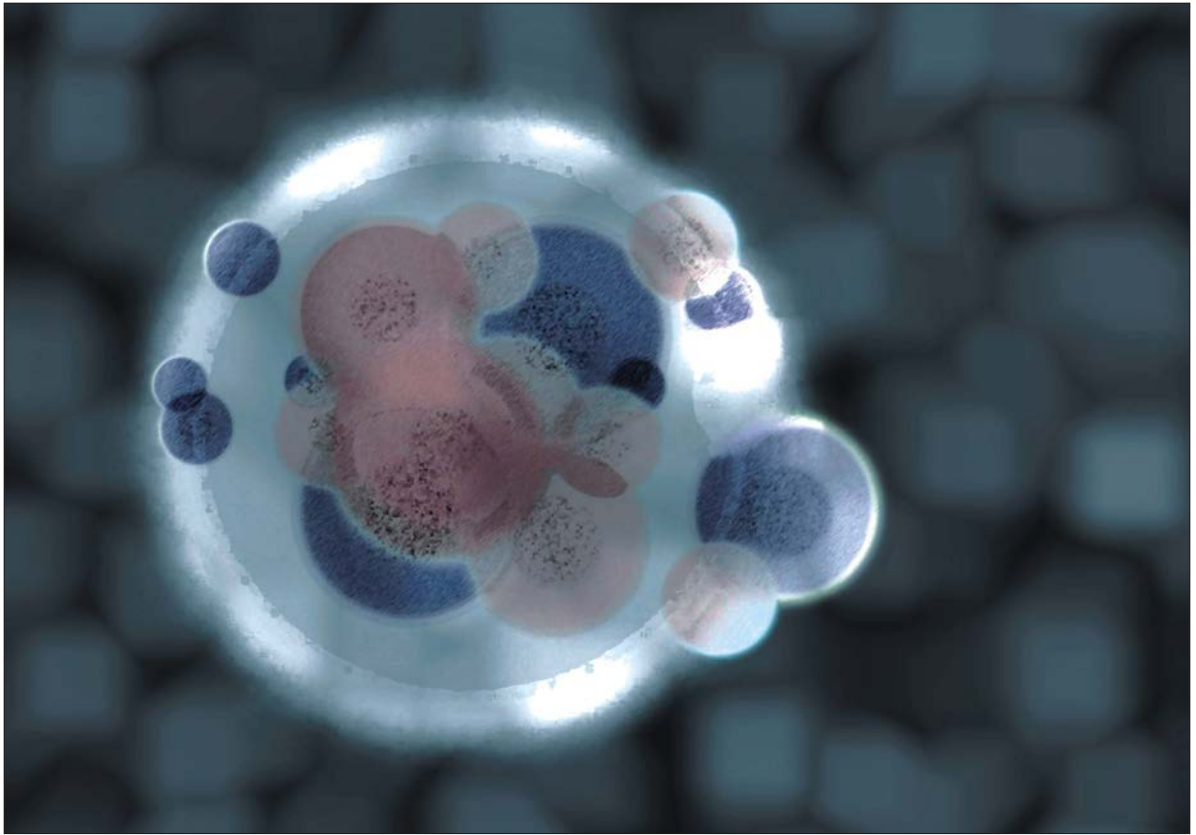




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Energy Dispersive X-Ray Analysis on Preservatives Treated Tropical Bamboo Species

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Abstract: The technique used in treating bamboo culms with preservatives are indisputably has some influences in determining the preservatives performance against insects and decaying fungi. The actual protection depends largely on the penetration, location and retention of the preservatives at the tissue and cell walls levels. At optimum retention levels the preservative performance should be comparable unless the preservative distribution, substrate susceptibility or fixation product had altered. In evaluating the performance of various treatment methods employed, the distribution of preservatives within the cell walls of treated bamboo must be consider. In this study, results of the energy dispersive x-ray analysis on *Gigantochloa scortechinii* treated with Copper Chrome Arsenic (CCA) and Ammoniacal Copper Quaternary (ACQ) are analyzed. The *G. scortechinii* samples were treated by soaking, vacuum pressure impregnating and high-pressure sap-displacement. Observation was carried out using the Transmission Electron Microscope (TEM) linked system to an Energy Dispersive X-ray Analyzer (EDXA). The system enable to detect preservative distribution at the cellular level and measured relative preservative content on the lumen surface, in the S₂ cell wall layer and in the middle lamella.

Key words: Preservatives treated bamboo, TEM-EDXA analysis, metallic elements distribution, bamboo cells

INTRODUCTION

The treatment techniques used in treating bamboo culms may have some influence on the preservative performance against decaying fungi and insects^[1]. The actual protections, however depends largely on the penetration, location and retention of the preservatives at the tissue and cell walls levels in the bamboo^[2,3]. At optimum retention levels the preservative performance should be comparable unless the preservative distribution, substrate susceptibility or fixation product had altered^[4]. In evaluating the performance of various treatment methods employed, one must consider the distribution of preservatives within the cell walls of the treated bamboo.

The bamboo (*Gigantochloa scortechinii*) culms used in the study were first treated by soaking, vacuum pressure impregnating and high-pressure sap-displacement. This study present the results of the energy dispersive x-ray analysis on *G. scortechinii* treated with Copper Chrome Arsenic (CCA) and Ammoniacal Copper Quaternary (ACQ) at 4% of concentration.

This study was designed to determine whether or not there were differences between the preservative distribution achieved at the tissue and cell walls levels

after each treatment processes. The Transmission Electron Microscope (TEM) linked to an energy dispersive x-ray analyzer that was used to detect preservative distribution at the cellular level and measured relative preservative content on the lumen surface, in the S₂ cell wall layer and in the middle lamella.

MATERIALS AND METHODS

An experiment using TEM linked system to an energy dispersive x-ray analyzer was adopted in order to detect the presence of preservative and their distribution at the cellular level and measured relative preservative content on the lumen surface, in the S₂ cell wall layer and in the middle lamella. This study was conducted in the year 2003 to 2004 at the University Malaysia Sabah and Forest Research Institute Malaysia.

Prior to that, bamboo culms of *G. scortechinii* were harvested from the Forest Reserve Area in Nami, Kedah, Malaysia. These bamboo samples were then taken to the University Malaysia Sabah for preservative treatments with CCA and ACQ at 4% concentration using soaking, vacuum pressure impregnating and high-pressure sap-displacement processes. The samples were then air-dried for 3 weeks.

Small match sticks (0.5x0.5x10.0 mm) were cut from the treated *G. scortechnii* culm sample blocks of selected blocks treated with CCA and ACQ by soaking, vacuum pressure impregnating and high-pressure sap-displacement. Thirty six match sticks blocks consisting one age group, 3 treatments methods, 2 type of preservatives and 6 replicates were prepared and analyzed.

The match sticks selected at the middle portion of the treated culm samples were prepared and selected using the procedure adopted by Newman^[5] with some modification. All samples were analyzed on a PHILLIPS 400T with a Link QX 200 analyzer. The specimen holder was fitted with a Beryllium (Be) low background windowed detection. Operating and analysis conditions were standardized as follows; beam current 17pA, accelerating voltage 80 kV, live time 200 sec and magnification 17,000 times, spot size 200 nm, specimen angle 20°.

The types of cells chosen for the x-ray analysis were a vessel, 3 fibres and a parenchyma cells. The vessel was chosen because this is where the major uptake of preservative was expected to take place. From here the preservative was are distributed to the surrounding cells and it was considered that cells near the vessel might absorb more preservative than cells further away. The cells analyzed were all located in one vascular bundle (Fig. 1). The first fibre was located in the first layer of fibres around the vessel. The second fibre was in the third layer around the vessel and the third fibre was located in the second layer in the free fibre strand closest to the vessel. The parenchyma was located in the third layer of the parenchyma ground tissue closest to the vessel. These cells were all about in one line to the chosen vessel. The location and types of cells selected for this investigation are shown as in Fig. 2.



Fig. A,B: Vascular bundles

Fig. 2: Location of cells within each vascular bundle for the TEM-EDXA study

Sample fixation, embedding and cutting:

The match sticks were:

Fixed in:	3% glutaraldehyde in 0.1M phosphate buffer (pH 7.2)	
Dehydrated in:	0.1M phosphate buffer (pH 7.2)	3x20 min
	50, 60, 70, 80 and 90% ethanol	1x10 min each
	100% ethanol	2x15 min
	Propylene oxide	2x10 min
Embedded in:	Propylene oxide: Spurr (2:1)	1 h
	Propylene oxide: Spurr (1:1)	1 h
	Propylene oxide: Spurr (1:2)	48 h
	Spurr (1)	30 h
	Spurr (2)	30 h
Cast in:	Spurr (3)	Spurr: ERL 4206 (10.0 g)
		DER 736 (4.0 g)
		NSA (26.0 g)
		DMAE (0.4 g)
Cutting:	Diatome 45° diamond knife	
	6° clearance angle	
	1 mm sec ⁻¹ cutting speed	
	Sections relaxed under chloroform	

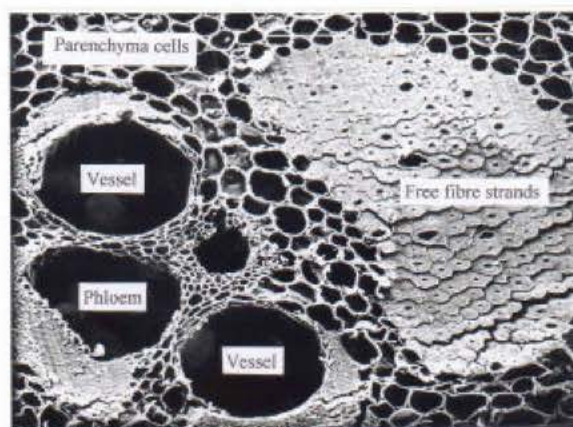
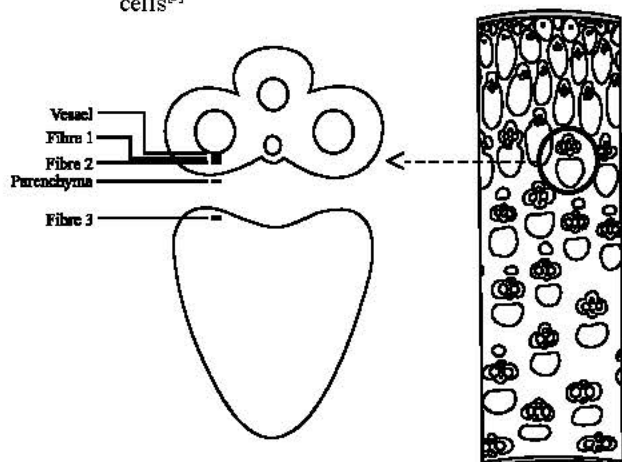


Fig. 1: A typical vascular bundle found in *G. scortechnii* showing vessels, phloem, parenchyma and fibre cells^[5]



B

C: Bamboo culm wall

RESULTS

Considerable variation was recognised between analyses carried out on different cells and in order to overcome this, where, possible analyses were conducted on cell walls that were nearer to the vessels. The estimated concentrations of the preservative of the preservative elements present were determined using a peak-to-background ratio P/B, where P and B are, respectively the areas of the peak and of the spectral background, within the same range of the energies. For each peak a number of selected channels of interest were used for the area determination:

40 for Cu K α , 44 for Cr K α and 24 for As L α

The background area was determined from a background line by linear interpolation of the value of the background spectrum in the extremes of each peak. In this condition, for each characteristic line of each active element, the P/B ratio is related with the concentration of the element in the block. Thus, for each active element it is possible to make a semi-quantitative comparison between the several fields of analysis.

The means values for the P/B ratio obtained for copper, chrome and arsenic elements are presented in Table 1 to 6. As already referred, this energy dispersive x-ray technique used gives only a semi-quantitative information of the concentration of the active elements in the treated *G. scortechinii*.

The results of the TEM-EDXA analyses on blocks treated with ACQ by soaking, vacuum pressure and high pressure sap-displacement processes are presented in Table 1 to 3. Only copper could be analyzed since it is the only metallic element found in the ACQ formulation.

The results obtained shows that the Peak to Background counts decreased from the vessel to the fibre 1, fibre 2, fibre 3 and parenchyma. Vacuum pressure process recorded highest count and soaking process followed closely behind (Table 2). High pressure sap-displacement recorded the lowest P/B counts (Table 3).

The results of the TEM-EDXA analyses on blocks treated with CCA by soaking, vacuum pressure and high pressure sap-displacement processes are presented in Table 4 to 6.

Similar observation were made in the P/B counts in the CCA treated bamboo culms where counts decreased from the vessel to the fibre 1, fibre 2, fibre 3 and parenchyma. Vacuum pressure process recorded highest count and soaking process followed closely behind (Table 5). High pressure sap-displacement recorded the lowest P/B counts (Table 6).

Table 1: Mean elemental peak: Background ratios in 2 year-old *G. scortechinii* treated with ACQ soaking treatment

Cell	Location	Peak: Background Copper
Vessel	Lumen	4.38
	S ₂	3.84
	Lamella	3.77
Fibre 1	Lumen	2.96
	S ₂	2.45
	Lamella	3.04
Fibre 2	Lumen	2.34
	S ₂	2.36
	Lamella	2.78
Parenchyma	Lumen	1.81
	S ₂	1.19
	Lamella	2.75
Fibre 3	Lumen	2.25
	S ₂	2.06
	Lamella	2.67

Table 2: Mean elemental peak: Background ratios in 2 year-old *G. scortechinii* treated with ACQ by vacuum pressure treatment

Cell	Location	Peak: Background Copper
Vessel	Lumen	6.27
	S ₂	6.08
	Lamella	4.71
Fibre 1	Lumen	3.76
	S ₂	3.16
	Lamella	4.30
Fibre 2	Lumen	3.31
	S ₂	2.96
	Lamella	3.36
Parenchyma	Lumen	2.68
	S ₂	1.98
	Lamella	2.76
Fibre 3	Lumen	2.92
	S ₂	2.79
	Lamella	3.48

Table 3: Mean elemental peak: Background ratios in 2 year-old *G. scortechinii* treated with ACQ through high pressure sap-displacement treatment

Cell	Location	Peak: Background Copper
Vessel	Lumen	3.53
	S ₂	3.25
	Lamella	3.14
Fibre 1	Lumen	2.42
	S ₂	2.19
	Lamella	2.92
Fibre 2	Lumen	2.15
	S ₂	1.91
	Lamella	2.25
Parenchyma	Lumen	1.42
	S ₂	1.18
	Lamella	1.97
Fibre 3	Lumen	1.62
	S ₂	1.30
	Lamella	1.66

Mean of 5 replicates

DISCUSSION

Complimentary TEM-EDXA studies conducted on matched transverse faces for the distribution of ACQ and CCA elements gave results showing that the copper,

Table 4: Mean elemental Peak: Background ratios in *G. scortechinii* treated with CCA by soaking treatment

Cell	Location	Peak: Background		
		Copper	Chrome	Arsenic
Vessel	Lumen	4.49	1.73	2.30
	S ₂	3.83	0.99	1.44
	Lamella	3.33	0.82	0.95
Fibre 1	Lumen	2.58	0.96	1.14
	S ₂	1.68	0.63	0.91
	Lamella	3.83	1.21	2.06
Fibre 2	Lumen	2.25	0.95	1.19
	S ₂	1.76	0.37	0.61
	Lamella	2.61	1.15	1.44
Parenchyma	Lumen	1.14	0.69	0.75
	S ₂	1.77	0.35	0.36
	Lamella	3.14	0.99	1.07
Fibre 3	Lumen	2.60	0.90	0.94
	S ₂	2.43	0.66	0.84
	Lamella	2.77	0.96	1.02

Table 5: Mean elemental peak: Background ratios in *G. scortechinii* treated with CCA by vacuum pressure treatment

Cell	Location	Peak: Background		
		Copper	Chrome	Arsenic
Vessel	Lumen	6.39	1.84	2.37
	S ₂	5.84	1.73	1.90
	Lamella	4.46	1.66	1.70
Fibre 1	Lumen	3.56	1.54	1.94
	S ₂	3.05	1.49	1.91
	Lamella	4.17	1.95	2.34
Fibre 2	Lumen	3.13	0.81	0.92
	S ₂	2.67	0.58	0.70
	Lamella	4.12	1.12	2.12
Parenchyma	Lumen	2.69	0.39	0.50
	S ₂	2.17	0.31	0.42
	Lamella	3.66	0.99	1.85
Fibre 3	Lumen	2.73	0.69	0.71
	S ₂	2.35	0.51	0.62
	Lamella	3.47	0.49	0.85

Table 6: Mean elemental peak: Background ratios in *G. scortechinii* treated with CCA by high pressure sap-displacement treatment

Cell	Location	Peak: Background		
		Copper	Chrome	Arsenic
Vessel	Lumen	3.45	1.73	2.51
	S ₂	3.12	1.72	1.49
	Lamella	2.66	1.35	1.30
Fibre 1	Lumen	2.44	1.54	1.28
	S ₂	1.65	0.92	0.98
	Lamella	3.12	1.68	1.72
Fibre 2	Lumen	1.98	1.27	1.10
	S ₂	1.86	0.60	1.40
	Lamella	2.42	1.39	1.66
Parenchyma	Lumen	1.95	0.84	0.85
	S ₂	1.86	0.39	0.67
	Lamella	2.76	1.09	1.64
Fibre 3	Lumen	2.35	0.94	1.40
	S ₂	2.14	0.72	0.87
	Lamella	2.48	1.06	1.34

Mean of 5 replicates

chrome and arsenic reading were found in the more lignified regions. The highest counts were generally recorded for the middle lamella followed by the lumen and

the lastly the S₂ regions of the fibres and parenchyma cells in *G. scortechinii* blocks. Similar findings were also observed by Newman^[5] Daniel and Nilsson^[6] and in their separate studies on CCA treated *Betula papyrifera*, *B. verrucosa*, *B. papyrifera* and *Pinus nigra* var. *maritima*, respectively. For the vessels the highest counts were recorded in the lumen areas where, the liquid absorption took place followed by the middle lamella and the S₂ layers.

The qualitative results gathered during the TEM-EDXA experiment shows that the preservatives are not evenly distributed in the bamboo culm walls. The P/B ratios of the metallic elements tended to decrease as the cells were located further away from the vessels. As we know the vessels played an important role in preservatives treatment. The penetration of liquids into the bamboo culms takes place through the vessels in the axial direction, from end to end. From the vessels the liquids are distributed to the surrounding fibres and parenchyma cells.

The parenchyma cell, which was located second last away from the vessel, were observed to have the lowest count for the preservative elements. The third fibre located further away from the vessel recorded a higher count number than the parenchyma. This indicated that the cell wall thickness is an important factor in influencing absorbing and retaining the preservative solutions.

Comparison between different treatment processes indicated that vacuum pressure gives higher number of count for the metallic elements followed by soaking treatment and high pressure sap-displacement, respectively.

CCA treated blocks recorded a higher count number for the preservative elements than the ACQ treated blocks. The distribution of copper in ACQ treated blocks however was slightly more uniform than between the middle lamella, S₂ and lumen in the CCA treated blocks. This was clearly shown in Table 1 to 3 and Fig. 2 to 4 where, there were slight differences in copper count between lumen, S₂ layers and the middle lamella of the fibres and parenchyma cells wall as well as the vessel.

It may be concluded from the present study that:

- The highest amount of metallic elements were recorded in the middle lamella followed by the lumen and S₂ layers for the fibres and parenchyma.
- The highest readings were recorded in the vessel lumen areas which is considered the primary site of preservative absorption and uptake.
- The lowest counts were recorded for the parenchyma S₂ cell wall layers where the cell wall thickness were thinner compared to that of fibre.

- Vacuum pressure treated blocks recorded the highest counts. This was followed by soaking and high-pressure sap-displacement, respectively.
- CCA treated blocks recorded the higher mean counts than the ACQ treated blocks
- ACQ treated blocks recorded a slightly more uniform counts for the presence of copper between the lumen, S₂ and middle lamella.
- The P/B ratio values tended to decrease with the radial distance from the vessel though occasionally the behavior was not consistent.

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