of the lid. Each lid had an inlet and outlet port that allowed for ventilation of soil headspace air using greenhouse air; each lid also had a port for watering. A pump was used to push greenhouse air through a polyvinyl chloride trap filled with activated charcoal to remove Hg into a polyvinyl chloride plenum with headspace inlet ports for 30 pots. With this system, plant headspace was flushed continuously through an outlet port tube that carried the air outside the ecopod.

Throughout the experiment, newly emerging foliage was aged and tagged every two weeks. The oldest foliage was on the bottommost part of the branch/stem, and the youngest foliage was at the end of the branch/stem. Also, the health of each plant was assessed every two weeks by measuring its height, number of leaves, leaf color, and general appearance.

At one week, foliage was harvested from the ambient air pods to serve as initial controls for the experiment. One to two leaves were sampled from 18 of the 30 trees in the control pods (three plants from each soil group). Following this, the oldest leaves were collected every three weeks throughout the experiment from all 90 trees. Clean-handling protocols were applied during a harvest; leaves were removed using a clean razor blade and were placed on clean chemical wipes that were labeled with the pot number. The petioles were removed from the leaf, the leaves weighed, and the length and width measured. Total leaf area was calculated based on an established allometric relationship between leaf dimensions and leaf area

<table>
<thead>
<tr>
<th>1.4 ng Hg/m³ air</th>
<th>14.9 ng Hg/m³ air</th>
<th>68.5 ng Hg/m³ air</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 µg/g soil</td>
<td>0.62 µg/g soil</td>
<td>25.6 µg/g soil</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic showing the experimental design of six ecopods with 15 quaking aspen planted in one of three soil exposures in each ecopod. Ovals within each ecopod represent pots, each of which contained a single quaking aspen tree.

Point was adjusted based on the ambient light as measured with a photon-flux sensor (Li-COR, Lincoln, NE, USA). To ensure consistent lighting of each ecopod for 14.5 h/d, four 150-W, full-spectrum light bulbs on timers were arranged above the pods. In the pods, CO₂ concentrations were monitored using a Li-COR 6252 infrared CO₂ analyzer and were maintained at ambient concentrations (398.5 ± 153.5 µmol/mol). In addition, relative humidity (General Eastern RH-2, Plainville, CT, USA) and light (Hamamatsu G1118 photodiodes, Bridgewater, NJ, USA) were measured continuously.

Air Hg concentrations in two replicate pods were maintained at 1.4 ± 0.4 ng/m³, which is within the range of reported ambient background concentrations (0.5–2 ng/m³) [17]. To achieve this, greenhouse air was circulated through an internal scrubbing unit that consisted of 13 filters containing activated charcoal.

Two ecopods were maintained at an air Hg concentration of 14.9 ± 1.7 ng/m³ and two at 68.5 ± 4.5 ng/m³. Gaseous Hg added to these pods was generated using elemental Hg permeation tubes (VICI Metronics, Poulsbo, WA, USA) in glass housing with air-intake and -outlet ports that were submerged in constant-temperature (35°C) water baths. Air entering the glass housing was scrubbed of Hg, and flow was controlled using mass flow controllers to ensure a constant output to the ecopods. Bath temperature, water concentrations, and airflows were monitored weekly. The Hg concentration in the air of each ecopod was measured weekly using a Tekran® 2357A-mercury analyzer (Toronto, ON, Canada). This instrument samples air and traps Hg onto a cartridge containing gold adsorbent. The amalgamated Hg is desorbed and detected using cold-vapor atomic fluorescence spectrometry [18]. The system sampled two pods for 10 min each per hour. The sample lines were moved to sample different ecopod pairs approximately every 2 d.

Within each ecopod, 15 quaking aspen were grown, with five in each of the soil exposure concentrations (Fig. 1). The aspen were potted in polyvinyl chloride pipe (diameter, 0.15 m; length, 0.44 m) that was capped at the base. All pots were weighed empty and then with soil and a one-year-old aspen whip. Soil weights were adjusted so that they were similar (8.89 ± 0.05 kg). This was done so that approximately 14% (w/w) soil moisture could be initially established using pot weight.

Harvested leaves were lyophilized for 48 h in a freeze-drier...
(Virtis Benchtop 3L, Gardiner, NY, USA) and stored at −30°C before analysis. Mercury content in leaves was determined using a Milestone® (Monroe, CT, USA) direct mercury analyzer 80 atomic absorption spectrophotometer [19]. National Institute of Standards and Technology (Gaithersburg, MD, USA) number 1515 apple leaves (Hg concentration, 0.44 ± 0.004 µg/g) and number 268.59 San Joaquin soil (Hg concentration, 1.4 ± 0.08 µg/g) standards were used to confirm instrument calibration. Acceptable error on standards was less than 5%. Triplicate analysis of leaves showed minimal sample variance (n = 42, coefficient of variance = 3.6%).

At the end of the 16-week experiment, a plant mass balance of Hg was done using 36 trees (two trees from each soil concentration within each ecopod). The Hg concentration and the mass of leaves, stems, and roots were determined for each plant. Root samples were carefully washed in 18.2-MΩ/cm deionized water to ensure that all root samples were free of soil particles. The total leaf area for each of the trees was measured.

The experiment was a split-plot design with five triweekly repeated measures. Air Hg concentration served as the main plot in the design, and soil Hg exposure concentration served as the split plot. The main plot (air treatment) had two replications (ecopods). The mean response for air Hg–soil Hg treatment combination (average of five plants/treatment) was used in the analysis to avoid problems with subsamples.

Statistical analysis entailed the use of PROC MIXED in SAS® Version 8.2 [20]. Statistical significance was tested based on α = 0.05. Correlation among repeated measurements was modeled by different correlation structures, and AR(1) or first-order autoregressive correlation structures gave the best fit based on the Akaike information criterion statistic. Relative importance of air concentration versus soil concentration was determined by slicing the interaction and then testing the air-concentration effects versus the soil-concentration effects using the F values.

RESULTS AND DISCUSSION

Plant exposure to Hg in air and/or soil at concentrations of as much as 68.5 ng/m³ (air) and 25.6 µg/g (soil) for 16 weeks did not have any observable negative physiological effects. Plant height and leaf production did not differ significantly for plants grown in high-Hg concentrations in soil and air versus those grown in Hg-free soil and ambient air (Fig. 2). Additionally, no observable changes in leaf color or toxicity damage was noted in plants growing in Hg-contaminated mediums (n = 10). It is known that mill tailings used to amend Hg-contaminated soils contain trace amounts of zinc, sulfur, and iron, among other essential plant nutrients. We do acknowledge that the presence of these micronutrients may have had some effect on Hg uptake by plants, but this was not measured in the present experiment.

Leaf rinses that were analyzed for Hg did not show significant leaf-surface deposition occurring within the ecopods. Differences in leaf-wash concentrations for plants exposed to high atmospheric Hg concentrations versus ambient air Hg concentrations were not statistically significant (0.04 ± 0.11 vs 0.02 ± 0.06 ng/m², n = 6, p = 0.34). Maserti and Ferrara [21] found that the Hg concentrations of washed and of unwashed leaves were similar. They suggested that this resulted from Hg uptake occurring primarily via stomatal pores or by passive absorption processes.

Figure 3 shows uptake in foliage over time as a function of both soil and air Hg concentrations. In general, foliar Hg concentration increased with air Hg concentration. We found no significant differences in foliar Hg concentration between consecutive sampling times for most exposures at low and intermediate concentrations of Hg in soil. However, for plants grown in an air Hg concentration of 68.5 ng/m³, all but one consecutive time-step exhibited higher concentrations of foliar Hg regardless of the soil exposure concentration. Table 1 compares leaf Hg concentrations measured at 12 weeks. It also examines leaf Hg concentration as a function of soil Hg concentration for the same air exposure concentration. In eight of the nine comparisons, final leaf Hg concentrations were significantly different, suggesting that soil concentration can influence leaf Hg concentration regardless of air Hg concentration.

Local deposition velocities (cm/s) calculated using foliar Hg concentrations at 12 weeks were not significantly different for air Hg concentrations of 14.9 and 68.5 ng/m³ at any soil exposure concentration, and these velocities ranged from 0.002
to 0.039 and from 0.002 to 0.017 cm/s, respectively. In ambient air for all soil Hg concentrations, local deposition velocities ranged from 0.007 to 0.224 cm/s. Lindberg et al. [22] reported deposition velocities in the range of 0.006 to 0.12 cm/s for field studies in a deciduous forest.

Table 2 illustrates that air Hg concentration was the most significant contributor to Hg in quaking aspen foliage based on tests of effect slices. This test compares the effect of all air concentrations on foliar Hg concentrations at each soil exposure concentration, and vice versa. The influence of air Hg concentration on the amount of Hg in foliar tissue as a function of soil Hg exposure is shown in lines one through three of Table 2, and the effect of soil Hg concentration on tissue amount as a function of air exposure is shown in lines four through six. The magnitude of the $F$ value reflects the significance of the factor being tested. The large $F$ values in lines one through three indicate that the effect of atmospheric Hg concentration on foliar Hg accumulation was much greater than the effect of soil Hg concentration (lines 4–6). This suggests that air Hg concentration was the primary factor controlling foliar Hg concentrations. The data also indicate that soil Hg concentration has a greater influence on foliar Hg concentration when the air Hg concentration is lower. Plants may move Hg from the soil to the atmosphere, and this is thought to occur via the transpiration stream [9–11]. Mercury transported to the leaf from the soil could accumulate in the leaf, as could Hg entering the stomata or being absorbed passively to the plant surface from the atmosphere. The experimental design that we used in this study did not allow us to distinguish Hg from these two sources. However, the foliar Hg data suggest that both soil and air concentrations influence foliar concentrations and that air Hg concentrations are more significant than soil Hg concentrations.

Mercury concentrations in root, stem, and leaf parts were measured at the end of the experiment ($t = 16$ weeks). Root Hg concentrations increased significantly with increases in soil Hg concentration for all air Hg concentrations that we studied (Fig. 4). Concentrations of Hg in the roots did not vary much in response to differences in Hg concentrations in the air (Fig. 4). This outcome indicates that Hg in the roots was derived primarily from the soil. This is consistent with the findings of Cocking et al. [7], who measured Hg in tissues of Asclepias, a vascular plant species, grown in soils with Hg concentrations of 10 to 20 µg/g. Those authors found that root-tissue concentration correlated strongly with soil Hg concentration. In the plant stem, air Hg concentrations of 68.5 ng/m³ significantly influenced stem Hg concentration; however, the Hg concentration in the soil did not. In a vegetation-sampling study at an abandoned Hg mine site in Italy, Bargagli et al. [23] compared Hg concentration in branch wood versus needles of Cedrus atlantica. They found that whereas Hg accumulated in the needles over time, it did not accumulate in the branch wood. Soil Hg concentrations at the sampling site were reported to be 43.1 ± 9.3 µg/g; atmospheric Hg concentrations in the area were not measured. Bargagli et al. suggested a lack of transfer of Hg from needles or that branch wood is involved in transfer processes and may have fewer Hg accumulation sites. Data from Godbold and Hüttermann [8] suggested that tree seedlings selectively exclude Hg from the stem during root uptake. Those authors proposed that although significant concentrations of Hg may occur in the roots, very little translocation to the stem occurs, because in the root, Hg binds tightly to cell walls.

Table 2. Test of effect slices results for the effect of individual soil Hg concentrations against the effect of all air Hg concentrations, and vice versa

<table>
<thead>
<tr>
<th>Hg exposure level in soil</th>
<th>Conc. of Hg in air (ng/m³)</th>
<th>Denominator df</th>
<th>F ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>—</td>
<td>4.41</td>
<td>209.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Intermediate</td>
<td>—</td>
<td>4.41</td>
<td>221.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>High</td>
<td>—</td>
<td>4.17</td>
<td>143.0</td>
<td>0.0001</td>
</tr>
<tr>
<td>—</td>
<td>1.4</td>
<td>11.4</td>
<td>45.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>—</td>
<td>15</td>
<td>11.2</td>
<td>53.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>—</td>
<td>68.5</td>
<td>11.4</td>
<td>13.4</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*The numerator degrees of freedom (df) was 2, and the denominator (error) df is listed. The denominator df was computed with the Satterthwaite adjustment in the SAS MIXED® model procedure (Cary, NC, USA).
ambient air and air Hg concentration of 14.9 ng/m³. In both our findings, Hg in the soil significantly influences Hg concentrations in foliar tissue (Fig. 4 and Tables 1 and 2). In both our study, it appears that leaves accumulate atmospheric Hg as a function of the air concentration. Thus, the leaves can function as a pathway for moving atmospheric Hg to the forest floor. This result indicates that litterfall from deciduous forest canopies can serve as a source of new Hg to ecosystems. Thus, forests may act as sinks for atmospheric elemental Hg, thereby affecting the biogeochemical cycling of Hg. This information is also critical for watershed management decisions regarding Hg-impacted ecosystems. Häkanson et al. [26] and Munthe et al. [27] suggested that Hg input to forest soils is an important source of Hg to surface waters, even though only a small fraction (0.01–0.04%) of the catchment store of Hg and methylmercury may be released annually. Based on our data, we suggest that atmospheric Hg assimilated in foliage is a potentially significant source of new Hg for forested catchments.

These data have important implications for the transport and fate of Hg at the level of forest ecosystems. Hanson et al. [9] showed that foliar surfaces were dynamic exchange surfaces that, depending on atmospheric Hg concentrations, may function as a source or as a sink. Plants transmit Hg from the soil to the air via the transpirational stream [9–11], but from our study, it appears that leaves accumulate atmospheric Hg as a function of the air concentration. Thus, the leaves can function as a pathway for moving atmospheric Hg to the forest floor. This result indicates that litterfall from deciduous forest canopies can serve as a source of new Hg to ecosystems. Thus, forests may act as sinks for atmospheric elemental Hg, thereby affecting the biogeochemical cycling of Hg. This information is also critical for watershed management decisions regarding Hg-impacted ecosystems. Häkanson et al. [26] and Munthe et al. [27] suggested that Hg input to forest soils is an important source of Hg to surface waters, even though only a small fraction (0.01–0.04%) of the catchment store of Hg and methylmercury may be released annually. Based on our data, we suggest that atmospheric Hg assimilated in foliage is a potentially significant source of new Hg for forested catchments.

Acknowledgement—This study was made possible by a grant from the U.S. Environmental Protection Agency EPSCoR Program. We would like to thank L. Sotoodeh at the Desert Research Institute for her help and support. Thanks also go to J. Benesch, A. Burt, B. Hewitt, M. Markic, C. Sladek, and M. Wyman. Special thanks go to G. Cramer, D. Johnson, W. Johnson, and S. Lindberg for their vital input. We also thank the reviewers for their time in making this publication possible.

REFERENCES