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Spatial Distribution of Trace Elements in Black Spruce by Imaging Microprobe Secondary Ion Mass Spectrometry

J.H.E. BAILEY and D.W. REEVE

Imaging Microprobe Secondary Ion Mass Spectrometry (SIMS) has been used to determine the spatial distribution of metal ions in black spruce, Picea mariana *(Mill.). Calcium, manganese, chromium, iron and zinc were found in high concentration in several morphological features: the torus, middle lamella, cell corners, the ray cell wall and a ray cell deposit. A profile of the metal ion intensity across a double cell wall was determined.*

INTRODUCTION

The concentration and distribution of inorganic elements in wood is of interest in a wide variety of research areas. A small concentration can result in a relatively large biological and/or chemical effect. The inorganic content of wood is often collectively described as the "ash content", which is generally 0.1–0.5% of the o.d. weight of wood. Potassium and the alkaline earth metals, calcium and magnesium, constitute 70–80% of the total ash content. The remainder is composed of a variety of elements. For example, 32 elements were identified in grand fir [1]. A wealth of information can be found in the literature on the inorganic constituents in wood and bark, however, there are only a few reports on their morphological distribution [2-13]. Most of these studies have employed ashing techniques, raising questions as to the precision of these measurements due to the difficulty in separating the various morphological regions to give quantities of tissue large enough and pure enough for accurate ashing. Several authors have employed techniques such as TEM-EDXA (transmission electron microscopy–energy dispersive X-ray analysis) [14], PIXE (proton-induced X-ray emission spectroscopy) [15-17] and LAMMA (laser microprobe mass analyzer) [18]. SIMS has the unique combination of high spatial resolution and high sensitivity.

The present research was initiated to investigate the spatial distribution of metal ions that influence catalysis of hydrogen peroxide reactions in order to better understand and, perhaps, to improve hydrogen peroxide brightening of mechanical pulps. The following describes the use of Imaging Microprobe SIMS, in generating elemental maps representative of the different morphological features in a black spruce fibre. The method of sample preparation is given in detail.

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SAMPLE PREPARATION

Samples of black spruce heartwood and sapwood, including both springwood and summerwood, were cut into strips approximately 4 mm x 2 mm x 0.5 mm. Each sample was soaked for 0.5 h in water, then 0.5 h in 50/50 ethanol/water, and then 2 h in ethanol. This was followed by the introduction of propylene oxide in a 25/75 ratio with ethanol. The samples were soaked for 0.5 h. The concentration of propylene oxide was increased to 50%, 75% and finally 100%. Samples were soaked for 1 h in each. Approximately 23 g of resin were freshly prepared from 5.03 g of diglycidyl ether of polypropylene glycol (DER), 5.03 g of vinylcyclohexene dioxide (ERL), 12.8 g of nonenyl succinnic anhydride (NSA) and 0.80 g of dimethylaminoethanol (S-1). Infiltration with resin was achieved by successively soaking the samples for 5 h in 25/75 and then 50/50 resin/propylene oxide, followed by 12 h in 75/25 resin/propylene oxide and then 100% resin. In order to avoid exceeding the 3-day shelf life of the resin, a fresh mixture was prepared for the remainder of the procedure; 4.77 g DER, 5.13 g ERL, 12.4 g NSA and 0.64 g S-1. The samples were placed under vacuum in 100% resin for 48 h, after which they were cured at 60°C for 16–24 h.

The resin blocks were cut and mounted for transverse or tangential sectioning. Facing was achieved with the use of an ultramicrotome equipped with either a glass knife or a diamond knife. Once facing was complete, the blocks were washed with ethanol and allowed to dry. A carbon paint was applied to each block leaving only the sample surface exposed. Immediately prior to analysis, samples were annealed for a minimum of 12 h in a small oven maintained at approximately 40°C and 30 psi. Each block was carbon painted to the sample holder to ensure electrical grounding.

quite the pretreatment

MATERIALS

All solvents were used as purchased from Aldrich Chemical Company. A Spurr resin kit TK4 was purchased from Marivac Ltd., Nova Scotia and a release agent was supplied by Buehler. Water was obtained from a Barnstead Nanapure II system. Mounting, trimming and facing of samples were performed at the Electron Microscopy Laboratory in the Medical Sciences Department at the University of Toronto. A Porter Blum MT-2 ultramicrotome was used for

 I'm not sure about all the soaking here.

TABLE I
TRACE ELEMENT CONCENTRATION IN BLACK SPRUCE, BY NEUTRON ACTIVATION ANALYSIS (NAA) AND INDUCTIVELY COUPLED ARGON PLASMA—ATOMIC EMISSION SPECTROSCOPY (ICAP)

Element	Technique	Concentration (ppm)	
		sapwood	heartwood
Calcium	NAA	970	1200
	ICAP	940	1200
Manganese	NAA	79	110
	ICAP	78	100
Iron	ICAP	32	21
Chromium	ICAP	1.8	1.8
Copper	NAA	n.d. [1.4]	n.d. [2.5]
	ICAP	3	3.2
Zinc	NAA	n.d. [230]	n.d. [380]
	ICAP	13	14
Potassium	NAA	n.d. [250]	n.d. [430]
	ICAP	690	260
Aluminum	NAA	31	5.3
	ICAP	14	15
Chlorine	NAA	130	44
Barium	ICAP	8.7	13
Magnesium	ICAP	130	150
Strontium	ICAP	4.4	6.3
Titanium	NAA	n.d. [4.3]	n.d. [6.2]
	ICAP	0.6	0.8
Silver	ICAP	n.d. [0.5]	n.d. [0.5]
Beryllium	ICAP	n.d. [0.05]	n.d. [0.05]
Cadmium	ICAP	n.d. [0.5]	n.d. [0.5]
Cobalt	ICAP	n.d. [5]	n.d. [5]
Molybdenum	ICAP	n.d. [5]	n.d. [5]
Nickel	NAA	n.d. [340]	n.d. [600]
	ICAP	n.d. [5]	n.d. [5]
Phosphorus	ICAP	50	n.d. [50]
Lead	ICAP	n.d. [5]	n.d. [5]
Thorium	ICAP	n.d. [5]	n.d. [5]
Vanadium	ICAP	n.d. [0.5]	n.d. [0.5]
Zirconium	ICAP	n.d. [5]	n.d. [5]
Iodine	NAA	n.d. [0.19]	n.d. [0.33]

n.d. - not detected
[ ] - detection limit

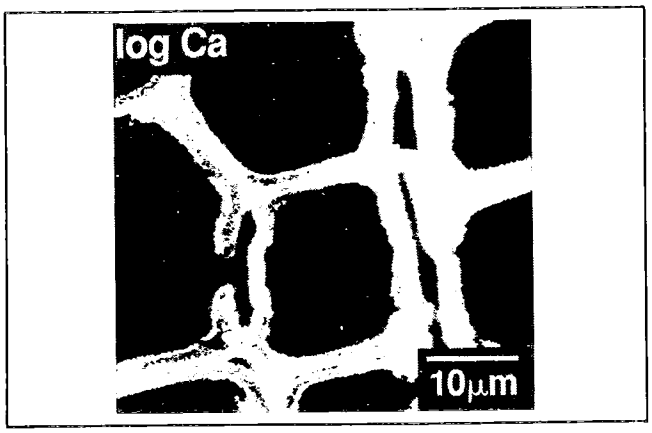

Fig. 1. Ca ion image from a transverse section of black spruce sapwood showing several tracheids, an intertracheid pit pair containing a torus, and a ray parenchyma cell in a 50 μm field of view.

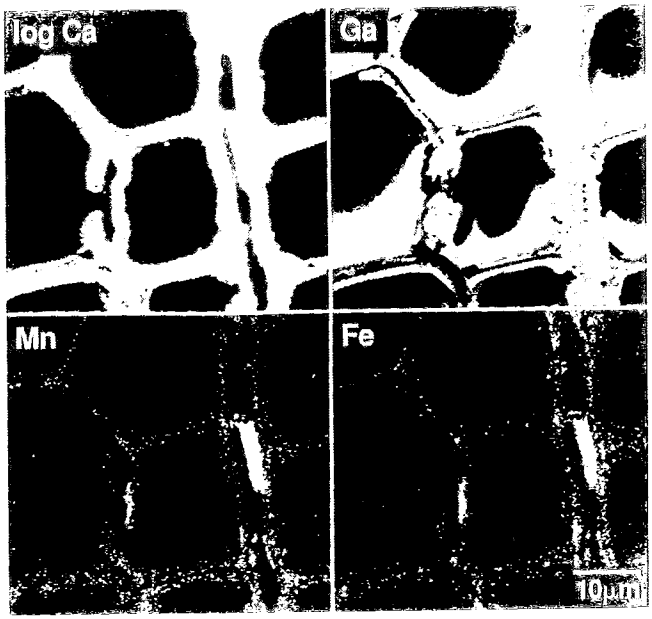

Fig. 2. Ca, Ga, Mn and Fe ion images from the transverse section of Fig. 1. The Ga image was generated by the injection of gallium ions into the sample.

facing samples. A DIATOME 3 mm diamond knife was used and glass knives were prepared as required.

ANALYTICAL METHODS

Elemental analysis on bulk samples was performed using Neutron Activation Analysis (NAA) and Inductively Coupled Argon Plasma—Atomic Emission Spectroscopy (ICAP). NAA analysis facilities at the University of Toronto were used. Samples were weighed and sealed in 1 mL polyethylene vials. A 10 kW, 300 s irradiation was used with a 2 min delay prior to 5 min of counting. ICAP analysis was performed by Barringers Laboratories of Mississauga, Ontario. Samples were digested in 16 M HNO_3 followed by evaporation and digestion in 0.8 M nitric acid prior to analysis.

Microprobe SIMS analysis was performed on a Perkin Elmer PHI 5500 series multitechnique instrument. The analyzer was a Balzers 16 mm rod quadrapole mass spectrometer. An FEI liquid gallium ion gun was used to produce a 25 keV and 300 nA ion beam, approximately 130 nm in diameter. A system vacuum of approximately 5×10^{-8} torr was maintained during analysis. The sputter rate was approximately 1.5×10^{-10} atoms/s. The practical spatial resolution approaches 0.2 μm and the sensitivity varies with the ion of interest. The instrument is located at the Institute for Microstructural Sciences, National Research Council of Canada, Ottawa, Ontario. SIMS analysis was performed by Dr. Don Mitchell.

RESULTS AND DISCUSSION

Samples of black spruce sapwood and heartwood were tested for Al, Ca, K, Mn, Ni, Zn, Ti, Cu, Cl and I by NAA and for 23 elements by ICAP analysis. The results are given in Table I. With the exception of iron and potassium, all elements determined were more concentrated in the heartwood than in the sapwood. A. Wong determined the content of ten elements in black spruce wood by atomic absorption [19]. He reported 1054 ppm Ca, 227 ppm Mn, 80 ppm Cd, 258 ppm K, 15 ppm Fe and 109 ppm Mg, most of which are similar to the present results with the exception of cadmium which was unusually high in Wong's analysis. Another example of the wide range of results noted in the literature comes from a study of black spruce by TEM-EDXA [14]. The authors report values for Cu and Zn consid-

erably higher than ours, while values for Ca and Fe are lower. We attribute these variations to the various environmental conditions of each area of tree growth.

Ion images were obtained on a Perkin Elmer PHI 5500 series multitechnique instrument using an FEI liquid gallium ion gun. A Ca image obtained from a transverse section of black spruce sapwood is shown in Fig. 1. The field of view is 50 μm square and is composed of 256 x 256 pixels. The logarithm of the Ca signal intensity is displayed because of the high-intensity ray cell deposit. Enrichment can be clearly seen in the torus of an intertracheid pit pair, middle lamella, cell corner, ray cell wall and ray cell deposit. These findings correlate well with Saka and Goring's study on the distribution of inorganic constituents in black spruce wood by TEM-EDXA [14]. Calcium was detected in all morphological regions of the fibre, with localized concentrations reaching 8300 ppm in the torus. The torus plays an important role in both the transport of elements through the cell and in the screening of toxic substances which enter the cell. It has been demonstrated by enzymatic degradation of softwoods that the pit membrane region is high in pectin content [20-22]. Enrichment in the middle lamella may be related in part to the organic composition of the cell wall components. It has been stated that, in addition to cellulose and hemicelluloses, the compound middle lamella also contains a high concentration of pectin and glycoprotein [23-24]. Pectin is a good chelator of Ca^{2+} and acts as a selective binder for Ca^{2+} ions in unlignified tissue. Westermark has proposed a mechanism of lignification in the plant cell walls in which calcium stabilizes a superoxide radical for phenolic coupling of coniferyl alcohol [25]. No reaction occurs without stabilization of the superoxide radical by the presence of calcium. Westermark and coworkers also found that, during the lignification process, pectin is removed or degraded, generating a high concentration of Ca^{2+} ions [26].

Figure 2 contains elemental maps obtained for gallium, manganese and iron for the same field of view as in Fig. 1. The Ga image is generated by the injection of gallium ions into the sample during sputtering and gives a representation of the topology of the analyzed surface. The pattern of enrichment of manganese and iron is similar to that of calcium with enrichment in the torus, middle lamella, cell corners, ray cell wall and the rectangular deposit in the ray cell. Distribution studies on Mn to date have dealt only with annual growth ring and sapwood/heartwood variations [8, 11, 27]. In Saka and Goring's study, Mn was undetectable by TEM-EDXA [14]. This is the first report of its kind that establishes the presence of Mn in several morphological features of a wood fibre. In addition to calcium, manganese and iron, the torus was also found to be highly enriched in chromium and zinc. In the work of Saka and Goring, copper was reported to be highly concentrated in the torus [14]. In our study, copper

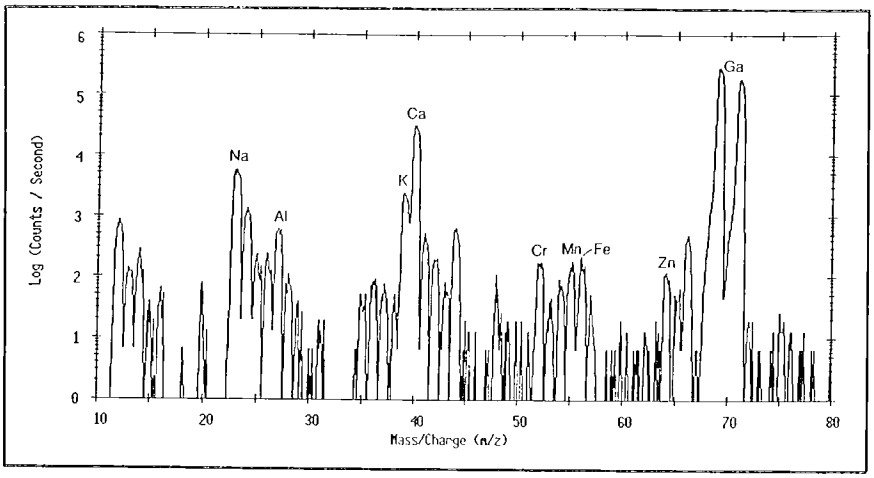

Fig. 3. Positive mass survey of the ray cell deposit seen in Fig. 1.

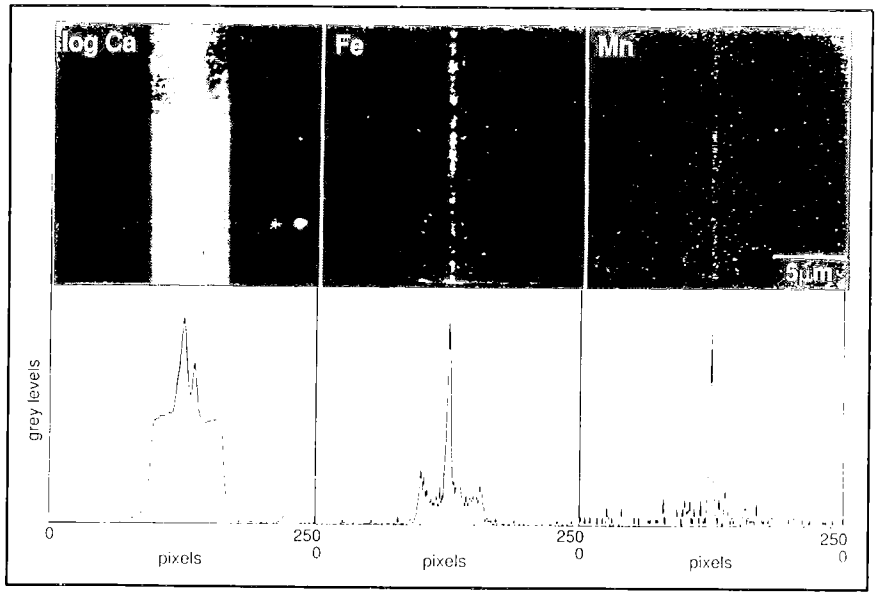

Fig. 4. Ion images obtained for Ca, Fe and Mn from a tangential section of a double cell wall of black spruce heartwood including the corresponding ion intensities, 20 μm field of view.

is not detected in any morphological feature analyzed.

A positive mass survey was taken of the ray cell deposit from Fig. 1. The mass survey of the ray cell, centred in a 33 μm field of view, is displayed in Fig. 3. The presence of several metal ions can be identified from the mass/charge ratios. Characteristic peaks include: Ca, 40; Fe, 56; Mn, 55; Zn, 64; Cr, 52; Al, 27; Na, 23 and K, 39. Images were obtained for calcium, iron, manganese, zinc, chromium, aluminum, nickel, copper and silicon. A high concentration of each element was seen in the ray cell deposit with the exception of nickel, copper and silicon.

Images were obtained for a tangential section of black spruce heartwood containing two adjacent tracheid cell walls. Counts were accumulated along a band 100 pixels wide running across the double cell wall. The grey levels were plotted against the horizontal pixel number. The images and a plot of grey levels vs. horizontal pixel number for calcium, iron and manganese are shown in Fig. 4. Calcium and, to a lesser extent, iron are of uniform concentration across the secondary wall. In contrast, manganese is not seen in the secondary wall. The middle lamella is enriched in all three elements. Fergus et al. determined the distribution of lignin in black spruce tracheids by UV microscopy [28]. Using densitometer tracking through the double cell wall, they concluded that the concentration of lignin in the middle lamella is considerably higher than in the secondary wall, across which it is of uniform concentration. This pattern is identical to that seen for calcium in our investigation. This suggests that the distribution of calcium and to a lesser extent iron is associated with that of lignin.

CONCLUSIONS

Imaging Microprobe SIMS can be used to determine the spatial distribution of metal ions in wood. Eight elements were identified in different morphological features. Calcium, manganese, iron, chromium and zinc were found in the tori, middle lamella, cell corners and ray cell wall. A ray

fine, but what about annual resolution?

cell deposit was found to contain Ca, Mn, Fe, Cr, Zn, Al, Na and K. The distribution of calcium, iron and manganese correlates well with that reported for lignin across a double cell wall.

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ABSTRACT: Imaging Microprobe Secondary Ion Mass Spectrometry (SIMS) has been used to determine the spatial distribution of metal ions in black spruce, *Picea mariana* (Mill.). Calcium, manganese, chromium, iron and zinc were found in high concentration in several morphological features: the torus, middle lamella, cell corners, the ray cell wall and a ray cell deposit. A profile of the metal ion intensity across a double cell wall was determined.

RÉSUMÉ: Nous avons fait appel à un spectromètre de masse aux ions secondaires à microsonde à formation d'images pour déterminer la répartition spatiale des ions métalliques dans l'épinette noire, *Picea mariana*. De fortes concentrations de calcium, manganèse, chrome, fer et zinc ont été découvertes dans plusieurs caractéristiques morphologiques: le tore, la lamelle médiane, les coins de cellule, la paroi du rayon médullaire et un dépôt du rayon médullaire. Nous avons par ailleurs déterminé le profil d'intensité des ions métalliques à travers la double paroi d'une cellule.

KEYWORDS: TRACE ELEMENTS, DISTRIBUTION, PICEA MARIANA, MASS SPECTROSCOPY, SPECTROMETERS, IONS, METALS, CELL WALLS, RAY CELLS, BORDERED PITS, MIDDLE LAMELLAE.