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Mappings of Chemicals Distribution in Preservatives Treated Tropical Bamboo *Gigantochloa scortechinii.*

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ABSTRACT

The mappings of chemicals in preservatives treated bamboo *Gigantochloa scortechinii* were studied. The chemical's distribution in the bamboo indisputably influences their durability against the attacks of the insects and fungi. This largely depends on the chemicals penetration, location and retention at the tissue and cell walls levels in the bamboo. At optimum retention levels, the preservative performance should be comparable unless the preservative's 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 considered. The results from the energy dispersive x-ray analysis on the bamboo treated with Copper Chrome Arsenic, and Ammoniacal Copper Quaternary 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 linked system to an energy dispersive x-ray analyzer. The system enables to detect preservative distribution at the cellular level and measured relative preservative content into the lumen surface, in the S_2 cell wall layer and in the middle lamella.

Keywords: Chemicals Treated Bamboo, Energy Dispersive X-ray Analyzer, Chemicals Distribution, bamboo cells.

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INTRODUCTION

Preservatives treated bamboo behaves differently from timber in their distribution of chemicals. The distribution of chemicals in the treated bamboo indisputably influences the preservatives performance against insects and fungi [1-2]. The actual protections, however, depends largely on the chemicals penetration, location and retention at the tissue and cell wall's levels in the bamboo [3]. At optimum retention levels, the preservative performance should be comparable unless the preservative distribution, substrate susceptibility or fixation product had altered [2][4][6]. In evaluating the performance of bamboo through various treatment methods employed, consideration must be given to the distribution of chemicals within the cell walls of the treated bamboo.

Three methods of preservatives treatment were employed in treating the bamboo in this study. These are soaking, vacuum pressure impregnating and high-pressure sap-displacement. This paper present the results from the energy dispersive x-ray analysis on *Gigantochloa scortechinii* culms treated with Copper Chrome Arsenic (CCA) and Ammoniacal Copper Quaternary (ACQ) at 4% of concentration.

This study was designed to determine the presence of different chemicals in the preservatives after each treatment at the tissue and cell walls levels. Transmission electron microscope (TEM) linked to an energy dispersive x-ray analyzer was used to detect the chemicals distribution at the cellular level. It was also used in measuring the relative chemicals content in the lumen surface, in the S_2 cell wall layer and in the middle lamella.

MATERIALS AND METHODS

This study was conducted during the year 2012 to 2013 at the University Malaysia Kelantan (UMK) and Forest Research Institute Malaysia (FRIM). The TEM linked system to an energy dispersive x-ray analyzer was used in detecting the presence of various chemicals and their distribution at the cellular level and measured relative preservative content into the lumen surface, in the S_2 cell wall layer and in the middle lamella.

Prior to that, bamboo culms of *G. scortechinii* were harvested from the FRIM Natural Bamboo Plot Areas in Jeli, Kelantan, Malaysia. These bamboo culms were then taken to the UMK and FRIM for preservative treatments with CCA and ACQ at 4% concentration using soaking process. The samples were then air-dried for three weeks. Procedure developed by Razak at el. [7] was used with some modification in this study.

Small match sticks (0.5 x 0.5 x 10.0 mm) were cut from the treated *G. scortechinii* 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, three 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 [8] with some modification. All samples were analyzed on a PHILLIPS 400T with a Link QX 200 analyzers. The specimen holder was fitted with a Beryllium (Be) low background windowed detection. Operating and analysis conditions was standardized as follows; beam current

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17pA, accelerating voltage 80kV, 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 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 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 (Figure 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 Figure 2.

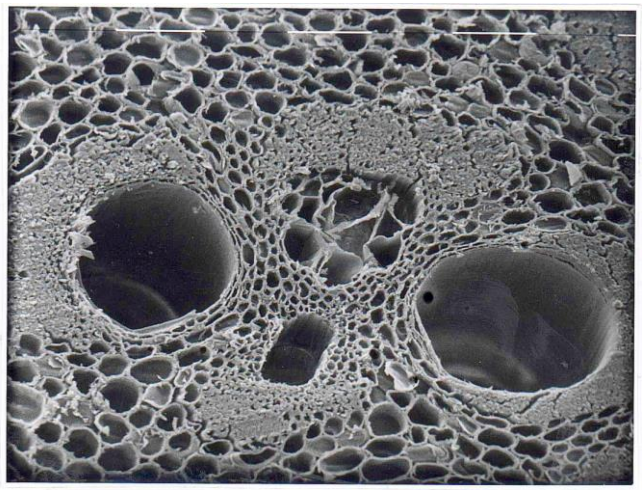
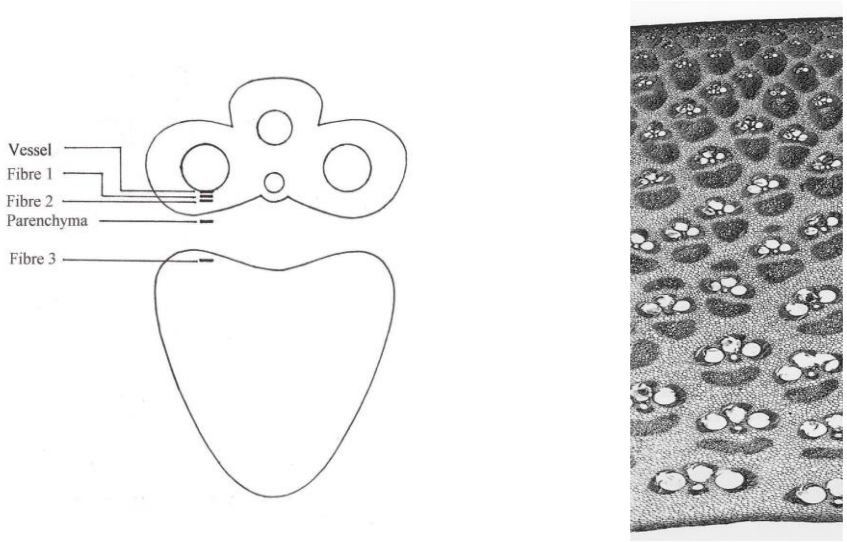


Figure 1: Vascular bundles of bamboo found in *G. scortechinii* taken at the center of the duct cross-section with two large metaxylem and phloem cells surrounding the fibers and parenchyma.



A bamboo vascular bundles B Bamboo culm wall

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

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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)		3 x 20 min
	50% / 60% / 70% / 80% / 90% ethanol		1 x 10 min each
	100% ethanol		2 x 15 min
	Propylene oxide		2 x 10 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 g)
		DER 736	(4 g)
		NSA	(26 g)
		DMAE	(0.4 g)
Cutting :	Diatome 45° diamond knife		
	6° clearance angle		
	1 mm per second cutting speed		
	Sections relaxed under chloroform		

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 under 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 in the area determination (Eqn. 1).

40 for Cu Kα, 44 for Cr Kα and 24 for As Lα …….. (1)

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 to 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 in the P/B ratio obtained for copper, chrome and arsenic elements are presented in Tables 1 to 6. As already referred, this energy dispersive x-ray technique used gives only a semi-quantitative information on 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 Tables 1 to 3. Only

copper could be analyzed since it is the only metallic element found in the ACQ formulation.

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

Type of 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.

Type of 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

Mean of 5 replicates.

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

Type of 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.

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

Type of 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

Mean of 5 replicates.

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).

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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 Tables 4 to 6.

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

Type of 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

Mean of 5 replicates.

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.

Similar observations 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). Bamboo treated through the high pressure sap-displacement recorded the lowest P/B counts (Table 6).

DISCUSSION

Complimentary TEM-EDXA studies conducted on the match transverse faces for the distribution of ACQ and CCA elements gave results showing that the copper, chrome and arsenic reading was found in the more lignified regions. The highest counts were generally recorded for the middle lamella followed by the lumen and the lastly the S_2 regions of the fibres and parenchyma cells in *G. scortechinii* blocks. Similar findings were also observed by Daniel and Nilsson [1], Razak et al. [6] Newman [8] and Othman et al. [9] in their separate studies on CCA treated, *Betula papyriferra, 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 S_2 layers.

The results gathered during the TEM-EDXA experiment shows that the preservatives are not evenly distributed in the cross-section of 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. The vessels played an important role in preservative's treatment [4]. The penetration of liquids into the bamboo culms takes place through the vessels in the axial direction, from the end to end [7]. 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 that 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 the 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, S_2 and lumen in the CCA treated blocks. This was clearly shown in Tables 1 to 3 and Figures 2 to 4 where, there were slight differences in copper count between lumen, S2 layers and the middle lamella of the fibres and parencyma cells' wall as well as the vessel.

CONCLUSION

The middle lamella recorded the highest counts of the metallic elements followed by the lumen and S_2 layers for the fibres and parenchyma cells. The vessel lumen areas recorded the highest reading counts and the vessel are considered the primary site of preservative absorption and uptake. The lowest counts were recorded for the parenchyma S_2 cell wall layers where the cell wall thickness was 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. The CCA treated blocks recorded the higher mean counts than the ACQ treated blocks. The ACQ treated blocks recorded a slightly more uniform count for the presence of copper between the lumen, S_2 and middle lamella.

The P/B ratio values tend to decrease with the radial distance from the vessel though occasionally the behavior was not consistent.

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