SEM X-RAY ANALYSIS OF PENTACHLOROPHENOL IN TREATED WOOD[1]

Research Note

Joseph B. Zicherman
Assistant Specialist, University of California, Forest Products Laboratory, Richmond, CA 94804

(Received 27 January 1975)

ABSTRACT

The distribution of pentachlorophenol preservative in cell walls of Douglas-fir pressure treated either by the Cellon[2] process, or with pentachlorophenol in light petroleum oil, was studied using scanning electron microscopy-energy dispersive X-ray analysis (SEM-EDXA). Results indicate that both preservative treatments caused pentachlorophenol to be deposited throughout the gross structure of the wood and within cell walls as well. Extraction with benzene failed to remove the pentachlorophenol deposited within cell walls, as indicated by SEM-EDXA results, although the preservative had been applied with nonpolar organic carriers.

Additional keywords: *Pseudotsuga menziesii*, Cellon process, energy-dispersive X-ray analysis (EDXA), preservative distribution, (SEM), wood preservation.

INTRODUCTION

Observation of water-borne preservatives in wood cell walls has been accomplished using several methods. For example, Rudman (1966) and Davies (1968) used transmission electron microscopy (TEM), while Petty and Preston (1968) and Chou and coworkers (1973) made use of electron probe microanalysis. Recently Greaves (1974) utilized scanning electron microscopy-energy dispersive X-ray analysis (SEM-EDXA) to locate such materials. Attempts to directly observe pentachlorophenol (PCP) applied by nonpolar treating solvents using TEM (Walters and Côté 1960) and SEM (Resch and Arganbright 1971), however, have been largely inconclusive. The problems in electron microscopic imaging of PCP involve its solubility in commonly used embedding media and its relative lack of contrast. Water-borne preservatives (generally metal salts) show high relative contrast in a wood substrate and are insoluble in common embedding media. In a recent TEM study by Wilcox and Parameswaran (1974), crystal deposits were observed throughout the cell walls of ultrathin sections of Cellon-treated Douglas-fir that had been fixed with $KMnO_4$. The electron-dense crystals observed were interpreted as a reaction product of PCP with the $KMnO_4$.

Using SEM at this laboratory, Resch and Arganbright (1971) were unable directly to observe PCP in Cellon-treated specimens. Similar results occurred in the initial phases of this study, where an SEM examination of specimens pressure-treated with PCP by the Cellon process and PCP in petroleum oil (PO) did not show any surface deposits.

Other techniques, such as electron probe microanalysis and the extraction of specimens with nonswelling solvents followed by quantitative analysis for PCP, have indicated its presence within cell walls (Resch and Arganbright 1971; Arganbright 1973; Leutritz 1971). A micro-bioassay technique has shown that at conventional treatment levels, PCP toxicity with respect to decay microorganisms remains after extraction

[1] The author expresses his appreciation for the assistance of Dr. D. G. Arganbright of this Institution and Dr. R. Falk and Dr. Dan Hess of the University of California, Davis, in preparing this study, and to NSF grant GB-29653 for the purchase of the SEM-EDXA equipment used.

[2] Cellon is a registered trademark of the Koppers Company.

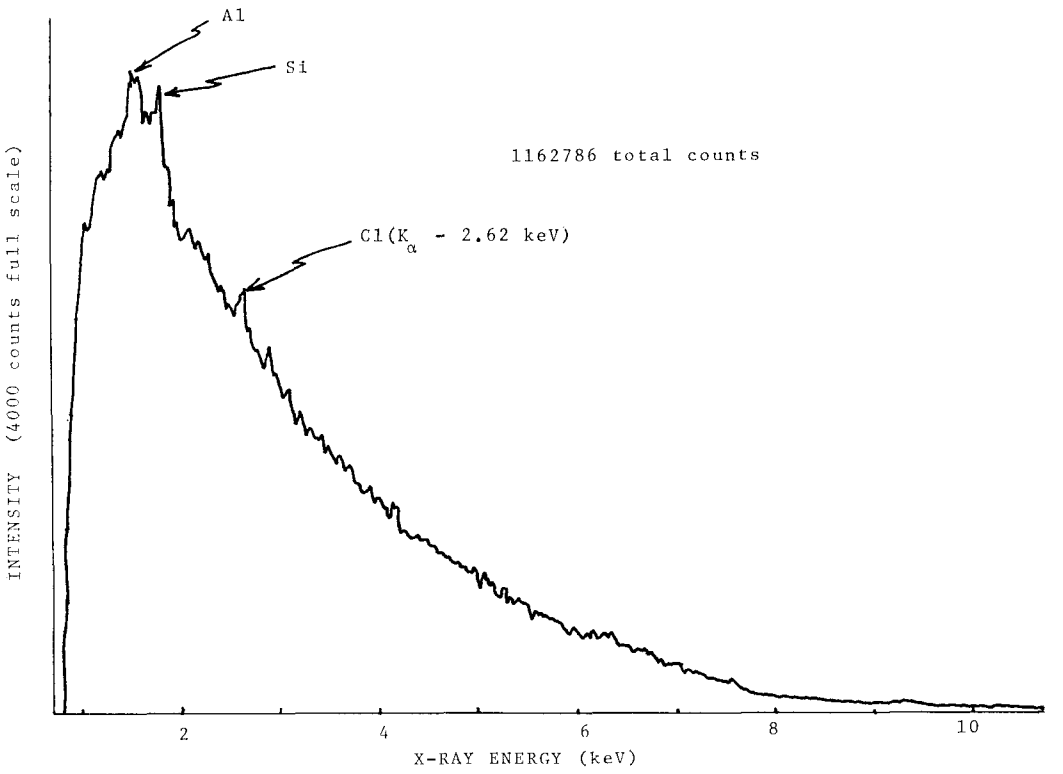

FIG. 1. X-ray energy spectra for control. 600-second analysis; 260×; 0° tilt angle (all angles are nominal)

treatment of wood with nonswelling solvents (Wilcox 1975).

Given the lack of success in directly imaging PCP in wood by TEM and SEM, it was decided to utilize SEM-EDXA. This technique is based on emission of characteristic X-ray energies when an element is bombarded by an electron beam. In the case of the SEM-EDXA system, an electron beam scanning the specimen causes the X-ray emission and a suitable detector and computer system are used to sense and process X-ray data. System output can be presented as a map for an element over the area scanned, a line scan showing the X-ray counts generated by an element over a single profile traced across a surface, or by numerical data for examinations of specific areas. X-ray element maps can be combined with secondary electron micrographs to provide a measure of spatial distribution for the element under study.

The subject of X-ray analysis for general biological materials has been reviewed in depth by Russ (1974) and discussed as a diagnostic tool in wood science by Gray and Côté (1974). The reader is referred to these sources for further discussions of the technique.

In this study SEM-EDXA was used to examine the occurrence of PCP in wood after pressure treatment with the nonswelling carrier solvents used in (a) the Cellon process and (b) pentachlorophenol applied from a petroleum oil carrier.

MATERIALS AND METHODS

Sample materials of Douglas-fir (*Pseudotsuga menziesii*) were taken from 2 inch × 4 inch studs, pressure treated either by the Cellon process (PCP dissolved in liquefied butane) or PCP dissolved in light petroleum oil (PCP-PO). Several wafers (6 mm longitudinal dimensions) from each

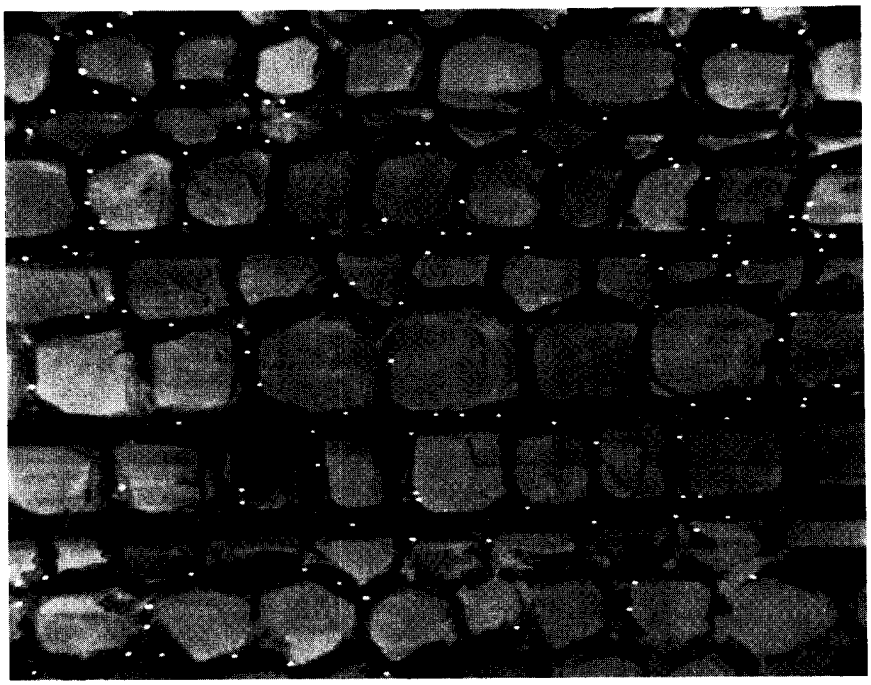

FIG. 2. Element map for Cl control. 600-second analysis; 260×; 0° tilt angle.

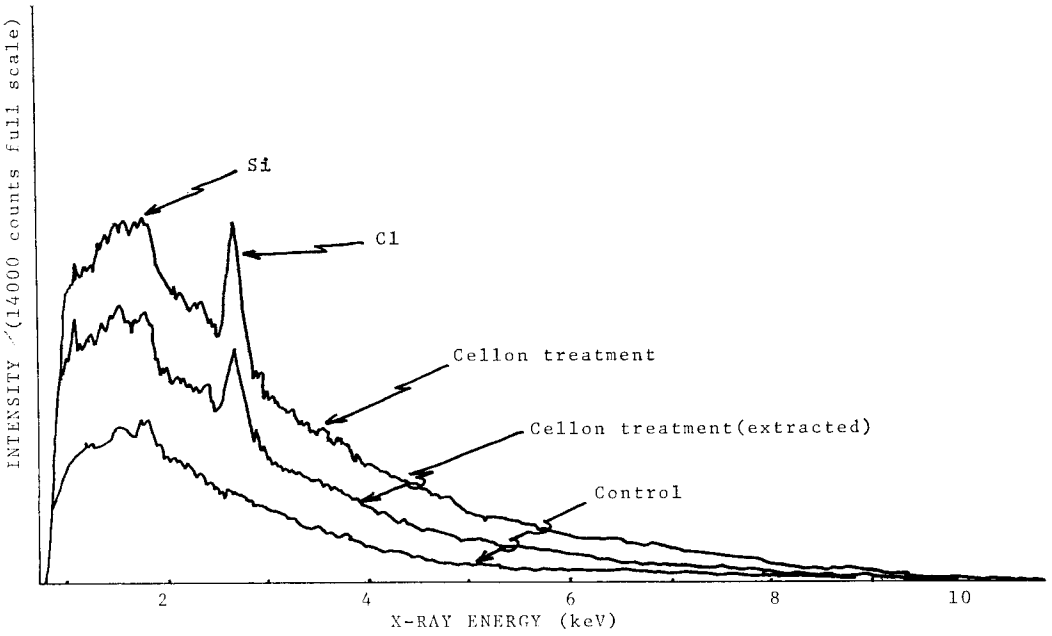

FIG. 3—Curve A—Control spectra. (400-second analysis; 220×; 40° tilt)
Curve B—Cellon-treatment spectra.
Curve C—Benzene-extracted, Cellon-treatment spectra.

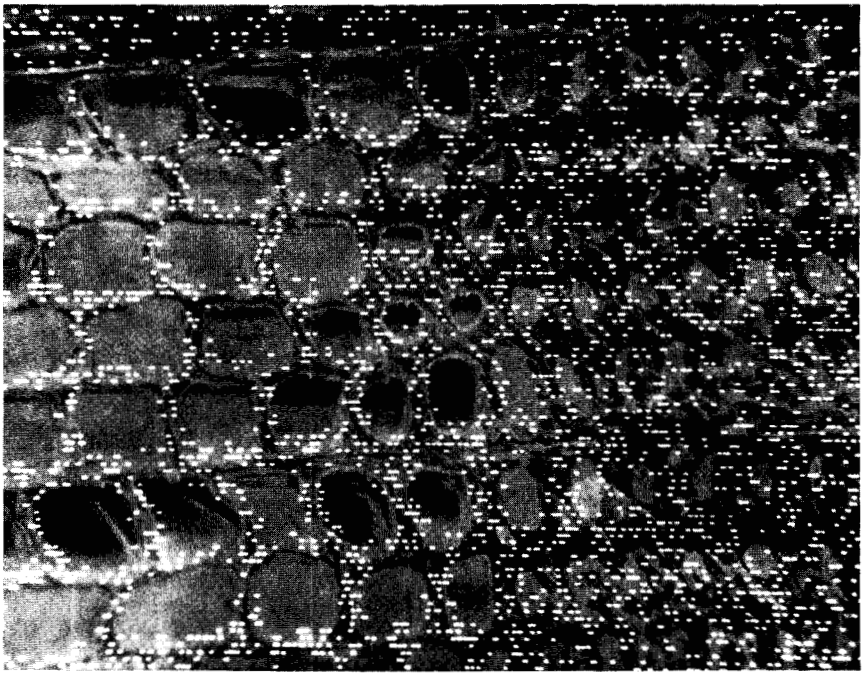

FIG. 4. Cl element map, Cellon treatment. 400-second analysis; 250×; 40° tilt.

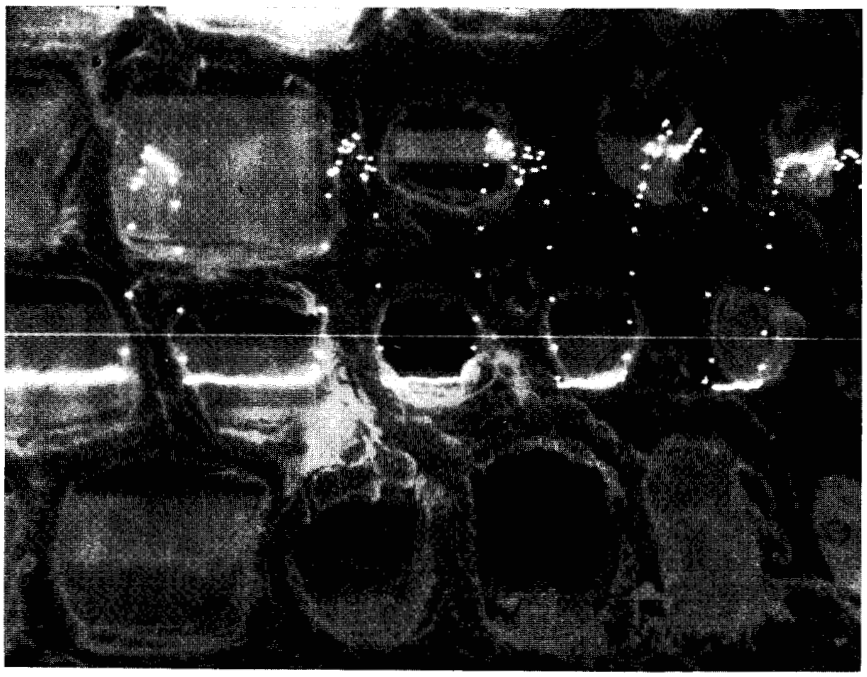

FIG. 5. Cl line scan, Cellon treatment. 400-second analysis; 625×; 40° tilt.

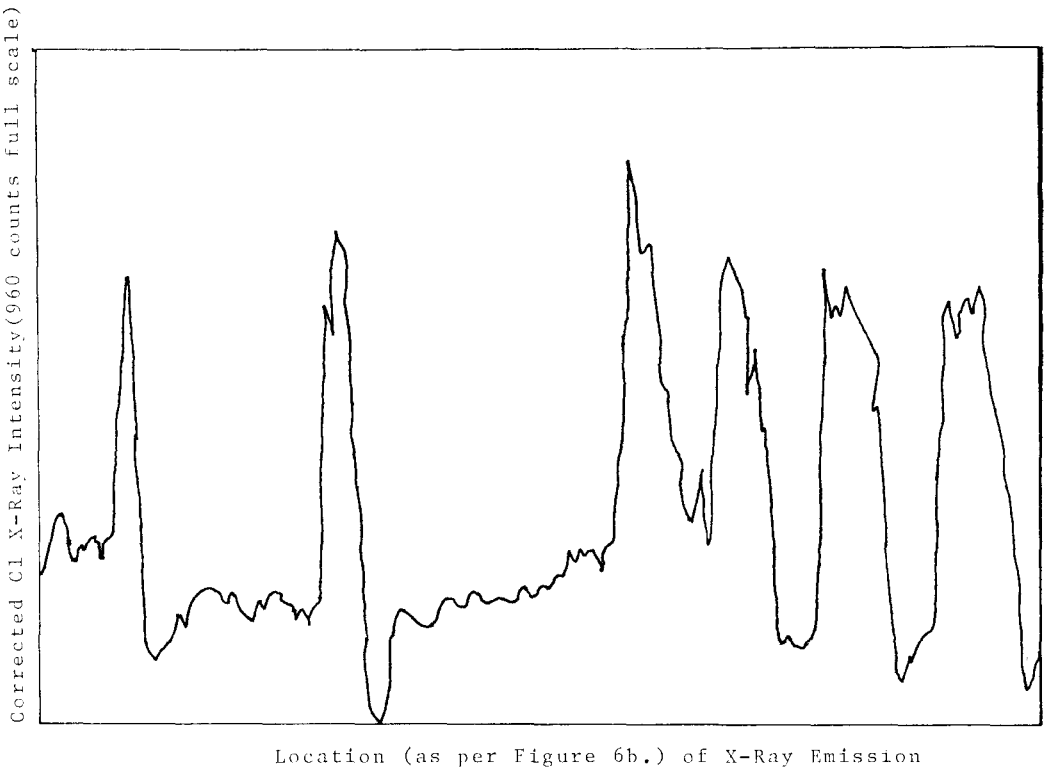

FIG. 6A. Corrected Cl line scan for benzene-extracted, Cellon-treated specimen shown in Fig. 6B. 400-second analysis; 570×; 0° tilt.

treatment type were extracted by reflux in benzene for 24 h (Arganbright 1973). General treatment effectiveness was assessed, using duplicate specimens of extracted and unextracted PCP-PO-treated wood. These materials were ground, ashed, dissolved in acid and quantitatively analyzed for PCP content by titration for chloride ion. Average PCP contents (weight basis) were 2.8% for nonextracted and 0.7% for extracted samples.

Since common embedding media might solubilize the PCP in microscopy specimens, their use was avoided. Instead, 5-mm cubes were prepared by cutting viewing surfaces with a razor blade as described by Exley and co-workers (1974). After mounting on stubs, specimen blocks were coated with a thin layer of carbon using standard vacuum-evaporation techniques. The conductive metal coating normally used in SEM studies of wood was not used since it would interfere with the X-ray analysis.

SEM-EDXA was performed on a Cambridge Stereoscan S-4 equipped with a 10-mm^2 Kevex Si(Li) X-ray detector having a resolution of 157 eV at 5.9 keV. A 0.3-mil Be window protected the detector. Analyses were performed at 10 keV; emission current was maintained at 150μA and the detector was positioned 48 mm from the sample. A Quanta-Metrix[3], Model 80-S computer system was used for data processing. CRT modulation for X-ray element mapping was modified in order to allow continuous modulation (as opposed to pulse rate modulation) when a preset count rate of Cl K_α X-rays was exceeded. In addition a "contrast-factor" function of the computer-based system was used to enhance element

[3] Finnegan Corporation, Sunnyvale, CA.

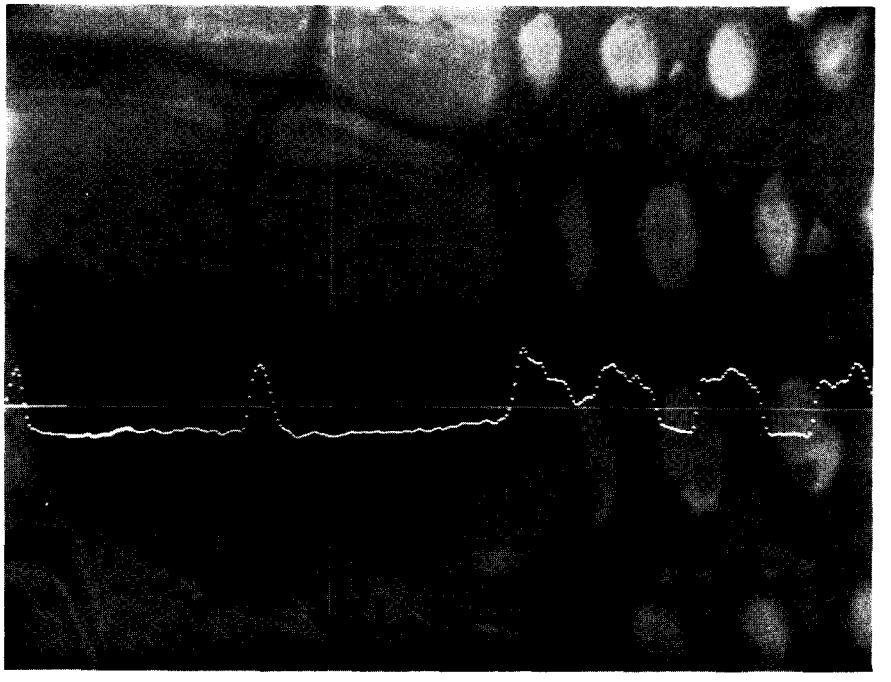

FIG. 6B. Cl line scan, benzene-extracted, Cellon-treated specimen. 400-second analysis; 570×; 0° tilt.

maps (not to be confused with an "image-enhancer" allowing for CRT modulation only above preset count rates) which removed background from X-ray maps produced. While such a system produces minimal modulation for Cl-free regions, it also rejects real X-ray counts permitting only a qualitative interpretation of data.

RESULTS AND DISCUSSION

To insure that no contamination of specimens had occurred in preparation, X-ray spectra of surfaces of both cut and fractured controls were run. The resulting spectra were not significantly different. The spectra of X-ray energies emitted by a control is presented in Fig. 1, with the area scanned to produce that spectra being shown in Fig. 2. An element map for Cl X-rays only is superimposed on the secondary electron mode image shown in Fig. 2. These results give an indication of levels of Cl occurring naturally in controls for treated specimens studied.

The energy spectra shown in Fig. 3, taken under identical operating conditions, compare the effect of both the Cellon treatment and the benzene extraction of Cellon-treated wood. The curves indicate the most Cl, when compared to background, to be present in the unextracted material, although Cl is still present in excess of naturally occurring levels in the extracted specimen. This confirms the observation that significant quantities of PCP, which are inaccessible to subsequent extraction, are added to the wood by the nonswelling treating solvents used. Figure 4 is a micrograph showing preservative occurrence in a Cellon-treated specimen through an element map for Cl. Figure 5 also shows a Cellon-treated specimen but incorporates a line scan for Cl. In this case the horizontal line indicates the profile scanned in the EDXA with the X-ray data generated rising and falling about that line as the scan encounters cell wall and lumen areas.

Figure A is a line scan profile for Cl in

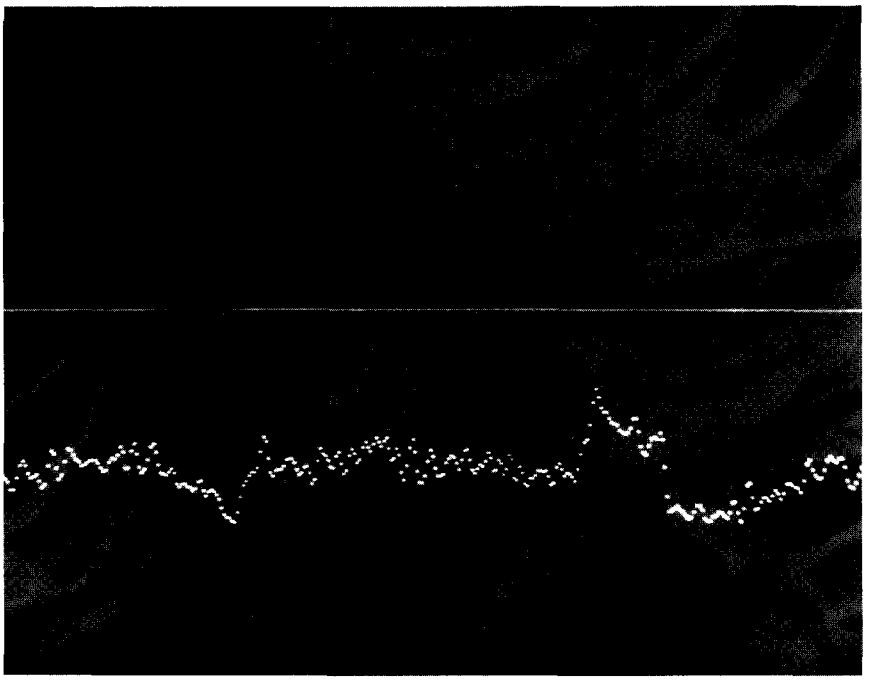

FIG. 7. Cl line scan, Cellon-treated specimen. 400-second analysis; 1000×; 40° tilt.

an extracted Cellon specimen that has been corrected for background. The correction was performed by collecting background count data in the computer memory above (2.42 keV) and below (2.69 keV) the chlorine K_α peak (2.62 keV). These data were then averaged and subtracted from the 512 corresponding point counts for Cl across the profile scanned. The original, uncorrected line scan (Fig. 6B) shows the relative occurrence of chlorine in the extracted, Cellon-treated wood.

Two signal minima occur in Fig. 6A immediately to the right of the first two springwood compound cell walls traversed. These minima are felt to result from specimen topography, which blocks off X-rays generated there (generally background radiation, also called brehmmstrahlung) and prevents their reaching the detector that was positioned to the upper left of the field of view. Such topographical effects are known in SEM/EDXA research and must be very carefully considered when attempting to interpret results. Figure 7, a radial view of a Cellon-treated specimen, presents a good example of such effects. In scanning over a pit aperture (arrow), the X-ray signal drops at the aperture and in the surrounding area as well. When traversing the excised cell-wall section (center right), signals increase most at the left edge as they reach the detector with greater efficiency. Such a peak, generated by a single scan of a rough surface, cannot be used to indicate a higher level of PCP in the wall as opposed to surrounding areas. Topographical effects in biological specimens were discussed by Hess et al. (1975) using rice hulls as well as a pure copper granule that had been acid-etched to yield a rough, but homogeneous surface. In both materials, variations in X-ray signals were shown to be topographical in nature.

The results found for the PCP-PO treatment again showed the preservative to be present within cell walls both before and after extraction with benzene. Figure 8, the energy spectra for unextracted and extracted PCP-PO treated specimens, also

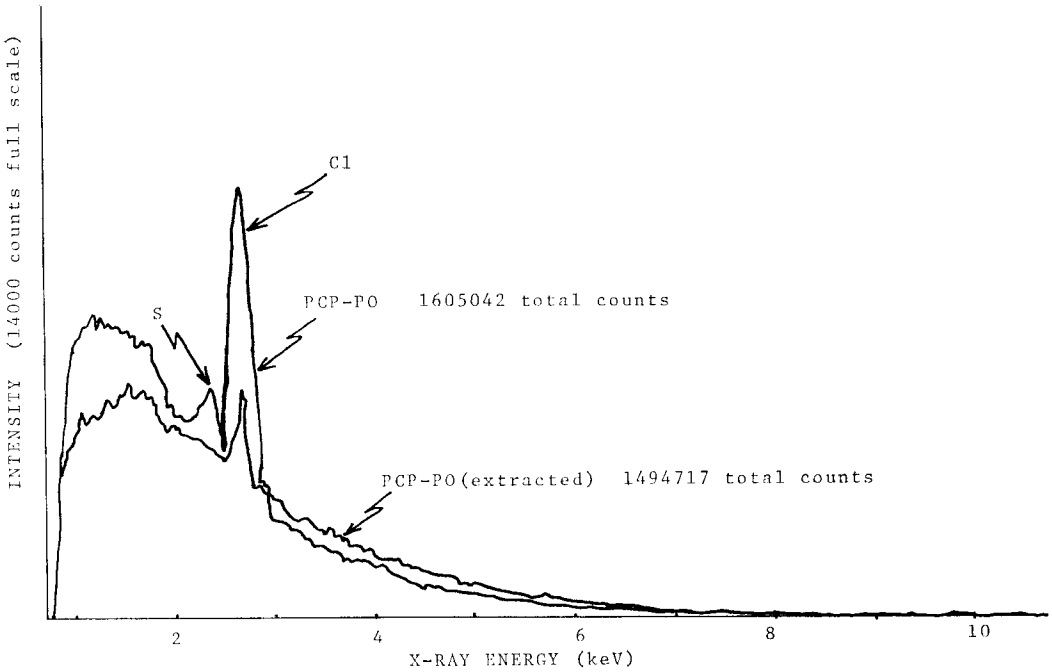

FIG. 8. Energy spectra for (a) PCP-PO treatment, (b) benzene-extracted PCP-PO treatment. 400-second analysis; 220×; 40° tilt.

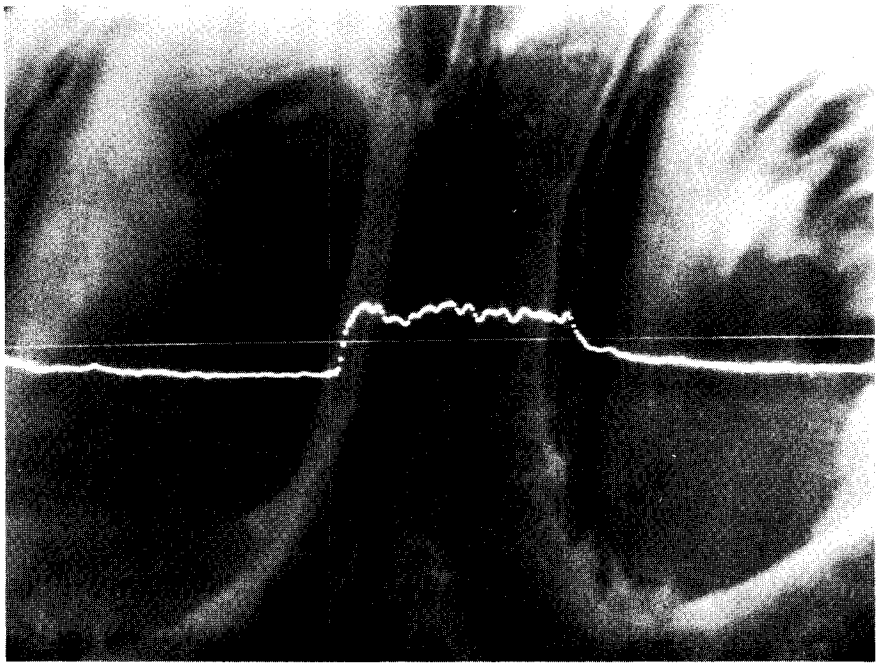

FIG. 9. Cl line scan, benzene-extracted PCP-PO treatment. 400-second analysis; 2400×; 40° tilt.

shows a moderate amount of sulfur in the unextracted specimen. This is likely the result of sulfur present in the light petroleum oil carrier. After extraction, the peak disappears. A line scan (Fig. 9) of a single compound cell wall from the PCP-PO treated, benzene-extracted materials demonstrates the persistence of the preservative in the specimens.

CONCLUSIONS

Significant amounts of PCP are added to the cell walls of wood by nonpolar treating solvents. It would be useful to specify the distribution of the treating chemical across cell walls, but given the topographical characteristics of the specimens examined, the inherent roughness of the wood and irregularities in the prepared surfaces, such characterization was not possible. The use of an embedment and fixation schedule system that will not dissolve or relocate the PCP after treatment is a logical approach for additional work on this problem of specimen preparation.

Definition of the mechanism by which the preservative, carried in nonpolar solvents, enters the cell wall has yet to be made, given current theories of cell-wall porosity and chemical composition. Some explanation of this process may lie in the treating pressures and (with respect to Cellon) the molecular size of the butane carrier. However, the petroleum oil carrier is composed of higher molecular weight hydrocarbons and one would not predict its entry into the cell wall. This appears to have occurred if PCP presence is any criterion. Further studies using SEM-EDXA techniques may help solve this general problem and, in any case, should be useful in detailing distribution of preservative compounds.

REFERENCES

ARGANBRIGHT, D. G. 1973. Interactions between pentachlorophenol solutions and wood. Wood Fiber 4(4):252–258.

CHOU, C. K., J. A. CHANDLER, AND R. D. PRESTON. 1973. Microdistribution of metal elements in wood impregnated with a copper-chrome-arsenic preservative as determined by analytical electron microscopy. Wood Sci. Technol. 7(2):151–160.

DAVIES, G. W. 1968. Electron microscopy and cell-wall porosity. Appita 21(4):117–121.

EXLEY, R. R., B. G. BUTTERFIELD, AND B. A. MEYLAN. 1974. Preparation of wood specimens for the scanning electron microscope. J. Micros. 101(1):21.

GRAY, R. L., AND W. A. CÔTÉ, JR. 1974. SEM/EDXA as a diagnostic tool for wood and its inclusions. IAWA Bull. 3:6–11.

GREAVES, H. 1974. Electron probe X-ray analysis of selected anatomical structures in copper-chrome-arsenic-treated wood. Wood Sci. 7(2):164–168.

HESS, D., R. FALK, AND D. BAYER. 1975. The influence of specimen topography on X-ray microanalysis element mapping. Am. J. Bot. 62 (3): 246–253.

LEUTRITZ, J. 1971. Stabilization of pentachlorophenol as indicated by extraction from wood samples with different solvents. Proc. Am. Wood Pres. Assoc. 67 (198–202).

PETTY, J. A., AND R. D. PRESTON. 1968. Electron probe microanalysis of metals in cell walls of conifer wood treated with preservatives. Holzforschung 22(6):174–177.

RESCH, H., AND D. G. ARGANBRIGHT. 1971. Location of pentachlorophenol by electron microprobe and other techniques in Cellon-treated Douglas-fir. For. Prod. J. 21(1):38–43.

RUDMAN, P. 1966. Studies in wood preservation. Holzforschung 20(2):60-67.

RUSS, J. L. 1974. X-ray microanalysis in the biological sciences. J. Submicro. Cytol. 6:55–79.

WALTERS, C. S., AND W. A. CÔTÉ. 1960. The distribution of pentachlorophenol in the microstructure of basswood. Holzforschung 14(6): 183–189.

WILCOX, W. W. 1975. Micro-bioassay of pentachlorophenol within wood cell walls. For. Prod. J. 25(10):37–43.

WILCOX, W. W., AND N. PARAMESWARAN. 1974. Method for electron microscopical demonstration of pentachlorophenol. Holzforschung 28 (4):153–155.