# A BIOASSAY TO DETECT PRESERVATIVE RETENTION IN HARDWOODS AND SOUTHERN PINES.

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SUMMARY

*In this paper the author discusses and suggests a new method of evaluating preservative retention in treated woods by means of bioassays.*

*Twelve North American species were tested with different retention levels of pentachlorophenol (PCP) and copper-chrome-arsenate (CCA) preservatives. The results observed in this work show that among other fungi,* Aspergillus niger *responds to both preservatives, with good relationship to the PCP retention for all wood species, and with good relationship to someCCA treated species.*

RESUMO

*Neste trabalho o autor discute e sugere um novo método de avaliação da retenção de preservativos em madeiras tratadas, pelo uso de ensaios biológicos.*

*Doze espécies norte americanas foram testadas com diferentes níveis dos preservativos pentaclorofenol (PCP) e cobre-cromo-arsênico (CCA). Os resultados observados neste trabalho mostra que entre outros fungos,* Aspergillus niger *responde à ambos preservativos, com boa correlação à retenção do PCP para todas as espécies de madeira utilizadas, e com boa correlação para algumas espécies tratadas com CCA.*

## 1. INTRODUCTION

As a new method of determining the solution concentration absorved by the treated wood, some bioassays have been tested for residual pentachorophenol (PCP) in wood materials exposed to weather (1,2). Results from these experiments have shown that the fungus **Aspergillus niger** responds to PCP preservative, indicating a probable good relationship between the amount of retained preservative in the wood and the circular area free from sporulated mycelium around preserved specimens, observed on agar substrate cultures. However, nothing has been done to investigate these effects against preservative retention values, which are used to evaluate the amount of chemicals absorbed by wood during treatment processes. A study on preservative retention and fungi response for different preservatives can be very useful for further evaluations, once proved that they have satisfactory relationship for practical purposes.

## 2. OBJECTIVES

The objectives of this experiment were to investigate responses of fungi exposed to preservative-treated wood specimens under laboratory conditions, the identification of the best fungus to be used for several wood species as well as the determination of calibration lines whenever it would be possible. Results from this investigation were expected to bring a valuable contribution as far as the evaluation of chemical retention is concerned in order to substitute other time consuming analitical methods.

## 3. MATERIAL AND METHODS

### 3.1. Preliminary Work

A preliminary trial was made using 5 x 5 x 6 mm treated specimens of hard maple sapwood which were exposed to **Aspergillus niger, Gloephyllum trabeum, Coriolus versicolor** and **Poria placenta** fungi (65% RU, 27°C.). With every individual fungus, five levels of CCA and PCP preservative retentions were tested and six specimens per retention level were used. The aproximated retention levels utilized are presented in the table below:

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TABLE 1: Approximate preservative retention levels utilized in the preliminary work.

| | C.C.A. Retention* ($kg/m^3$) | PCP Retention* ($kg/m^3$) |
|---|---|---|
| Level 1 | 0,04 | 0,02 |
| Level 2 | 0,08 | 0,04 |
| Level 3 | 0,16 | 0,08 |
| Level 4 | 0,32 | 0,16 |
| Level 5 | 0,64 | 0,32 |

Potato-dextrose agar was used as cultura medium to grow fungi in the incubation room. The assay dishes were filled up to a depth of about 7mm with culture medium, made by mixing 33,6gm of potato dextrose agar to 1000ml of distilled water. After cooking the culture medium was sterilized (15psi, 120°C) and poured into the petri dishes. After inoculating with **A. niger** in water suspension, the cultures were stored in a refrigerator up to the time of laying down the treated specimens on the agar surface.

For every individual treatment, wood blocks were randomly taken as a following step, and 3 specimens per dish and 10 dishes per treatment were allocated according to statistical standards. Afterwards the cultures were transferred to the incubation room (27°C, 65% RU) to provide the fungus development.

### 3.1.1. PREPARATION OF THE TEST BLOCKS

One block of hard maple sapwood with dimensions of about 400 x 20 x 20mm was selected. Eeach test block was rip-sawn into three individual pieces. The core was used for moisture content (%) and specific gravity determinations on an oven dry basis. The two shell pieces obtained per block were passed through a planer in order to get smooth surfaces and a thickness of 5mm.

Afterward, they were rip-sawn into a width of 5mm and the rough surfaces planed. As a final procedure, by end-cutting the specimens they were dimensioned into 5 x 5 x 6mm, and irregularities on the wooden blocks caused by the sawing action were manually corrected with a shaving blade.

The freshly cut specimens were readily weighted on an analitical balance (initial weight = A), treated and reweighted (final weight = B).

Wood volume ($cm^3$) was calculated using the following formula:

$$\text{Wood volume} = \frac{A}{(1 + \text{m.c., in decimal}) \times (\text{wood specific gravity})} \quad [cm^3]$$

Calculations for preservative retention ($kg/m^3$) were made using the formulas:

$$\text{CCA retention} = \frac{(B\text{-}A) \times (\text{Amount of chemical in preservative solution, in gm/l.})}{\text{Wood volume, in } cm^3} \quad [kg/m^3]$$

* Refers to values obtained by the wood specific gravity and the weight of the individuals, at 1 x $10^{-5}$ gm of accuracy.

$$\text{PCP retention} = \frac{(B\text{-}A) \times (\text{Amount of chemical in preservative solution, in gm/liter} \times 1.15473}{\text{Wood volume in cm}^3} \quad [\text{kg/m}^3]$$

Were: 1.15473 = 1/0.866 = corretion factor for toluene density, and
0.866 gm/cm³ = density of toluene.

All treated blocks were allowed to dry in the climatization room for at least 4 weeks before their transference to the culture media.

### 3.1.2. TESTS

Continous observation on the performance of the different treatments in the preliminary test was used to select the most appropriate fungus to be used in the complementary work. The following variables were observed with more emphazis, namely:

a. Contamination of the fungus culture being used;
b. Fungus response to the treated material;
c. If any, shape and size of the total zone of effect (TZE)* — to measure the size, a 15mm scale lens prossessing 1/10mm accuracy was used.
d. Time consumed by each fungus, to spread over the substrate until evalution was possible; and.
e. The correlation factor between the total zone of effect and preservative retention for every particular fungus.

### 3.2. COMPLEMENTARY WORK

In order to determine the calibration lines for preservative retention versus total zone of effect, with **A. niger** selected in the preliminary work nine hardwood- and one softwood species were submitted to a similar test as described before. Thirty 5 x 5 x 6 mm individuals were used per treatment in this step, i.e., per fungus/preservative. A variation in preservative concentration was used to provide different preservative retentions, in order to estimate further relationship between TZEs and the amount of chemical retained by the wood.

The test block preparations were the same as described in the preliminary work, see iten 3.1., except in the case of red oak, where heartwood was also included in order to observe differences in preservative performance.

In order to compare the accuracy of measurements of retention determinations by weight means, retention was additionally determined on basis of the wood-block dimensions for Southern pine, for both CCA and PCP preservatives.

During the preliminary work, a new inoculation method was tested with success and adopted in the second step. This method consists of simply taking an **A. niger** culture dish after eliminating the loosened spores by beating the dish up-side down. This action was continued over culture dishes to be inoculated, where only a very fine layer of spores was wanted.

## 4. RESULTS

Results from the preliminary work showed that among the fungi used, **Aspergillus niger** was the most appropriate fungus to be used in the complementary work. All other fungi didn't present adequate results whenever response fungus-preservative was concerned. To summarize, the following observations were made from the preliminary step (table 2):

* To TZE is meant the ratio value measured from the specimem to the point where sporulated hyphae are present.

TABLE: 2 Fungal behavior with hard maple wood blocks treated with CCA and PCP preservatives +

| Fungus | Preservative | Observations |
|---|---|---|
| **A. niger** | CCA | response present, no contamination, circular TZE |
| **G. trabeum** | CCA | All contamined |
| **C. versicolor** | CCA | All contamined |
| **P. placenta** | CCA | All contamined |
| **A. niger** | PCP | response present, no contamination, circular TZE |
| **G. trabeum** | PCP | no contamination, assymmetry of the TZE |
| **C. versicolor** | PCP | All contamined |
| **P. placenta** | PCP | All contamined |

+ Observations made on 30 specimens/fungus/preservative.

The minimum amount of preservative in the wood that presented response for **A. niger** was about 0.25Kg/m³ for CCA and about 0.015 Kg/m³ for PCP preservative.

Between the two preservatives all PCP treated specimens revealed to have an excellent correlation coefficient as far as the fitness of the particular preservative retentions and the line describing the total zone of effect was concerned. Those treated with CCA had a tremendous variation from species to species. Relationships for PCP preservative were found to be in the range from 0.7219 to 0.9003. Their equations and correlation factors are presented on table 3, and their respective calibration lines presented by fig. 1.

Since CCA preservative treated specimens had too much variation in and among wood species, an individualization to report some of the results is necessary. Equations for Aspen and Southern pine treated specimens showed good correlation coefficients, whereas hard maple presented a lower relationship. Their equations and correlation coefficients are presented on table 4, and their respective calibration lines presented by fig. 2.

Because of the excellent symmetry of the area in study, TZEs measurements for Aspen and S. pine were easily obtained, hard maple, however, did not produced a well defined shape. Therefore it was more difficult to estimate the TZEs values. Basswood was the only CCA — treated wood species that presented a semitransparent TZE as those observed in specimens treated with PCP, i.e., mycelium not sporulated around the specimens. Most wood species used in this experiment presented a complete transparent TZE surrounded by sporulated hyphae. For basswood specimens treated with CCA a very poor correlation factor between retention and TZE was obtained ($r = 0.2823$), and no graph or equation are presented. It was not possible to calculate relationships for other species because there was no preservative — fungus response for most of the lower levels of preservative retentions. Among the species included in this group, birch started showing some response with a retention of 3.14 kg/m³, ash with 5.0 kg/m³, beech with 5.24 kg/m³, hicory with 5.14kg/m³ and, red oak sapwood as well as heartwood with 7.57 kg/m³. Elm showed no response at all to a retention of 7.5 kg/m³ of CCA preservative.

**TABLE 3:** Equations and correlation factors observed between TZEs and PCP retention levels.

| wood | Equation | Correlation coefficient |
|---|---|---|
| Ash sapwood | $y = -4.7655 + 1.1673 \times$ | 0.8363 |
| Aspen sapwood | $y = -6.4455 + 1.4314 \times$ | 0.8518 |
| Basswood sapwood | $y = -5.4247 + 1.5603 \times$ | 0.8090 |
| Beech sapwood | $y = -3.8426 + 1.0870 \times$ | 0.8371 |
| Yellow birch sap. | $y = -3.0139 + 1.1490 \times$ | 0.8613 |
| Elm sapwood | $y = -3.1757 + 1.2288 \times$ | 0.8110 |
| Hard maple sap. | $y = -4.6172 + 1.2048 \times$ | 0.7219 |
| Hickory sapwood | $y = -1.9424 + 0.8298 \times$ | 0.8733 |
| Red Oak sapwood | $y = -2.0107 + 1.1678 \times$ | 0.8769 |
| Red Oak heartwood | $y = -1.5595 + 1.0964 \times$ | 0.8516 |
| Southern pine sap. | $y = -3.5564 + 1.3691 \times$ | 0.8856 |
| Southern pine sap. | $ya = -3.8715 + 1.4691 \times$ | 0.9003 |

Where

y = Retention in kg/m³, determined by volume on basis of the wood specific gravity.

ya = Retention in kg/m³, determined by volume on basis of the specimens dimensions.

x = total zone of effect, in mm.

**TABLE 4:** Equations and correlation factors observed between TZEs and CCA retention levels.

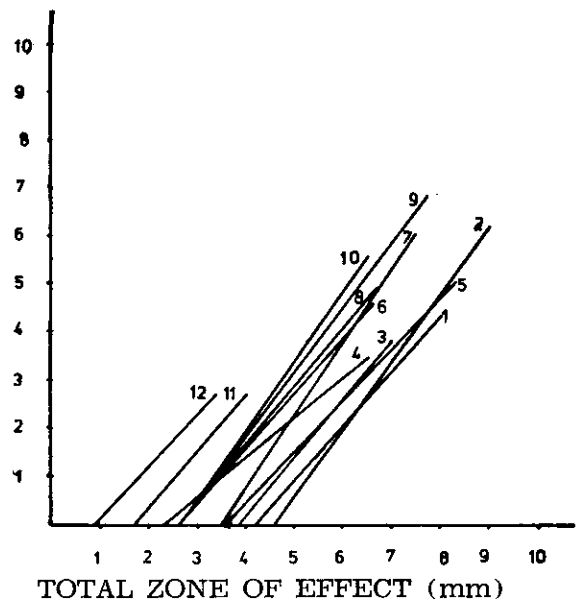
| wood | Equation | Correlation factor (r) |
|---|---|---|
| Aspen sapwood | $y = -0.6443 + 2.4149 \times$ | 0.8725 |
| Hard Maple sap. | $y = 0.1706 + 4.3975 \times$ | 0.6402 |
| Southern pine sap. | $y = 0.5513 + 3.1596 \times$ | 0.7569 |
| Southern pine sap. | $ya = 0.5144 + 3.3602 \times$ | 0.7495 |

Where

y = Retention in kg/m³, determined by volume on basis of the wood specific gravity.

ya = Retention in kg/m³, determined by volume on basis of the specimens dimensions.

x = total zone of effect, in mm.




( 1) Ash sapwood;
( 2) Aspen sapwood;
( 3) Hard Maple sapwood;
( 4) Hickory sapwood;
( 5) Beech sapwood;
( 6) Yellow Birch sapwood;
( 7) Basswood sapwood;
( 8) Elm sapwood;
( 9) Southern Pine sapwood;
(10) Southern Pine sapwood;
(11) Red Oak sapwood;
(12) Red Oak heartwood

FIG. 1: A general view of the relationship between retentions and TZEs, observed by bioassays with **Aspergillus niger** fungus and penta-treated specimens.

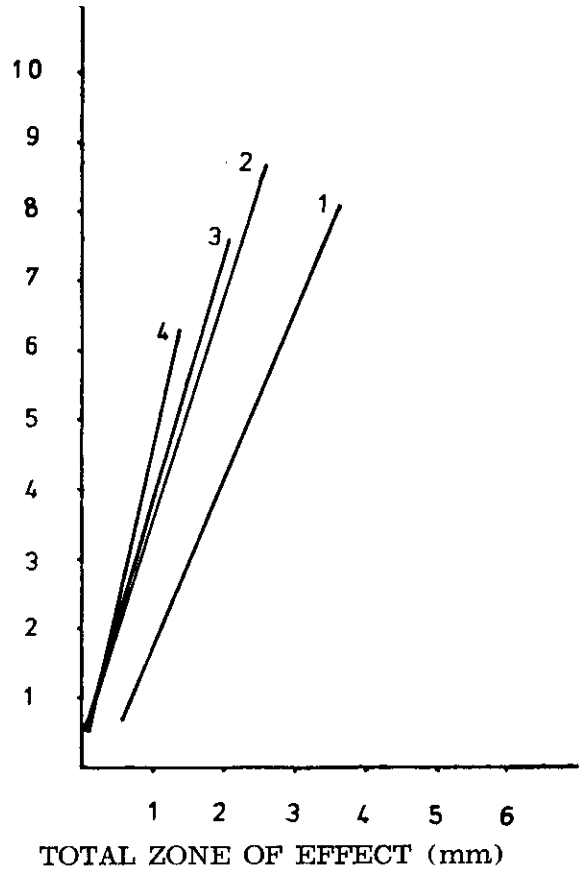



FIG. 2: A general view of the calibrations lines for Bioassay analysis by **Aspergillus** niger fungus on CCA-treated specimens of Aspen (1); Southern Pine (2) & (3); and Hard Maple (4).

## 5. DISCUSSION AND CONCLUSION

The use of the bioassy method described in this work shows to be reasonably accurate and of easy approach to estimate the amount of penta preservative retained in treated wood. However, it must be considered that a significant correlation gain between preservative retention and the total zone of effect could be obtained, by determining what are the causing agents that induce variations among wood species and introducing them in further experiments into multiple regression analysis. Unfortunately, variations occuring among correlation coefficients reveal different behaviors for every wood species and cannot be explained by this experiment due to lack of data. Whereas reasonably good correlations between CCA preservative retention and TZE were observed for aspen and S. pine, other wood species did not present satisfactory correlations. This could be explained as a result of interactions existing among wood components and/ or structure, fungus and the utilized culture media. Not all wood species theated with CCA did present satisfactory results when tested with the described fungus — culture medium combination. Therefore further trials using other culture media, other fungi or a combination of both could be done.

Aspen and S. pine which presented acceptable results can be readily used in identical bioassays as those tested, because their estimation shall have no deviation from the expected values within the probability specified by their particular correlation factors.

Comparisons between determinations of wood volume showed opposit degrees of accuracy between preservative treatmens. However, the determinations on the basis of wood specific gravity for PCP treatment were presented with a relative correlation gain of 1.4%, whereas the loss observed for those treated with CCA was of only 0,07%. Since PCP treatments presented greater correlation factors than for those with CCA it implies that the data for PCP is much more confident, and that the wood block volumes were better estimated throughout the wood specific gravity rather than by measurements of block dimensions.

**Aspergillus niger** was chosen as the most appropriate fungus to be used in this experiment, due to its fast growth, symetry of the TZE, facility of measurement, and because of the absence of infections by other fungi.

Considering the facility of using **A.** niger, it is suggested that new experiments should first be conducted to solve the non correlation problem observed for CCA preservative, by varying the culture medium, the time of fungal exposure, and other variables, in preference to the fungus substitution.

The inoculation method utilized in the secondary step appears to be appreciably better than in-water suspension, whenever powdery spores on the culture media are reached.

## 6. LITERATURE

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