

Lead Mobility within the Xylem of Red Spruce Seedlings: Implications for the Development of Pollution Histories

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ABSTRACT

Development of Pb pollution histories using tree ring analyses has been troubled by possible mobility of Pb within stem xylem. In a 2-yr study, we exposed red spruce (*Picea rubens* Sarg.) seedlings to Pb during one growing season, with Pb excluded in either the previous or following growing season. Lead levels within xylem rings and bark were subsequently determined using atomic absorption analyses. Lead concentrations in tissues from exposed seedlings were significantly higher than controls, and significant Pb concentrations were found in xylem formed during periods of Pb exclusion. Routes of Pb movement are uncertain, but were most likely acropetal from temporary storage sites in the roots.

MORE THAN 4.5 million metric tons of Pb are added to the world's atmosphere yearly (Nriagu, 1978). Atmospheric Pb is eventually removed by precipitation scavenging and dry deposition; Nriagu (1978) cites annual Pb deposition rates ranging from about 1 mg m⁻² in the Sierra mountains of California to more than 1200 mg m⁻² near a Pb smelter in Missouri.

Because Pb is toxic to plants and animals (Forbes and Sanderson, 1978; Posner et al., 1978; Koeppe, 1981), it is important to monitor its abundance in the environment. The analysis of heavy metals in tree rings has received much attention as a pollution-monitoring tool because most trees in temperate regions are long-lived, stationary, accumulate heavy metals, grow on a regular seasonal basis, produce datable annual growth increments, and can be nondestructively sampled (Lepp, 1975). Use of trees for pollution monitoring assumes that trees take up and incorporate pollutants in relative proportion to their environmental abundance. Under this assumption, pollutant concentrations within a series of tree rings should provide documentation of temporal patterns of pollutant availability. For example, Scherbatskoy and Matusiewicz (1988) recently used results of this technique as evidence of temporal increases in heavy metal availability within the Green Mountains of north-central Vermont.

Numerous researchers have attempted to develop Pb pollution histories using tree-ring analyses, but results have not been consistent. For example, in some studies, Pb concentrations in annual rings were consistent with the expected pattern of Pb availability (Rolfe, 1974; Ward et al., 1974; Kardell and Larsson, 1978; Symeonides, 1979; Berish and Ragsdale, 1985). In other studies, it did not (Szopa et al., 1973; Barnes

et al., 1976). In yet other studies, the agreement between measured and predicted Pb varied with tree species (Baes and Ragsdale, 1981; Hampp and Holl, 1974).

Lack of association between predicted and measured levels may have been the result of mobility of Pb within sampled trees. For the purpose of using annual rings to document the chronology of its relative abundance, Pb can be considered functionally mobile if for any reason it accumulates in an annual ring not formed during the year of Pb uptake. If Pb is functionally mobile, concentrations in a particular ring may not reflect environmental abundance during that year. The question of mobility is a major obstacle preventing the wide acceptance of tree-ring analysis for chronicling Pb pollution (Szopa et al., 1973; Hampp and Holl, 1974; Lepp and Dollard, 1974a,b; Rolfe, 1974; Barnes et al., 1976; Dollard et al., 1976; Kardell and Larsson, 1978; Symeonides, 1979; Baes and Ragsdale, 1981; Robitaille, 1981).

Our research was conducted as part of an intensive program, "Survey of Forest Effects of Atmospheric Deposition in the Northeast—Chemistry of Tree Rings"; the study reported herein examines the nature and extent of apparent inter-growth-ring mobility of Pb within red spruce (*Picea rubens* Sarg.) seedlings.

MATERIALS AND METHODS

Lead Treatments

Our study was designed to allow detection of apparent Pb movement within xylem of red spruce seedlings both inward toward the pith, and outward toward the bark. This necessitated the inclusion of three treatments: (i) a treatment consisting of first-year Pb exposure followed by a year of Pb exclusion; (ii) a treatment consisting of first-year Pb exclusion followed by a year of Pb application; and (iii) control seedlings that never received Pb. Additionally, to confirm that Pb applications were resulting in tissue-Pb concentrations large enough to statistically discriminate between control and treated seedlings, we harvested a control and a Pb-treated seedling pair from each replication following the first year of Pb application. Inclusion of these two first-year analyses resulted in a total of five treatments within the study.

Lead is thought to be deposited to forested ecosystems predominantly in particulate form (Smith and Siccama, 1981). Much of this Pb is retained within organic soil layers in the forest floor (Johnson et al., 1982). However, some must be in exchangeable chemical form(s) available for root uptake, because detectable levels of Pb have been found in xylem of numerous forest species (Rolfe, 1974; Heinrichs and Mayer, 1980; Morrison and Hogan, 1986). Clearly Pb is incorporated within stem tissue under natural conditions, and one must assume that it is initially taken up and transported within xylem in a soluble form. The focus of the present study was not to determine proportions of the Pb pool available for uptake, nor to identify the specific chemical forms of Pb absorbed, but rather to determine if Pb, once within a plant, remained confined to xylem produced during periods of Pb uptake. Thus, we chose to administer Pb hydroponically, in the form of a soluble salt, in concen-

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trations sufficient (as determined empirically) to result in Pb concentrations great enough to discriminate from control plants, and allow for detection of even a small fraction of the incorporated Pb if it moved into Pb-free xylem.

Lead applied hydroponically in the form of dissolved PbNO_3 results in Pb incorporation within red spruce xylem (Schaberg, 1985). Results of a preliminary study indicated that 2.0 mg L^{-1} Pb (as PbNO_3) was sufficient to allow for adequate Pb incorporation in the xylem of treated plants, and that at this concentration the constituents of Ingestad (1959) nutrient solution did not precipitate or appear to interfere with adequate Pb uptake.

Seedling Growth and Imposition of Treatments

A total of 100 red spruce seedlings, initiated from a bulked collection of seeds from high-elevation trees in the Green Mountains of Vermont, were grown in sulfuric acid-washed and distilled water-rinsed, 10-cm pots containing Pb-free perlite in an indoor growth-facility at The University of Vermont, School of Natural Resources. Average temperature and relative humidity in this facility were 21°C and 60%, respectively. Artificial lighting of approximately $150 \mu\text{E s}^{-1} \text{ m}^{-2}$ was provided during an 18-h photoperiod.

Seedlings were arranged in the growth facility in 20 replications, each containing five seedlings, with each seedling randomly assigned to one of the five treatments discussed above. Seedlings were watered to excess every other day with either standard Ingestad (1959) nutrient solution (control seedlings and treatment seedlings during periods of Pb exclusion) or with the same solution plus 2.0 mg L^{-1} Pb (as PbNO_3). Contamination of the bark surface by splashing of watering solution was minimized by placing a 2-cm plastic collar around the base of each seedling stem, extending just below the surface of the perlite.

To evaluate inter-growth-ring mobility of Pb, the seedlings had to produce clear definable annual rings. Therefore, following the first growing season, the photoperiod was reduced to 12 h until the seedlings had set bud. Seedlings that had been treated with Pb the first growth period, but from which Pb was to be excluded during the second, were removed from their pots, their roots washed in distilled water to remove any Pb-containing perlite, and repotted into acid-washed, 10-cm pots containing fresh Pb-free perlite. With the exception of those seedlings harvested after 1 yr, all seedlings (treatments and controls) were then placed in a walk-in cooler with temperature set to 4°C and light levels of approximately $40 \mu\text{E s}^{-1} \text{ m}^{-2}$ for 2 mo to satisfy dormancy requirements (Safford, 1974). Seedlings were then returned to the growth-facility under an 18-h photoperiod to initiate bud break and subsequent growth for the second growing season.

Seedling Harvest and Tissue Separation

After initiation of bud-set following each of the two growing seasons, seedlings from the appropriate treatments were harvested by severing the stem at the rooting media surface with an acid-washed razor blade. Following the first growing season (1986), a 10-cm stem section was removed from each harvested seedling immediately above the plastic stem collar. Bark was carefully removed, and the exposed xylem surface was lightly scraped with a razor blade to remove any residual phloem from the xylem surface. Bark and xylem from each seedling were placed in acid-washed vials and dried in an oven at 85°C .

Following the second growing season (1987), an analogous 10-cm segment was removed from the stem of each harvested seedling. Bark was removed as described above. Then, under a large magnifying lens, the two annual xylem rings were separated by removing the outer ring from the inner in a series of small tangential cuts. To avoid contam-

ination, xylem is close proximity to the ring boundaries was discarded. As with seedlings harvested following the first growing season, pith was not isolated and was thus treated as first year xylem tissue. The resulting three tissues (first- and second-year xylem, and bark) were placed in acid-washed vials and dried in an oven at 85°C .

Tissue Digestion and Analyses

Seedling tissues were prepared for Pb analysis using a modification of a dry/wet ashing procedure described by Jackson (1958). Specific ashing methods were as follows: (i) 0.5 g of oven-dried plant material were placed into an acid-washed, fused-silica crucible; (ii) crucibles were placed in a muffle furnace at 400°C overnight; (iii) resulting ash was covered with 2 M HNO_3 , and this liquid was evaporated on a hotplate at approximately 75°C ; (iv) crucibles were again placed in a muffle furnace at 400°C overnight; (v) the ash was covered with 2 M HCl and evaporated on a hotplate at approximately 75°C ; and (vi) the ash was taken up in 0.1 M HNO_3 and stored in acid-washed vials prior to analysis.

Lead concentrations were then quantified using a Perkin-Elmer HGA 500 graphite furnace and Model 560 atomic absorption spectrometer. Based on analyses of pine needles from the National Bureau of Standards (Standard Reference Material 1575) using the above methods, the achieved Pb-recovery was 96.6%.

To comply with statistical assumptions of equality of variances among treatment groups, all tissue Pb concentrations were transformed using a $\log_{10}$ transformation. Transformed values were then statistically analyzed following a randomized complete block design. Differences among specific treatment means were determined using Duncan's multiple range procedure. In all cases, differences among means were considered statistically significant if $P \leq 0.05$. Treatment means presented throughout this manuscript are anti-logs of the arithmetic means of log-transformed Pb concentrations for each treatment, and are thus in the original units (mg kg^{-1}).

RESULTS AND DISCUSSION

Distribution of Lead within Plant Tissues

Seedlings Harvested in 1986.—Two groups of seedlings were harvested in 1986: control seedlings and first-year, Pb-treated seedlings. Mean Pb concentrations (followed by their standard errors) in the xylem and bark of control seedlings were 0.8 ± 1.2 and $1.1 \pm 1.2 \text{ mg kg}^{-1}$, respectively (Fig. 1); these concentrations were not significantly different. Mean Pb concentrations in treated-seedling xylem and bark were 10.5 ± 1.1 and $15.1 \pm 1.3 \text{ mg kg}^{-1}$, respectively. Bark concentrations were significantly greater than xylem concentrations, and concentrations in both tissues were significantly greater than in analogous tissues of control seedlings.

Seedlings Harvested in 1987.—Three groups of seedlings were harvested: control seedlings, seedlings treated with Pb during the first year but not the second, and seedlings treated with Pb the second year but not the first. Control seedlings' mean Pb concentrations in first-year xylem, second-year xylem, and bark were 4.0 ± 1.2 , 3.8 ± 1.2 , and $4.6 \pm 1.1 \text{ mg kg}^{-1}$, respectively (Fig. 1). Comparable mean concentrations for seedlings treated with Pb during their first year of growth were 34.7 ± 1.1 , 15.8 ± 1.1 , and $11.0 \pm 1.1 \text{ mg kg}^{-1}$, respectively. Comparable mean concentrations for seedlings treated with Pb during their second year were

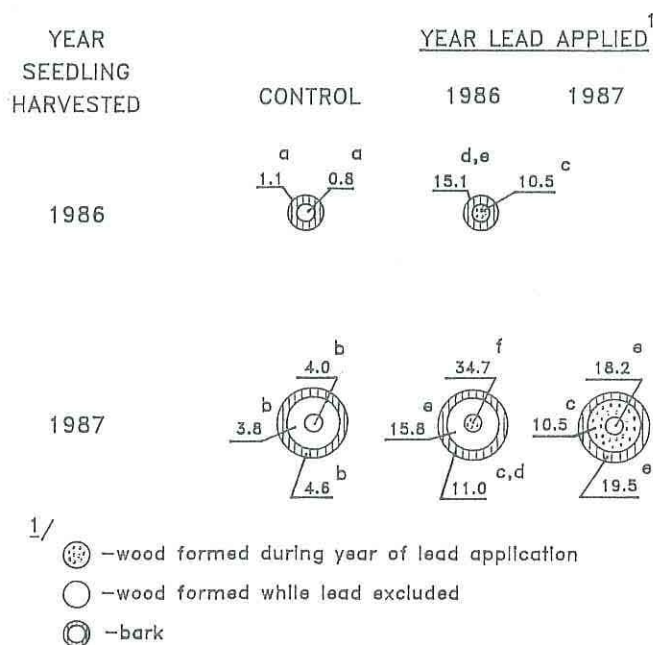


Fig. 1. Concentrations (mg kg^{-1}) of Pb within stem cross-sections of red spruce seedlings treated with 2 mg L^{-1} Pb (as PbNO_3). Presented values are anti-logs of arithmetic means for each treatment of $\log_{10}$ -transformed observations. Values not followed by the same letter are different ($P \leq 0.05$) as determined by Duncan's multiple range procedure.

18.2 ± 1.3 , 10.5 ± 1.2 , and $19.5 \pm 1.3 \text{ mg kg}^{-1}$, respectively. Lead concentrations in all tissues from seedlings treated with Pb in either year were significantly greater than their analogous control tissues. Specific differences among Pb concentrations of treated-seedling tissues are given in Fig. 1.

Relationships among Tissue Lead Concentrations

Lead concentrations within control seedlings (all tissues) averaged 0.9 mg kg^{-1} in 1986, and 4.1 mg kg^{-1} in 1987; within a harvest year, control tissues did not differ in their Pb concentrations (Fig. 1). Lead within control seedlings was most likely due to Pb particulates in ambient air and to the accumulation of Pb from the nutrient solution; Pb concentration of the Ingestad solution averaged 0.001 mg L^{-1} . Differences in Pb concentrations between 1986- and 1987-harvested control seedlings most likely resulted from additional incidental Pb accumulation by 1987-harvested seedlings during their second year of growth.

Mean Pb concentrations in tissues formed during the year of Pb application were strikingly similar for seedlings harvested both years, i.e., Pb concentrations in xylem from seedlings treated with Pb and harvested in 1986, and second-year xylem from seedlings treated and harvested in 1987 both averaged 10.5 mg kg^{-1} . The same pattern was also true for bark, i.e., bark of seedlings treated with Pb and harvested in 1986 had mean Pb concentrations of 15.1 mg kg^{-1} , whereas bark from seedlings treated and harvested in 1987 had Pb concentrations averaging 19.5 mg kg^{-1} .

Seedlings treated in 1986 and harvested in 1987 tended to have lower bark concentrations than did seedlings harvested the year of Pb treatment. This

might have been the result of Pb movement out of bark tissue, presumably inward into second-year xylem, or it could have resulted from dilution of Pb as new bark tissue was produced.

Evidence of the Functional Mobility of Lead

In seedlings that had been treated only during the first year, Pb concentrations in xylem formed during the second year averaged 15.8 mg kg^{-1} (Fig. 1). This was significantly greater than the 3.8 mg kg^{-1} within comparable tissues of control seedlings. And, for seedlings treated with Pb during the second year of growth, Pb concentrations in first-year xylem averaged 18.2 mg kg^{-1} , again significantly greater than the 4.0 mg kg^{-1} found in analogous xylem tissue of controls. For the purposes of this study, functional Pb mobility within xylem tissue was defined as the accumulation, for any reason, of Pb in annual rings not formed during periods of Pb exposure. Based on this definition, Pb was indeed functionally mobile. If radially oriented increment cores had been removed from stem xylem and analyzed for Pb concentrations, resulting Pb contents would have inaccurately predicted abundance of Pb in the ambient environment. For example, if increment cores had been obtained from seedlings treated only during 1987, they would have incorrectly indicated that environmental concentrations of Pb were higher in 1986 than in 1987 (Fig. 1).

Probably the major route of Pb movement into xylem formed during periods of Pb exclusion was acropetally from temporary storage sites in the roots. We did not analyze root tissue in this study, but in a previous study within our lab (Schaberg, 1985), root tissue of red spruce seedlings had Pb concentrations 10 times those found in stem tissue. And Koeppel (1981) states that in plants exposed to a Pb-containing rooting media, roots always contain higher Pb concentrations than above-ground tissues. Once inside plant tissue, Pb is believed to bind to cell wall cation exchange sites, at least temporarily in an exchangeable form (Brown and Slingsby, 1972). Dollard et al. (1976) postulate that, in terms of Pb's movement, the xylem behaves as an ion-exchange column, with movement of ions taking place by progressive binding to negatively charged sites within the xylem column.

Because of these relationships, root tissue of previously treated seedlings probably represents a significant source of Pb for subsequent acropetal transport. Seedlings treated and harvested in 1986 had xylem Pb concentrations averaging 10.5 mg kg^{-1} ; similarly treated seedlings harvested in 1987 had first-year xylem Pb concentrations averaging 34.7 mg kg^{-1} (Fig. 1). The most obvious explanation for the increased Pb in this tissue is that it originated in the roots and moved upward during the second year of growth. Because all xylem in these seedlings was functional (i.e., no non-conducting wood), acropetal transport of Pb from seedling roots into stem xylem would result in Pb distributions within stem xylem mimicking those expected from lateral Pb transport. For example, assuming that xylem formed in both years was fully conducting, second-year acropetal movement of root-xylem Pb originating from first-year applications

would result in Pb deposition and accumulation in second-year stem xylem; precisely that pattern expected from true lateral Pb movement.

Another possible explanation for the presence of Pb in xylem formed during periods of Pb exclusion is that Pb actually moved laterally through radially oriented xylem elements, most likely the rays. Although some Pb transport may have occurred in this fashion, exclusive movement along this route would not have produced our observed results. The more than three-fold increase in Pb content of the 1986-treated, first-year xylem (10.5 mg kg⁻¹ in 1986 to 34.7 mg kg⁻¹ in 1987) virtually precludes exclusive lateral Pb transport, and strongly implicates at least partial acropetal transport from root tissues.

Regardless of the route(s) of Pb movement within seedling xylem, our findings may have important implications in using tree-ring analyses as a tool for developing precise Pb pollution histories. Functional lateral movement of Pb certainly occurs in red spruce seedlings, at least among adjacent rings. But we do not know to what extent this movement occurs within larger stems with many more xylem rings. Our results imply that Pb moves acropetally from root tissue, becoming distributed within currently conducting xylem. If Pb moves in this manner, Pb levels within a given xylem ring reflect not only Pb uptake during the year of ring formation, but also are related to root Pb levels during the time period in which xylem within that ring remains functional. Presumably once xylem becomes nonconducting, no further acropetal transport occurs within that tissue. Consequently, Pb concentration within a xylem ring would depend on level of Pb uptake during the year of ring formation, subsequent rate of acropetal transport from roots, and the length of time xylem remains functional. The ultimate result of these dynamic factors operating in concert would be difficult to predict, but seem unlikely to result in xylem-ring Pb concentrations that are precise indices of yearly Pb levels in the ambient environment. However, lack of precise relationships does not necessarily preclude use of tree-ring analyses to develop general Pb trends. That is, differences in Pb within specific xylem rings resulting from differing Pb availability in the soil environment may be obscured by acropetal transport from root tissue; but, because of the eventual transition of conducting xylem to nonconducting heartwood, longer term trends might be preserved. Thus, although our findings limit the usefulness of tree-ring analyses as a tool for providing precise yearly Pb pollution histories, decisions concerning the degree to which they impact tree-ring analysis for pollution monitoring must await implementation of studies of much longer duration.

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