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Development of Solid Calibration Standards for Trace Elemental Analyses of Tree Rings by Laser Ablation Inductively Coupled Plasma-Mass Spectrometry

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Laser ablation sampling (LAS) in conjunction with inductively coupled plasma-mass spectrometry (ICP-MS) was used for the quantitative multielemental analysis of red maple (*Acer rubrum* L.) tree rings. Slight differences in ablation were corrected using ^{13}C as an internal standard. Calibration standards, consisting of individual tree rings from wood sections sampled from trees along a transect away from a metal smelter, were used to convert integrated signal intensities (cps) into concentrations (mg kg^{-1}). The relative standard deviation (RSD) of element distribution within individual tree rings was <15% for 11 elements analyzed. Calibration curves were calculated for ^{43}Ca, ^{55}Mn, ^{63}Cu, ^{64}Zn, ^{114}Cd, ^{202}Hg, and ^{208}Pb, as only these seven elements had different concentrations in the tree rings used as wood standards. Tree cores from red maple trees growing in contaminated soils adjacent to metal smelters and a discolored core from a tree growing in uncontaminated soil were analyzed by LAS ICP-MS. Differences in element concentration over time and between trees were found, but surprisingly, concentrations of Cu, Zn, Mn and Ca were up to five times greater in discolored regions of the tree core from the unpolluted site compared to cores from contaminated sites. Damaged or discolored tree cores should therefore be avoided in dendrochemical studies. LAS ICP-MS can be used for the quantitative analysis of *Acer* tree rings providing a rapid, nondestructive technique which involves minimal sample preparation and provides high spatial resolution with very low detection levels for many elements.

Introduction

Patterns in the chemical content of tree rings can be used to monitor changes in atmospheric metal deposition (*1–3*) and changes in soil chemistry (*4–6*). A number of analytical techniques have been applied to dendrochemical studies, most notably atomic absorption spectrometry (AAS) (*7, 8*), neutron activation analysis (NAA) (*9*), inductively coupled plasma-mass spectrometry (*3, 10*), and inductively coupled plasma atomic emission spectrometry (*11*). Scanning microprobe techniques such as particle induced X-ray emission spectrometry (PIXE) (*12*), X-ray chromatography (XRF) (*13*),

and secondary ion mass spectrometry (SIMS) (*14, 15*) have further enhanced our understanding of wood chemistry.

Laser ablation sampling (LAS) in conjunction with inductively coupled plasma-mass spectrometry (ICP-MS) allows the rapid, nondestructive analysis of many elements in tree rings with high spatial resolution (*16, 17*). Presently, the application of LAS ICP-MS (and SIMS) in dendrochemistry is restricted due to a lack of matrix matched standards (*15, 17*). Ideally, wood calibration standards should be used which have the same matrix as the wood tissue being sampled (*18*), although no solid commercial standards are currently available. Element concentrations within tree rings have also been found to vary tremendously (*19–22*), potentially limiting the use of solid wood as calibration standards for LAS ICP-MS. In particular, there is evidence that some metals are localized within small regions of individual tree rings (*21, 23*) and differences in element concentration between early-wood and late-wood are found in some tree species (*12*).

The spatial variability of Pb, Ca, Mg, and K within individual tree rings of sugar maple (*Acer saccharum* Marsh.), however, is low (*24*). The relative standard deviation in element distribution was less than 15% for these elements, allowing tree rings to be used as calibration standards for the analysis of sugar maple tree rings by LAS ICP-MS. Unlike many other tree genera (*25*), *Acer* spp. are considered useful monitors of both atmospheric metal deposition (*3, 26*) and of changes in soil chemistry (*3, 27*). The objective of the present study is to use individual tree rings of red maple (*Acer rubrum* L.) as calibration standards to convert signal intensities obtained from LAS ICP-MS into concentrations for a number of elements. Tree cores extracted from trees growing in contaminated soils adjacent to metal smelters and a discolored core from a tree growing in unpolluted soil were analyzed by LAS ICP-MS, and changes in element concentration over time were examined.

Experimental Section

Work was performed using a PE-SCIEX Elan 5000 in combination with a laser sampler (Perkin-Elmer SCIEX, Thornhill, Ontario, Canada). The laser sampler consisted of a frequency quadrupled Nd:YAG laser operating at 266 nm, a sample stage with three controlled stepper motors, and a video camera system for observation of the sample. The sampler was controlled by ALAS (automated laser ablation sampling) software (*28*). Changes were made to the commercial laser sampler model 320 converting the output to the UV 266 nm wavelength, which is more suitable for biological materials (*28*). The computer controlling the laser sampler was connected to the Elan via the flow injection analysis system (FIAS) control port to allow automatic triggering of the Elan.

Samples. Tree cores were extracted from three red maple (*Acer rubrum* L.) trees in the spring of 1997. Surface soil samples were also taken from the base of each tree using a stainless steel trowel and were transferred to the laboratory in plastic bags. Soil pH$_{aq}$, organic matter (loss on ignition at 400 °C for 16 h), and total (HNO$_3$-extractable) trace metal concentration were determined using standard analytical techniques (*29*). Precision and accuracy were confirmed by repeated analysis of reference San Joaquin soil samples (NIST). Tree cores were taken from the base (10 cm above ground) of individual, stunted red maple trees growing within 1 km of a Cu–Ni smelter, at Sudbury, Ontario, which closed in 1972 and within 0.5 km of a zinc smelter at Rouyn-Noranda, Quebec. As most trees at the contaminated sites were stunted

TABLE 1. Total (HNO₃-Extractable) Trace Metal Concentrations in Bulked Surface Soil (0–10 cm) Sampled beneath Three Red Maple Trees Used for Dendrochemical Analysis[a]

site	appearance of tree core	soil pH	% O.M.	Cr	Co	Ni	Cu	Zn	As	Cd	Pb
								mg kg⁻¹			
Rouyn-Noranda	healthy	3.3	23.9	38.0	19.4	44.0	1043	840	421	3.8	697
Sudbury	healthy	3.7	14.2	42.0	55.0	978	906	58.0	137	0.6	106
Rollet	discolored-blue/green	4.5	22.9	85.0	6.8	33.5	56.3	73.3	6.3	0.7	53

[a] % O.M. = % organic matter determined by loss on ignition at 400 °C for 48 h.

and had multiple stems, cores were taken from the base of the trees (*30*). The main living stems which reached breast height were unsuitable for obtaining tree cores. A third tree at Rollet, an uncontaminated site approximately 40 km south of Rouyn-Noranda, was sampled at breast height (1.5 m). This tree was growing in very moist conditions, and sections of the tree core were discolored blue-green and were analyzed to determine how tree chemistry is affected by fungal infection. Tree cores were extracted with an acid-washed (10% H₂SO₄) 5 mm stainless steel increment borer. The borer was washed three times with distilled deionized water (dd-H₂O) after each core extraction. Samples were immediately sealed in dry, plastic straws for transport to the laboratory and were stored at −10 °C prior to analysis.

Branches were also cut from red maple trees growing at varying distances from the smelter at Rouyn-Noranda. Trees were approximately 1, 3, and 10 km from the smelter. A branch from a red maple tree growing on Trent University campus at Peterborough, 150 km northeast of Toronto, was also cut. From each branch, 5 cm deep cross-sections were cut and sealed in plastic bags for transport to the laboratory. Individual tree rings were separated from each cross-section, using an acid washed (trace HNO₃) hand-saw and stainless steel knife. Each of the tree ring sections (approximately 0.2 cm × 1.0 cm × 3.0 cm) was divided in two; one-half was analyzed by ICP-MS after conventional wet acid digestion (*3*) and one-half was sampled by LAS prior to analysis by ICP-MS.

Acid Digestion. Wood sections were ground and washed for 10 s in 10% HNO₃, followed by three rinses in dd-H₂O prior to acid digestion. Duplicate samples were placed in prewashed (15% H₂SO₄) 50 mL borosilicate glass tubes, dried at 70 °C for 48 h, weighed, and then dissolved in 70% trace grade HNO₃ overnight and subsequently digested under reflux at 80 °C for 4 h. Samples were filtered through acid-washed (10% HNO₃) Whatman No. 42 filter papers and diluted to 12.5 mL with dd-H₂O. Solutions from three of the wood standards were analyzed for Ca, Mn, Cu, Zn, Cd, and Pb by ICP-MS (*3*), and solutions of all four wood standards were analyzed for Hg by ICP-MS (*31*). Precision and accuracy were tested through repeated analysis of ground hop-hornbeam reference wood samples (Canadian Forest Service). Precision was excellent with <5% difference between samples and recovery of elements was 97.8% for Ca (1200 mg kg⁻¹); 93.8% for Mn (55.3 mg kg⁻¹); 96.4% for Zn (6.05 mg kg⁻¹); 105% for Cu (2.50 mg kg⁻¹); 97.1% for Pb (2.04 mg kg⁻¹); 95.0% for Cd (0.40 mg kg⁻¹), and 96.1% for Hg (0.032 mg kg⁻¹).

Laser Ablation Sampling. The three tree cores and four wood sections used for calibration were sanded and washed in 10% trace HNO₃ for 10 s and rinsed with dd-H₂O prior to LAS. Individual tree rings were not visible through the video camera system attached with a microscope, and so points were selected at regular intervals along each core with three parallel transects taken per core. After analysis, ablation holes were matched to the year of formation in each of the tree core sections. As a result, not all rings were sampled and tree rings contained between three and nine ablation

TABLE 2. Integrated Signal Intensities (Counts per Second) from a Wood Sample Analyzed by LAS ICP-MS of Isotopes Not Used for Calibration Curves[a]

isotope	signal intensity (cps) from wood	background signal intensity (cps)
⁵²Cr	417 138 (12908)	49 621 (1405)
⁵⁸Ni	14 226 (2064)	494 (273)
⁷⁵As	9700 (901)	195 (58)
¹²⁰Sn	57 768 (4857)	2671 (450)

[a] Background values are also shown. Values are means (standard deviation) from six ablations.

holes. Each wood section used for calibration was ablated six times.

Elan 5000 Operating Conditions. The laser was operated in Q-switch mode (8 ns pulse) at 55 J (beam output 15 mJ) with a Q-switch delay of 240 μs, using 40 pulses at 10.0 Hz. Samples were ablated for 15 s, although only integrated signal intensities between 2 and 10 s were used as signal intensities were smooth during this time period. Samples were analyzed for ⁴³Ca, ⁵²Cr, ⁵⁵Mn, ⁵⁸Ni, ⁶³Cu, ⁶⁴Zn, ⁷⁵As, ¹¹⁴Cd, ¹²⁰Sn, ²⁰²Hg, and ²⁰⁸Pb in addition to ¹³C. The beam diameter at the sample surface was approximately 50 μm, and hole sizes were around 70 μm. The beam was focused by eye to allow for unevenness of the sample, and ¹³C was used as an internal standard to correct for slight differences in ablation. Relationships between integrated signal intensities and concentration were tested by simple linear regression (*32*).

Results and Discussion

Concentrations of Trace Metals in Soil. Surface soil collected from beneath the stunted trees at Sudbury and Rouyn-Noranda was contaminated with several trace elements (Table 1). At Sudbury, concentrations of Ni, Cu, As, and Pb were much greater than concentrations normally found in surface soils (*33*) and which were found at an unpolluted site at Rollet. At Rouyn-Noranda, soil was heavily contaminated with Cu, Zn, As, Cd, and Pb. Soil at all three sites was acidic; pH values ranged from 3.3 at Rouyn-Noranda to 4.5 at Rollet. At these pH values, it is likely that "plant-available" concentrations of trace metals are also elevated in the surface soil (*33*). Previous studies have found that trees growing in soils polluted with metals contain higher concentrations of these trace elements in their rings (*1–3*).

Trace Metal Concentrations in Wood. Signal intensities were obtained for all 11 isotopes analyzed by LAS ICP-MS (Table 2). Holes produced from LAS were approximately 70 μm in diameter, and there was no evidence of scorching at the wood surface. Peaks in signal intensity, commonly observed during ablation of other biological structures (*34*), rarely occurred during ablation of the maple wood. As a result, strong linear relationships ($r^2 > 0.99$) were obtained between normalized integrated signal intensity and concentration in wood for ⁴³Ca, ⁵⁵Mn, ⁶³Cu, ⁶⁴Zn, ¹¹⁴Cd, ²⁰²Hg, and ²⁰⁸Pb (Figures 1–3). In addition, the RSD for these isotopes was <15% in all wood calibration standards.
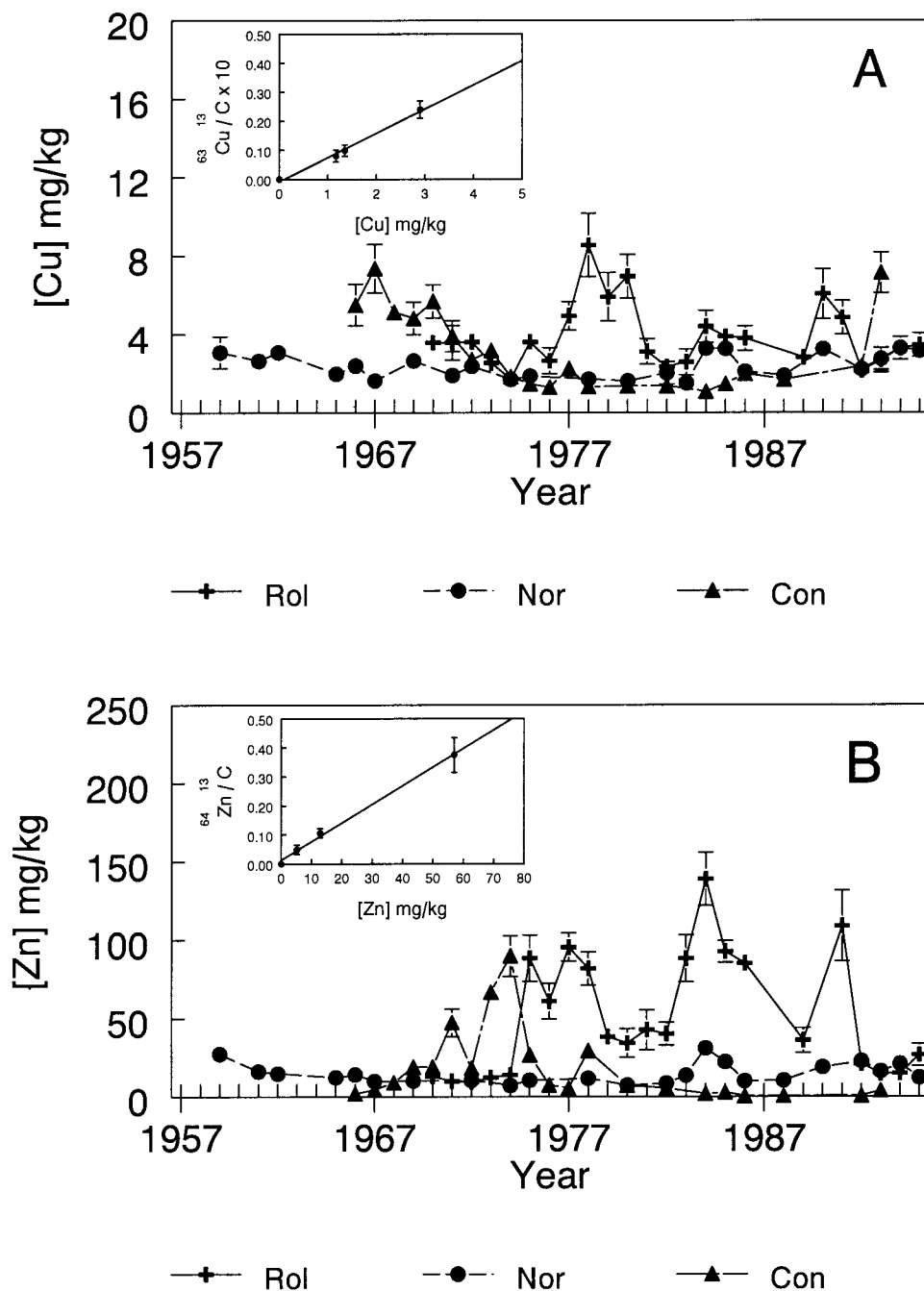
FIGURE 1. Copper (A) and zinc (B) concentrations in red maple tree cores from Rollet (Rol.), Rouyn-Noranda (Nor.), and Coniston, Sudbury (Con.) determined by LAS ICP-MS (B). Values are means ± standard deviation (n = 3−9). Relationships between normalized integrated signal intensities of ^{63}Cu and ^{64}Zn and respective Cu concentrations (mg kg^{-1}) (r^2 = 0.999) and Zn concentrations (mg kg^{-1}) (r^2 = 0.999) in wood calibration standards are included.

Calibration curves were not obtained for ^{52}Cr, ^{58}Ni, ^{75}As and ^{120}Sn because differences in concentration between the wood calibration standards were not found. Nevertheless, signal intensities were much higher than background levels, and RSDs of <15% were found for these isotopes, suggesting these elements may also be quantified in wood by LAS ICP-MS when suitable calibration standards are used. Potentially, LAS ICP-MS may now be applied to many elements present at a wide range of concentrations in maple wood. Analytical curves using a focused Nd:YAG laser are linear over 4 orders of magnitude (35), and previous studies have shown that linear relationships between signal intensity and element concentration in *Acer* wood exist for a number of elements (17, 24).

Copper concentrations were similar in rings from trees at Sudbury and Rouyn-Noranda formed since the mid 1970s (Figure 1). Concentrations were around 1.5 mg of Cu kg^{-1} during this period, but showed a tendency to increase toward the cambium, a natural radial tendency found in *Acer* (3). At Sudbury, however, Cu concentrations were much higher in tree rings formed in the late 1960s, but declined sharply during the early 1970s (from 7.0 mg of Cu kg^{-1} to 2.0 mg of Cu kg^{-1}), which coincides with the period when the Cu−Ni smelter at Coniston was closed. Although site histories should not be based on single trees, it is possible that this reflects a decrease in available Cu to the tree, due to either a reduction in uptake from soil or a reduction in Cu uptake through the bark or foliage (36). A similar reduction in Cu content of *Acer*
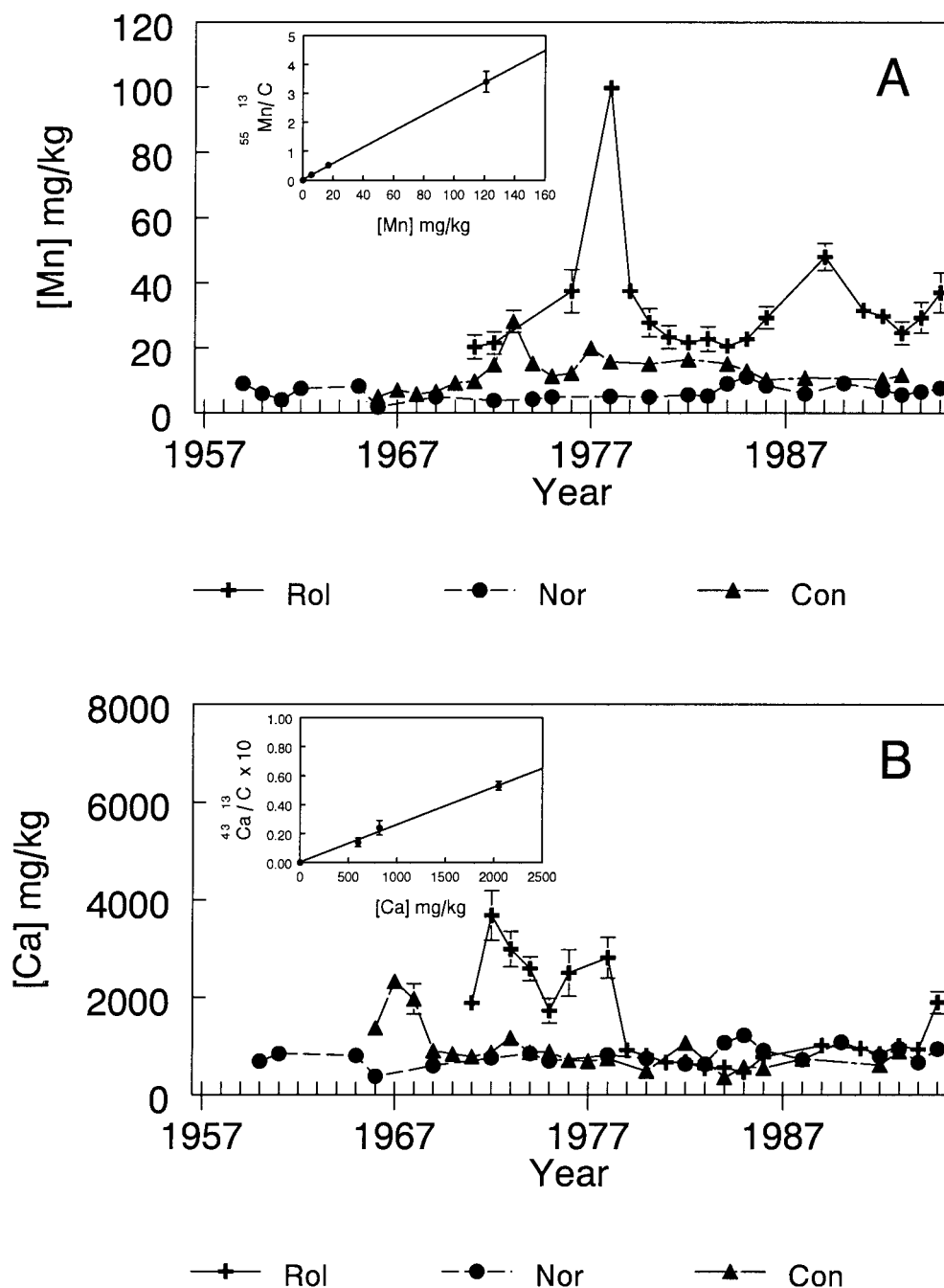
FIGURE 2. Calcium (A) and manganese (B) concentrations in red maple tree cores from Rollet (Rol.), Rouyn-Noranda (Nor.), and Coniston, Sudbury (Con.) determined by LAS ICP-MS (B). Values are means ± standard deviation ($n = 3-9$). Relationships between normalized integrated signal intensities of ^{43}Ca and ^{55}Mn and respective Ca concentrations (mg kg^{-1}) ($r^2 = 0.996$) and Mn concentrations (mg kg^{-1}) ($r^2 = 0.998$) in wood calibration standards are included.

pseudoplatanus L. (sycamore) tree rings was found in trees adjacent to a Cu refinery when emissions were reduced (*3*). Alternatively, the rings formed in the late 1960s were found toward the pith of the tree, and the elevated Cu may simply be due to natural radial tendencies or the young age of the tree when these rings were formed. More tree samples, from a number of different aged trees, are needed to clarify this relationship.

No pronounced radial tendencies in tree cores from Sudbury and Rouyn-Noranda were found for Mn, Ca, and Zn. Concentrations of Zn were higher in wood formed since 1980 at the Zn-contaminated Rouyn-Noranda site (around 20 mg of Zn kg^{-1}) compared to Sudbury. Sharp peaks in Zn concentrations (up to 95 mg of Zn kg^{-1}) in wood formed during the early 1970s were found in the tree core from

Sudbury, however (Figure 1). Soil at the Sudbury site is not contaminated by Zn, so the increase is more likely due to some aspect of tree physiology, rather than Zn pollution. Mn concentrations tended to be greater in tree rings at Sudbury than at Rouyn-Noranda in wood formed during the 1970s, although concentrations were similar in wood formed since 1983 (Figure 2). Similarly, Ca concentrations were comparable between trees from Sudbury and Rouyn-Noranda, with concentrations generally constant around 700 mg of Ca kg^{-1}, although a peak in Ca occurred in rings formed in the late 1960s at Sudbury (Figure 2).

Surprisingly, concentrations of Ca, Mn, Zn, and Cu were much higher in discolored areas of the tree core from Rollet, a site uncontaminated by trace metals. Concentrations were up to five times greater in discolored wood from Rollet than
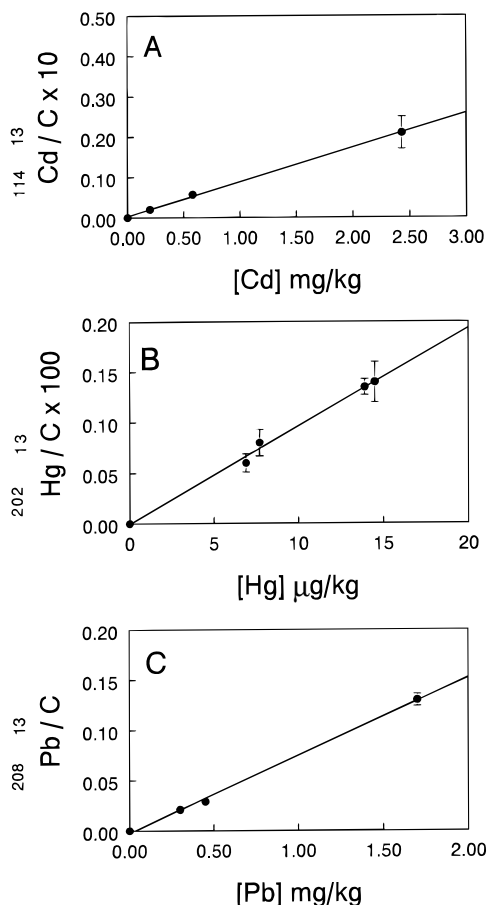
FIGURE 3. Relationships between normalized integrated signal intensities of [114]Cd and Cd concentrations (mg kg^{-1}) ($r^2 = 0.996$) (A); signal intensities of [202]Hg and Hg concentrations (μg kg^{-1}) ($r^2 = 0.996$) (B); and signal intensities of [208]Pb and Pb concentrations (mg kg^{-1}) ($r^2 = 0.999$) (C) in red maple wood calibration standards. Values are means $\pm$ standard deviation ($n = 6$).

in tree cores from the metal-polluted sites. The discoloration is most likely a result of physical damage and/or fungal infection (37−39). Infection of trees including red maple can occur after mechanical wounding of the tree in the root or buttress (38). Discoloration occurs prior to infection and is due to the physical effects of increased desiccation and aeration (38). Infection by a number of fungi and bacteria may occur (39−40), and the tree responds by forming a column boundary layer which restricts the spread of infection (38, 41−42). This green-colored column boundary layer and discolored wood in general have been shown to contain greatly elevated concentrations of several organic ions and inorganic anions and cations (37−38). Concentrations may be so high as to promote symptoms of cation deficiency in severely infected trees followed by visible signs of decline (38). In the present study, elevated concentrations of Ca, Mn, Cu, and Zn (all essential elements) are probably a result of fungal infection and indicate that decayed or discolored wood should be avoided in dendrochemical studies concerned with environmental monitoring.

Concentrations of Pb, Cd, and Hg in rings of sampled trees were low (data not shown), and no marked differences between trees were found. It is evident that low levels of trace metals are detectable in wood analyzed by LAS ICP-MS; concentrations as low as 0.1 mg of Cd kg^{-1}, 0.1 mg of Pb kg^{-1}, and 0.005 mg of Hg kg^{-1} could easily be detected in red maple wood. These concentrations are well below concentrations reported in the vast majority of dendrochemical studies, for many tree species growing in both rural

and urban areas (43−45). Although several studies have reported on changes in Pb and Cd in tree rings, far fewer studies with trace metals such as Hg exist, probably due to their low concentrations in wood. For instance, Hg concentrations in black spruce (*Picea mariana* Mill. B. S. P.) rings in trees growing in northern Quebec ranged from 13 to 37 ng g^{-1} (46). To detect Hg in wood, 0.4 g samples were required for analysis. Using LAS ICP-MS, low detection levels for many elements in extremely small wood samples may be achieved.

In conclusion, LAS ICP-MS may be used to quantitatively analyze elements in red maple tree rings. Variation in element distribution within individual tree rings was low for all 11 elements analyzed in the present study. Linear relationships between normalized integrated signal intensities and element concentration in wood were found over a wide range of element concentrations. To produce calibration curves, however, element concentrations must differ between wood calibration standards. Suitable wood calibration standards can be obtained by sampling trees in clean and contaminated areas and by sampling different aged wood, utilizing natural radial tendencies in concentrations from pith to cambium which are found for some elements. This technique is rapid, fairly inexpensive, involves minimal sample preparation, and is capable of high spatial resolution with extremely low detection limits. Potentially, LAS ICP-MS can be used to analyze wood of any tree species, although its application will depend on the element homogeneity within annual tree rings of the test species.

Acknowledgments

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