Mercury Accumulation in *Pinus nigra* (Austrian Pine)

Russell J. Hutnik[1], James R. McClenahen[2], Robert P. Long[3], and Donald D. Davis[4,*]

Abstract - The overall objective of this field study was to determine if *Pinus nigra* (Austrian Pine) could serve as a useful biomonitor to evaluate multi-year concentrations of total mercury (tHg) in vegetation within southwestern Pennsylvania. Austrian Pine has been widely planted as an ornamental, and formerly as a Christmas tree, and is now naturalized within the region. We collected needle samples annually during October 2004–2010 at 15–21 locations within a 5000-km^2 study area. Because Austrian Pine trees typically retain needles for 3 years, we collected samples from 3 needle-age groups: current year (~0.5 y old in October), previous year (~1.5 y old), and third-year (~2.5 y old), and analyzed them for total mercury (tHg). Across all years and plots, mean tHg concentrations among the 3 needle ages were significantly ($P < 0.05$) different from each other. Mean tHg concentration was greatest in the 2.5-yr-old needles (25.9 ± 3.4 ng/g), less in 1.5-yr-old needles (20.2 ± 3.2 ng/g), and least in the 0.5-yr-old needles (11.3 ± 2.3 ng/g). The greatest mean tHg content in the oldest needles indicates that Austrian Pine may sequester atmospheric Hg in/on its needles. Although the tHg concentrations within all 3 needle ages declined slightly during 2004–2010, downward linear trends were not significant, possibly due to the short sampling period (7 years). Needle tHg concentrations were significantly less in the northeastern portion of the study area, located farthest downwind from industrial sources of tHg, and may represent background tHg levels for conifers in the region. Results from this study suggest that any biomonitoring program involving conifers should consider needle age when developing sampling protocols. In addition, results suggest that abscised older pine needles may contribute substantially to the tHg soil burden beneath conifer stands. This is the first report from North America regarding tHg concentrations in/on various-aged Austrian Pine needles. Austrian Pine may prove useful as a biomonitor when evaluating spatiotemporal patterns of tHg accumulation within vegetation in eastern North America.

Introduction

Mercury (Hg) is a highly toxic element that is emitted from anthropogenic point sources such as steel mills, coal-fired power plants, and landfills, as well as incinerators that burn municipal, medical, and hazardous waste (Lindberg et al. 2001, USEPA 1997). Anthropogenic Hg may circulate in the earth's atmosphere, becoming part of the global Hg cycle (Schroeder and Munthe 1998), but eventually can be deposited into aquatic and terrestrial ecosystems. Most, if not all, total Hg (tHg) on or in vegetation comes from atmospheric deposition rather than uptake from the soil (Ericksen et

[1]School of Forest Resources, The Pennsylvania State University, University Park, PA 16802 (retired). [2]School of Natural Resources, The Ohio State University, Wooster, OH 44696 (deceased). [3]USDA Forest Service, Northern Research Station, Irvine, PA 16329. [4]Department of Plant Pathology and Environmental Microbiology, Penn State Institutes of Energy and the Environment, The Pennsylvania State University, University Park, PA 16802. [*]Corresponding author - ddd2@psu.edu.

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al. 2003, Grigal 2003). As such, patterns of atmospheric Hg deposition/accumulation in terrestrial ecosystems can be evaluated using plant biomonitors.

Since 1999, we have used biomonitors (e.g., leaves, lichens, moss, and soil) to elucidate patterns of pollutant deposition/accumulation in southwestern Pennsylvania (Davis et al. 2001, 2002, 2007; Hutnik et al. 1989; McClenahen et al. 1999, 2007, 2012, 2013). Non-deciduous coniferous plant species are especially useful biomonitors of Hg concentrations because many species retain needles for several years. Simultaneous collection of several years' needle complements from individual trees allows estimation of past annual Hg deposition/accumulation over multiple years. As such, needle samples may reflect annual Hg accumulations that have occurred for a decade or more for coniferous species that retain needles for longer times (Grigal 2003). Rasmussen (1995) reported that Hg concentrations within needles of the coniferous genera *Abies* (fir) and *Picea* (spruce) in Ontario generally increased from spring to fall within the same growing season. He also reported that Hg concentrations in *A. balsamea* L. (Balsam Fir) and *P. glauca* (Moench) Voss (White Spruce) in Ontario increased as needle age increased from 1–3 years. Regarding the coniferous genus *Pinus* (pines), Fleck et al. (1999) reported that Hg concentrations in 2-year-old *P. resinosa* Ait. (Red Pine) needles in Minnesota were approximately twice that of 1-yr-old needles. *Pinus nigra* Arnold (Austrian Pine), the pine species used in this study, is commonly planted as an ornamental or Christmas tree in northeastern US and southeastern Canada, and has become naturalized throughout our study area within southwestern Pennsylvania (D.D. Davis, pers. observ.).

The objectives of this study were to determine 1) if needles of Austrian Pine accumulated tHg and could serve as a biomonitor of Hg pollution in southwestern Pennsylvania, 2) if tHg concentrations varied with needle age, 3) which needle age would best characterize patterns of tHg pollution, and 4) if there were spatial patterns of tHg accumulation across the study area. To facilitate meeting these objectives, we used a robust sample size (n = 372), a 7-year sampling period (2004–2010), 3 needle-age groups (0.5-, 1.5-, and 2.5-yr-old needles), and a large sampling area (~5000 km^2).

Methods

Study area

The study area is located in a mainly rural area within the Appalachian Plateau physiographic province in southwestern Pennsylvania (Fig. 1). The area has a rolling, dissected topography: ridges oriented in a southwest–northeast direction, and elevations ranging from 300 to 800 m (McClenahen et al. 2013). Average annual precipitation is ~100 cm, the climate is continental, and prevailing winds are from the west–southwest. Laurel Ridge, one of the first ridges east of the Rocky Mountains that pollutant-laden air masses encounter as they travel towards the Northeast, is located within the study area. Forests are mainly *Quercus* (oak)-dominated mixed hardwoods with areas of *Fagus* (beech)-*Betula* (birch)-*Acer* (maple) northern hardwoods on the more mesic sites and at higher elevations. *Pinus strobus* L. (Eastern White Pine) and *Tsuga canadensis* (L.) Carr. (Eastern Hemlock) are the

predominant native conifers in the area, but occur only at scattered locations. However, non-native ornamental conifers including Austrian Pine have been planted as ornamentals and Christmas trees, and have become naturalized throughout the area, as well as much of northeastern US and southeastern Canada (USDA 2013). Small towns and villages dot the region. Farms are generally small, and agricultural crops (e.g., corn and hay) are grown mainly as animal feed to support the dairy industry.

The study area contains some industries, including steel mills and coal-fired electric generating stations, and is downwind from the Pittsburgh and industrial Ohio River Valley areas, which are regional sources of historical and current tHg emissions. The city of Johnstown, which has a 100-year history of pollut-

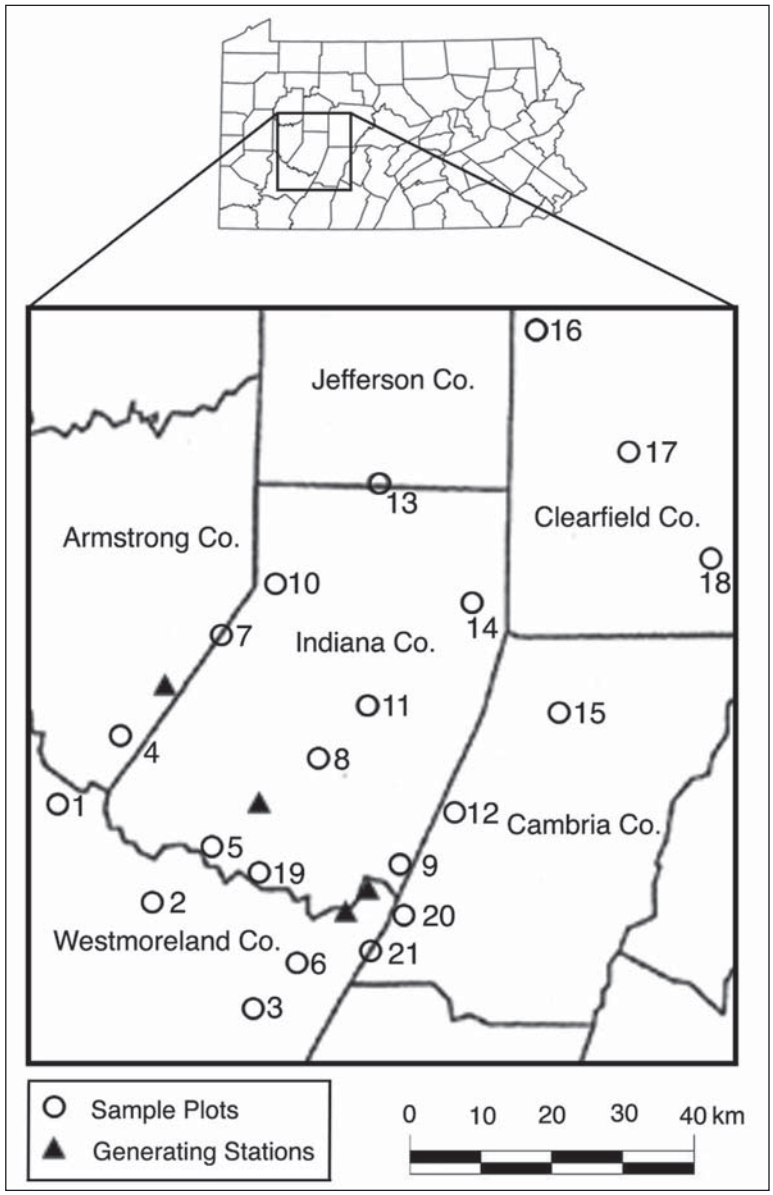

Figure 1. Location of 21 Austrian Pine sample plots (open circles) and 4 coal-fired power plants (solid triangles) within the study area in southwestern Pennsylvania. For spatial analysis, tHg (ng/g) needle data from plots 1–6, 7–12, 13–18, and 19–21 were combined into 4 area groups, respectively designated as areas 1–4.

ant emissions from steel mills and coke works (Brown 1989, McClenahen et al. 2013), lies at the southeastern edge of the study area. Although Johnstown is slightly downwind from the study area, occasional southeasterly wind may funnel air pollutants from Johnstown through the Conemaugh River Gap and into our study area (McClenahen et al. 2013). In addition, during nocturnal inversions, air emissions from Johnstown accumulate within the valley and flow downstream into the Conemaugh Gap (J.R. McClenahen, pers. observ.). Annual ambient Hg deposition near the study area is ~9–11 μg/m^2 (Lynch et al. 2001), some of which likely originates from sources outside of the study area (McClenahen et al. 2013). In recent years, industrial emissions have been reduced within and upwind from the study area, and ambient air quality in southwestern Pennsylvania has improved (McClenahen et al. 2013).

During the late 1960s and mid-1970s, R.J. Hutnik established Austrian Pine biomonitors on a grid-like network of 22 plots (Hutnik et al. 1989), mainly on low-elevation former agricultural fields within the study area, to evaluate sulfur (S) accumulation in pine needles. Although some plots have been abandoned, Dr. Hutnik and his colleagues have monitored 18 plots (plots 4–21 in Fig. 1) for decades and conducted a variety of field studies. Beginning in 2004, we utilized this overall network of 18 plots as the basis for our sampling design. However, larger Austrian Pines that shaded the plots had been routinely removed at 10–15-year intervals and replaced with new Austrian Pine seedlings. Thus, the number of plots that we sampled varied from year-to-year because we did not sample plots that contained only seedlings or young Austrian Pine saplings.

In order to balance the sampling design and to increase the number of samples, we established 3 new sampling sites (plots 1–3 in Fig. 1) in 2006 approximately 10 km upwind (southwest) from the most southwestern plots. Each of these new locations consisted of 5–10 individual landscape Austrian Pine trees because we found no pine plantations in the area. We established 15–21 plots in these latter 3 locations and sampled them annually.

For spatial analyses, we grouped plots into 4 areas, according to distance from the more industrialized regions as well as elevation. Area 1 included the 6 most southwesterly plots (plots 1–6 in Fig. 1) that were closest to, and immediately downwind from, the industrial Ohio River Valley and Pittsburgh regions. Area 2 consisted of the 6 plots (plots 7–12 in Fig. 1) located immediately downwind from four large coal-fired electric generating stations within the study area. Area 3 included the 6 most northeasterly plots (plots 13–18 in Fig. 1), which were farthest from industrial sources of pollution and represented possible background sites in the study area. Area 4 consisted of 3 plots (plots 19–21 in Fig. 1) that were located between Areas 1 and 2, but were on ridgetops as opposed to the valley floor where most other plots were located.

Sampling and Hg analyses

We collected needle samples annually during October 2004–2010. Rasmussen et al. (1991) reported that location of needle sampling within a conifer crown did

not influence Hg content, so we sampled only readily accessible lower branches. We wore particle-free latex gloves and removed needle samples by hand from the mid-portion of several branch whorls at 1–2 m height; needles on the main trunk were not sampled.

Austrian Pine trees within the region normally retain 3 years' needles. We collected ten needle samples from each of the 3 needle-age groups on each of 5 trees/plot and composited them by needle age. Thus, samples consisted of current-year needles (~0.5 yr old), previous-year needles (~1.5 yr old), and third-year needles (~2.5 yr old). Assuming no Hg uptake from the soil or Hg mobility from other age needles, current-year needles would reflect the tHg deposition/accumulation that occurred only during that first growing season. We hypothesized that the 1.5-yr-old and 2.5-yr-old needles would retain the first-year tHg, but would also accumulate additional tHg during the second and third year, respectively. This sampling procedure resulted in 372 samples analyzed for tHg over the duration of the study ([3 needle-age groups] x [7 years] x [average of 17.71 plots sampled/year]).

We air-dried, ground, and passed unwashed needle samples through a 20-mesh sieve, then stored them in sealed plastic bags. Samples were sent to Flett Research Ltd. (440 De Salaberry Avenue, Winnipeg, MB RLOY7, Canada), where they were oven-dried and analyzed for tHg as described by McClenahen et al. (2013). Analysis employed a nitric-sulfuric acid digestion followed by atomic fluorescence, which allowed a detection limit of ~2 ng tHg/g dry wt. Approximately 0.1–0.2 g of each dry sample was placed into acid-clean 20 mm x 150 mm test tubes and weighed to 0.0001 g. A typical sample lot was comprised of 20 samples, two matrix spike/matrix spike duplicates (~3–10 X tissue ng Hg), one sample duplicate, 3 analytical blanks, and duplicate certified reference materials ([CRM] NIST 1515 apple leaf CRM typical), for a total of 30 tubes. All dry samples were first wet with 0.5–1 ml DI water, then 10 ml of acid (1:2.5 HNO_3:H_2SO_4) was added to each test tube, including blanks, and the tubes were covered with acid-cleaned glass marbles. The acid was allowed to sit at room temperature in the covered test tube for 1 hr before a 6-hr digestion (covered) at 150 ˚C in an aluminum hot-block. When cool, 200 µL of BrCl was added and the digests brought up to 25.0 ml with low-Hg DI water. Aliquots of 0.1–1.0 ml were analyzed by a variant of EPA Method 1631 (USEPA 2013). Method-detection limits were ~2 ng Hg/g dry weight of tissue. Spike and reference recoveries typically were 100 ± 10%. We present all values on a dry-weight basis.

Data analyses

We evaluated normality of the Hg data using SAS Proc Univariate; data transformations were not required. To determine the effect of needle age on tHg concentration and to examine temporal trends in tHg concentrations, the 7-yr (2004–2010) mean concentration of tHg in the 3 needle ages was subjected to a fixed-effects 2-factor ANOVA with year (7) and needle age (4) as main factors. To examine spatial trends, area data were analyzed with a 2-factor fixed-effects ANOVA with year (7) and area (4), using tHg data only from the 2.5-yr-old needles. We

selected the 2.5-yr-old needles for the spatial analysis rather than the 0.5- or 1.5-yr-old needles because of their greater tHg content (see Results). Least squares means were evaluated using the Tukey-Kramer adjustment. Pairwise mean comparisons were used to test for location of significant differences in all analyses (SAS 2008). Significance in all statistical analyses was tested at $P < 0.05$.

Results and Discussion

Average tHg needle concentrations

The mean tHg concentration of Austrian Pine needles across all plots, sampling dates, and needle ages was 18.8 ± 7.3 ng/g tHg (n = 372; Table 1). This concentration is generally similar to mean tHg levels of unwashed needles for other coniferous species within North America (Fleck et al. 1999, Grigal 2003, Heyes et al. 1998, Rasmussen 1995, Rasmussen et al. 1991), but is considerably less than Hg concentrations in conifer needles sampled near large point-sources of Hg in Europe (Bargagli et al. 1986, Barghigiani and Bauleo 1992, Maserti and Ferrara 1991). However, these results do not agree with the preliminary finding of Smith (1972), who reported that the 1969 Austrian Pine needles collected from a single tree during fall 1970 (~1.5-yr-old needles) in urban New Haven, CT, contained 170 ng/g Hg. This discrepancy may have been related to Smith's small sample size (n = 1) compared to ours (n = 124) or to a greater tHg deposition in CT during the late 1960s.

Most Hg in plant tissues is accumulated from atmospheric deposition (Ericksen et al. 2003, Grigal 2003), thus, our results likely reflect atmospheric deposition from the upwind industrial Ohio River Valley and Pittsburgh regions, and point sources in southwestern Pennsylvania.

Influence of needle age

All pairwise comparisons of Hg concentrations among the 3 needle-age classes were significant. Least-squares-mean tHg concentrations across all locations and sampling dates were greatest in 2.5-yr-old needles (25.4 ± 1.8 ng/g), less in 1.5-yr-old needles (19.9 ± 1.7 ng/g), and least in 0.5-yr-old needles (11.2 ± 1.6 ng/g) (Table 1). Regarding annual accumulations, the 0.5-yr-old needle concentration of 11.2 ng/g tHg represented 44% of the 3-year total accumulation. The 1.5-y-old needles accumulated an additional mean 8.7 ng/g tHg (34% of the 3-year total). Finally, the 2.5-y-old needles accumulated an additional 5.6 ng/g tHg (22% of the 3-year total). These results suggest that the rate of apparent accumulation decreased annually during the 3 sampling years (44 > 34 > 22%) and may indicate that the number of sorbtion sites on the needles decreased over time as tHg occupied available sites.

Greater concentrations of tHg in older needles were also reported in Balsam Fir and White Spruce in Ontario (Rasmussen 1995). Similarly, Barakso and Tarnocai (1970) reported greater Hg concentrations in older conifer needles and recommended the use of second- and third-year needles in biogeochemical prospecting for Hg in British Columbia. Likewise, Fleck et al. (1999) reported that the Hg concentrations of 2-year-old Red Pine needles in Minnesota

Table 1. Total mercury (tHg) concentrations (ng/g) and standard deviations in 0.5-, 1.5-, or 2.5-yr-old needles from Austrian Pines sampled in 2004–2010. For clarity, raw means are presented in main table body, and Least Squares (LS) means, which were used in statistical analyses, are presented in the summary column and summary row. 7-year needle age tHg LS means followed by the same uppercase letter are not significantly different ($P < 0.05$) based on Tukey's pairwise means comparisons. Yearly tHg LS means for all 3-year needle ages followed by the same lowercase letters are not significantly different ($P < 0.05$) based on Tukey's pairwise means comparisons.

	Year of sampling							
Needle age (yrs)	2004	2005	2006	2007	2008	2009	2010	7-year LS mean
0.5	14.3 ± 4.1 (n = 15)	9.3 ± 1.8 (n = 18)	11.3 ± 2.9 (n = 21)	11.8 ± 3.3 (n = 20)	10.2 ± 2.0 (n = 18)	10.6 ± 2.0 (n = 17)	10.6 ± 2.3 (n = 15)	11.2 ± 1.6A (n = 7)
1.5	23.0 ± 5.4 (n = 15)	18.2 ± 4.5 (n = 18)	19.7 ± 4.9 (n = 21)	20.3 ± 3.7 (n = 20)	18.0 ± 3.6 (n = 18)	20.9 ± 3.5 (n = 17)	19.2 ± 3.6 (n = 15)	19.9 ± 1.7B (n = 7)
2.5	28.8 ± 6.5 (n = 15)	23.4 ± 5.3 (n = 18)	25.5 ± 5.4 (n = 21)	25.3 ± 4.3 (n = 20)	24.6 ± 4.6 (n = 18)	26.3 ± 5.3 (n = 17)	23.8 ± 5.2 (n = 15)	25.4 ± 1.8C (n = 7)
Annual LS means for all age groups	22.0 ± 7.3a (n = 3)	17.0 ± 7.1b (n = 3)	18.9b ± 7.1c (n = 3)	19.1 ± 6.8bc (n = 3)	17.6 ± 7.2b (n = 3)	19.2 ± 8.0bc (n = 3)	17.9 ± 6.7bc (n = 3)	18.8 ± 7.3 (n = 372)
Total number of plots sampled	15	18	21	20	18	17	15	124

were approximately twice that of 1-yr-old needles. Barghigiani et al. (1991) and Maserti and Farrara (1991) reported that *P. nigra* Arnold var. *laricio* Maire]), a Mediterranean variety of Austrian Pine (Calabrian Black Pine), had greater Hg concentrations in the 2- and 3-yr-old needles than in the 1-yr-old needles. Furthermore, Barghigiani and Bauleo (1992) reported that the Hg content of *Abies alba* Mill. (Silver Fir) in Italy generally increased as needle age progressed from 1–12 years. Because Austrian Pine retains only 3 years' healthy needles in Pennsylvania, we could not evaluate the temporal trend in our data beyond 3 years. However, our overall findings of increasing tHg content with Austrian Pine needle age are consistent with other reports, indicating that the increasing trend occurs in at least three genera of conifers: *Abies*, *Picea*, and *Pinus*. Also, our results suggest that any biomonitoring program involving conifers should take needle age in to consideration when formulating needle-sampling protocols.

The oldest needles of Austrian Pines annually fall to the forest floor as litter. Mercury in litterfall constitutes new annual input to a forest ecosystem, and the total Hg flux in litterfall varies among forest-cover types (Bushey et al. 2008, McClenahen et al. 2013, Rea et al. 2001, Risch et al. 2012). Annual variation in amount of litterfall may be more important than concentration of litterfall Hg in determining total dry Hg deposition (Risch et al. 2012). Most reports dealing with tHg in litterfall involve deciduous forests; we do not attempt to present an Hg budget for our study area, but we suggest that older fallen conifer needles likely represent a substantial contribution to the tHg burden in soil beneath conifer stands.

2004–2010 temporal trends

Although the tHg concentrations in all 3 needle-age groups declined somewhat during the 7-year period from 2004–2010, the slight downward trends for all 3 needle groups, as determined by linear regression (SAS 2008, regression analysis not shown), were neither significant nor greatly influenced by high 2004 levels. In 2004, the mean tHg concentration for all needle ages was significantly greater than for all other years, which were statistically similar (Table 1).

Figure 2 illustrates the difference in tHg content among the 3 needle ages over time, and also illustrates that the annual variation in tHg content among the 3 needle ages was synchronous. For instance, the sharp decline in tHg content from 2004–2005, due to unknown causes, occurred in all 3 needle-age classes.

The lack of a significant downward temporal trend in Hg concentrations within Austrian Pine needles from 2004–2010 is in contrast to our longer-term findings in the study area for other bioindicators, including freshly fallen oak litter, a corticolous moss, and the fermentation organic soil layer (McClenahen et al. 2013). Use of these latter bioindicators revealed significant decreases in tHg over time within the same region, possibly relating known emission controls to concomitant tHg reductions. However, the time period covered by our Austrian Pine dataset (2004–2010) was shorter than for our other biomonitors, and use of Austrian Pine needles may require a longer monitoring period to determine if statistically significant time trends can be discerned. We recommend using 3-yr-old needles rather than younger

needles in future biomonitoring efforts using Austrian Pine. The tHg concentrations were greatest for the oldest needles and were well above threshold levels thus minimizing non-detectable values.

Spatial trends

As stated above, only the oldest Austrian Pine needles were used to evaluate spatial patterns. The 6 most northeasterly plots (Area 3, plots 13–18 in Fig. 1), which were located farthest downwind from industrial sources of pollution, had a significantly lower mean tHg concentration (22.1 ± 1.9 ng/g) than needle samples from Area 1 (plots 1–6; 28.5 ± 4.0 ng/g), Area 2 (plots 7–12; 27.2 ± 1.9 ng/g), and Area 4 (plots 19–21; 25.5 ± 1.3 ng/g). Area 1 (27.9 ± 4.0 ng/g) also had a significantly greater ($P < 0.03$) tHg concentration than Area 4 (25.5 ± 1.3 ng/g). All other comparisons were not significantly different. These findings suggest tHg deposition may be similar and regional in nature within the 6 plots closest to, and immediately downwind from, the industrial Ohio River and Pittsburgh industrial regions (Area 1), the 6 plots immediately downwind from the four large coal-fired generating stations (Area 2), and the 3 ridgetop plots (Area 4). In contrast, tHg deposition in Area 3 may represent background with little tHg augmentation from industrial sources.

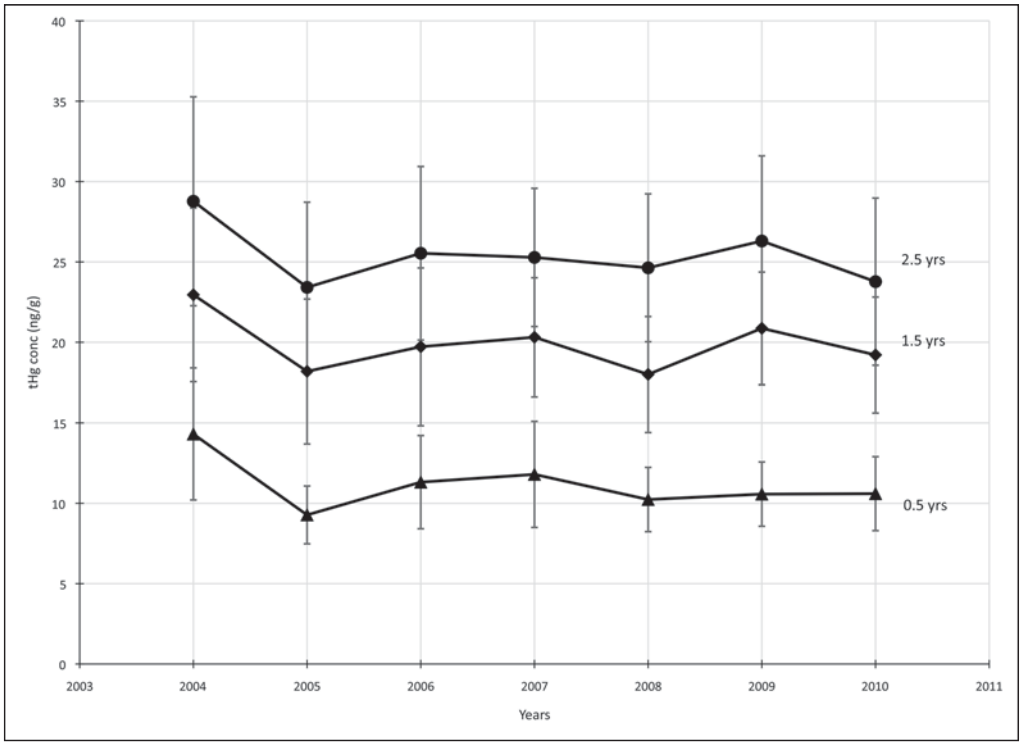

Figure 2. Means and standard deviations of tHg concentration (ng/g) for 0.5-, 1.5-, and 2.5-yr-old Austrian Pine needles during 2004–2010. Mean tHg contents for the 3 needle classes were significantly different ($P < 0.05$) from each other. Mean tHg contents for 2004 were significantly different from all other years, which were not significantly different from each other.

Conclusions

Mean tHg concentration was greatest in the 2.5-yr-old needles, less in 1.5-yr-old needles, and least in the 0.5-yr-old needles. Although the tHg concentrations within all 3 needle ages declined slightly during 2004–2010, downward linear trends were not significant. Needle tHg concentrations were significantly lower in the northeastern portion of the study area (Area 3), located farthest from industrial sources of tHg, and may represent the regional background levels of tHg for pine needles in this part of Pennsylvania. In contrast, the needle tHg concentrations in Areas 1, 2, and 4 may represent tHg levels typical of an industrialized region. Austrian Pine may prove useful when evaluating spatiotemporal patterns of tHg deposition and accumulation of tHg in northeastern United States and southeastern Canada. Biomonitoring programs involving conifers should consider needle age when researchers develop sampling protocols. In addition, because Austrian Pine annually sheds older needles, conifer litter may contribute annually to the tHg burden within the soil beneath conifer stands.

Acknowledgments

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