

**Effects of Permethrin Treated Wood on the Subterranean Termite
Reticulitermes flavipes (Kollar) and Comparison of Solvent
Extraction for HPLC Analysis of Permethrin in Wood**

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ABSTRACT

Permethrin is a common insecticide used in wood preservation. It is an effective synthetic pyrethroid that is considered to be less toxic to higher organisms than organochlorine insecticides. In wood preservation, it can be used in combination with fungicides such as 3-iodo-2-propynyl butyl carbamate (IPBC). Permethrin has a dual mode of action as it is a repellent and a contact insecticide. Although the efficacy of permethrin on termites has been examined, many of the studies looked at the effects of treated soil and not treated wood. In this study, we examined the effects of three levels of permethrin in treated southern yellow pine (SYP) on the subterranean termite, *Reticulitermes flavipes*. Termites were exposed to SYP blocks treated by the cooperator to low, medium and high levels. In a separate AWPAA E1 test, we treated blocks with 0.04, 0.07, and 0.25% permethrin (m/m). Complete mortality (100%) was observed at all treatment levels at the end of 28 days in both tests. Untreated and non-permethrin treated pine exposed in both tests showed low mortality and greater mass loss compared to treated blocks. Since an AWPAA standard for analysis of permethrin from wood does not currently exist, an experimental HPLC method was developed comparing two different solvent systems to remove permethrin from ground wood via sonication with subsequent analysis via HPLC. The results from these experiments may serve as preliminary data to develop a proposed AWPAA analytical standard for permethrin analysis in wood.

INTRODUCTION

Permethrin is a synthetic pyrethroid based on chemistry of naturally occurring pyrethrins. It was developed in an effort to produce less toxic pesticides with a favorable environmental profile to replace highly recalcitrant organochlorine insecticides (Freeman et al. 2007). Permethrin has been used in wood preservation since the 1980's because of its favorable environmental profile, its broad spectrum toxicity, its stability in timber and its dual mode of action as both a repellent and contact insecticide (Freeman et al. 2007). Effective levels of permethrin in wood are 0.07 - 0.1% m/m; the equivalent of approximately 0.02 pcf (Lloyd et al. 1998, Hunt et al. 2005, Freeman et al. 2007). Permethrin in wood can degrade in sunlight as well as ground contact and it is therefore only allowed in use of up to AWPAA Use Category (UC) UC3A, above ground protected use (Rutherford et al. 1983, Lloyd et al. 1998). It has been found to be effective in higher UC situations, including marine applications, when used as a secondary wood preservative with creosote where it is effective against marine borers (Cragg, 1989). For wood treatment, permethrin is commonly used in conjunction with a fungicide; generally, IPBC (3-Iodo-2-Propynyl Butyl-Carbamate). Aside from degradation in soil, permethrin can be degraded by UV light, but earlier research showed UV affects only 2-5 mm below the surface of treated timber (Hunt et al. 2005). Insects can also develop resistance to permethrin, however, because of its less toxic environmental profile and broad spectrum effectiveness, it is still used heavily as an insecticidal wood protectant.

Permethrin has been used extensively in Australasia where Australian wood protection entities have developed a standard for permethrin analysis in treated wood (Standards Australia, 2006). In this standard, permethrin is extracted from treated wood shavings using ethanol. In other methods and studies, organic insecticides including permethrin are removed from wood using methanol because of its wide polarity range (AWPAA 2015a, Šťávková et al. 2011). In North America, however a standard for permethrin analysis from wood currently does not exist.

The objectives of this study were to evaluate permethrin treated southern yellow pine (SYP) samples treated by a cooperator and samples treated in house at the Forest Products Laboratory (FPL) of known permethrin loadings against the subterranean termite *Reticulitermes flavipes* (Kollar). Secondly, since no AWPAA standard currently exists for the analysis of permethrin in wood, recovery of permethrin from wood was examined via High Pressure Liquid Chromatography (HPLC) comparing extraction with ethanol or methanol and two different calculation techniques to develop a reliable method for permethrin analysis from wood.

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MATERIALS AND METHODS

Termite Testing

Treated samples were provided for testing by the cooperator. A set of water treated controls was added by FPL for internal interest, comparison and test validity. The test was performed in accordance with American Wood Protection Association AWP A E1-15: "Standard Method for Laboratory Evaluation to Determine Resistance to Subterranean Termites (AWPA, 2015b)." The single choice method was used. The test samples consisted of 35 treated southern yellow pine samples consisting of five treatments of seven replicates each. Of these treatments, three sets of blocks (25 x 25 x 6 mm) had been treated with IPBC and permethrin levels that were designated as low, medium and high. The other two sets were no-permethrin treatments. Lastly, a set of deionized water treated southern yellow pine sapwood controls was added by FPL. All samples were conditioned at 33°C and 62% RH (12% EMC) until a constant weight was reached. Conditioned samples were placed into desiccators before being weighed and placed into test jars. An AWP A E1-15 method with the modification that 27 ml of deionized water was added to each test jar (to achieve 18% moisture content in the sand) was conducted. Screw top jars were filled with 150 grams of sand along with 27 ml distilled water and left for two hours to equilibrate. After two hours, treated and control blocks were added to the jars so that each jar received only a single block. Each sample was placed in each jar on top of the sand on a small square aluminum foil barrier to prevent chemical leaching. Foil and samples were placed so that two corners of the block touched the inner container wall. All treatments were replicated seven times. A total of 400 termites (396 workers + 4 soldiers) were introduced to each jar on the side opposite to the sample. Jars were placed in a conditioning chamber at 27°C and 75±2% RH for 28 days.

After this period, the number of surviving termites was counted and the samples were removed and brushed to clean off sand. Test samples were conditioned at 33°C and 63% RH (12% wood MC) then weighed to determine final weight loss. Samples were later rated according to the AWP A E1 rating system.

The mass loss was used as the measure of termite resistance (Excel, 2010). An ANOVA was used to test the effects of the treatments on termite mortality and mass loss. Means were compared using a t-test.

FPL USFS Permethrin Treatment, Termite Testing, and Preparation for Analysis

In a separate test, southern yellow pine (SYP *Pinus taeda* L) sapwood blocks (25 x 25 x 6 mm) were weighed and conditioned to a constant weight (33°C, 62 ± 3% RH). These blocks were vacuum and pressure treated with three levels of permethrin at concentrations of 0.04, 0.07 and 0.25% m/m. For control treatments, blocks were treated with deionized water. Blocks were vacuum/pressure treated by placing 12 blocks in a 300 ml beaker containing the treatment solution in a vacuum-pressure chamber. Vacuum was applied for 30 minutes then pressure was applied at (275.8 kPa) for one hour. After pressure treatment, blocks were dried using paper towels, weighed, and conditioned again at 33°C and 62±3% RH. A set of five blocks treated to each concentration was exposed to termites in a separate E1 no-choice test as described above. The resulting data were tabulated, subjected to an ANOVA and means were compared using an unpaired t-test.

Retentions were determined on a subset of three blocks that were separately ground to 20 mesh in a Wiley mill. The ground samples were assessed for permethrin content. Approximately 0.25 g of ground wood of each treatment was weighed and placed in a 5 ml vial. The vial and vial cap were weighed separately before the sample was weighed and added. Five milliliters of methanol were added to each vial. Another vial, cap and sample set were weighed and 5 ml of ethanol was added to the vial. For each loading of permethrin, we used three replicate samples for extraction with methanol and three replicates for extraction with ethanol. All samples were sonicated at 50°C for four hours. Vials were manually agitated every 30 minutes during the sonication. After this period, the samples were allowed to cool to room temperature and filtered through a 0.45µ filter for analysis. We also analyzed aliquots of the three treatment solutions (0.04, 0.07, and 0.25%) used for the wood treatment. A subset of three samples from the E1 test performed for the cooperator were also prepared for analysis via solvent extraction and HPLC.

HPLC Analysis: Permethrin Method

Analysis was performed using a Waters® 2695 Separations Module and a 996 Photodiode Array (PDA) detector. The Agilent HPLC system method for separation and quantification of permethrin used an Agilent Zorbax Eclipse XDB-C18, Analytical 4.6 x 150 mm, 5-micron column. The mobile phase was 75% Acetonitrile: 25% Water (CH₃CN:H₂O) with an isocratic flow rate of 10.0 mL/min. The oven temperature for the column was set at 45°C and 10.0 µL of sample was injected for analysis for 15.0 min using UV-detection at 215.0 – 400.0 nm to analyze the sample. All samples were run using a 500 ppm commercial standard permethrin (Chem Service, West Chester, PA).

Figure 1 shows the HPLC chromatogram for a 500 ppm standard used for calibration and analysis of the ppm permethrin in liquid and extracted samples. The peak results indicated that the total cis and trans isomers equaled 500 ppm permethrin showing the accuracy of the HPLC confirming the concentration of the standard.

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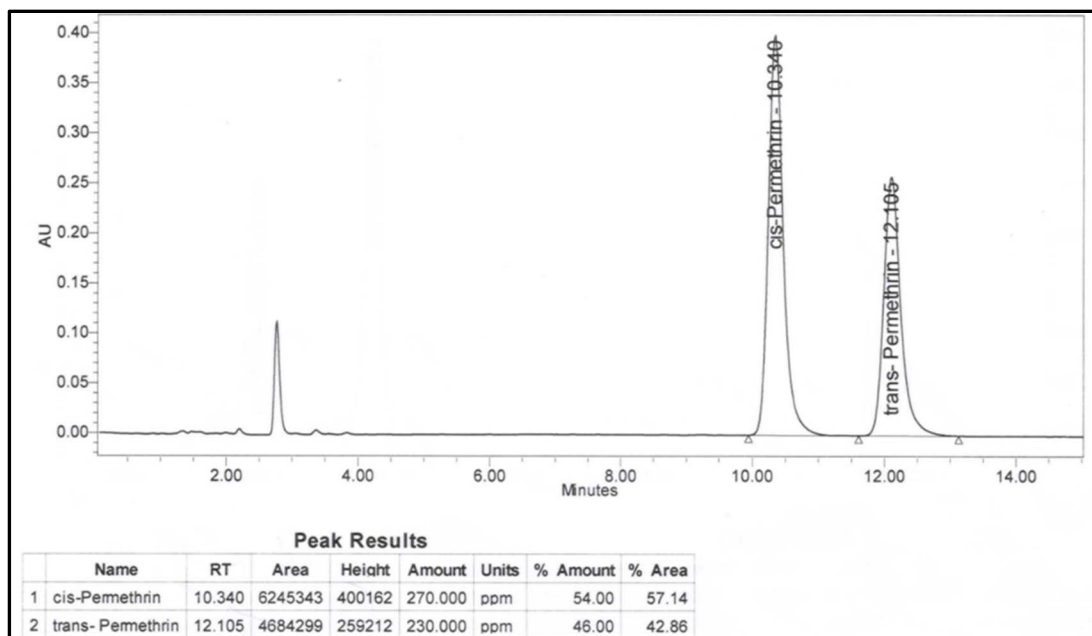


Figure 1. Chromatogram for a 500 ppm permethrin standard used in analysis of permethrin solutions and permethrin from wood extracted with ethanol.

The data obtained from the analysis of the 500 ppm standard was used to analyze the 0.04, 0.07 and 0.25% permethrin treatment solutions using the same HPLC method described above. Figure 2 shows the chromatogram for the 0.04% (400 ppm) permethrin solution. The total ppm for the permethrin isomers was 464.66 ppm or 0.0465%. The ppm results for the 0.07 and 0.25% treatment solutions were 679 ppm and 2608 ppm, respectively.

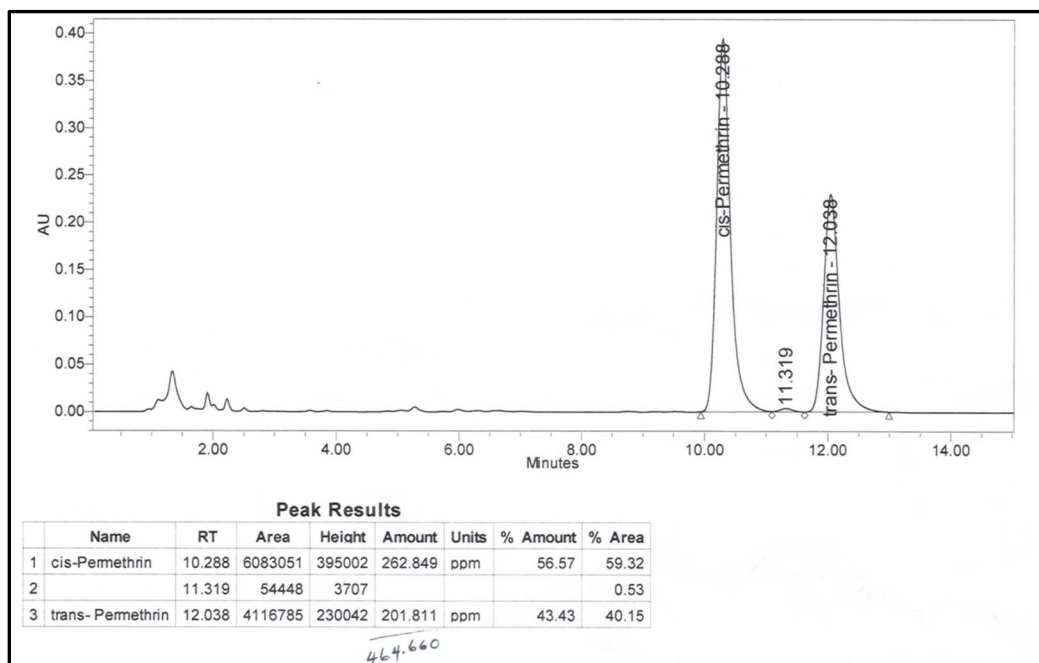


Figure 2. Chromatogram for a 0.04% (400 ppm) permethrin treatment solution used to treat wood blocks.

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Calculations and Example

Permethrin concentrations in the treatment solutions and wood were determined using the method described by the AS/NZS 1605.3 (Standards, Australia, 2006) for permethrin in wood and also by using a variation of the equations that account for solvent density described in the AWP A43-14 (AWPA, 2015a). Percent recovery from wood was based on treatment net solution uptake divided by the actual amount obtained from the extracted sample. Both methods use a variation in the following equation.

$$\% \text{ m/m} = \frac{C \times V}{W_s \times 10^4}$$

The method described in the AS/NZS 1605.3 used the total ppm obtained from HPLC analysis as the concentration multiplied by the volume of solvent used in the extraction. This was then divided by the pre-extracted sample dry weight multiplied by 10,000. In our case, we used the sum of the output for ppm of each isomer obtained by HPLC analysis multiplied by 5 then divided by 0.25 grams x 10,000.

AS/NZS 1605.3

$$\text{Total Permethrin (\%m/m)} = \frac{(\text{cis} + \text{trans}) \text{ permethrin} \times V}{\text{Sample dry weight} \times 10^4}$$

Where V = 5 ml sample volume

A similar method described in the AWP A43-14 used for calculating imidacloprid in wood accounts for solvent density in the volume factor. The weight of methanol or ethanol solvent was converted to volume by dividing the weight by the density of the mixture. In this case, the equation was the same except the volume factor is obtained by subtracting the cap, vial and sample from the sum of the cap, vial and sample initially weighed before the addition of solvent. This is then divided by the density of the solvent.

AWPA A43-14

$$\text{Total Permethrin (\%m/m)} = \frac{(\text{cis} + \text{trans}) \text{ permethrin} \times V}{\text{Sample dry weight} \times 10^4}$$

Where V =

$$\frac{W_B - W_A - W_S}{\text{Solvent density}}$$

$W_B = \text{cap} + \text{vial} + \text{sample} + \text{solvent}$

$W_A = \text{cap} + \text{vial}$

$W_S = \text{sample}$

$D_{\text{meth}} = 0.792$

$D_{\text{etoh}} = 0.789$

Figure 3 shows results for permethrin analysis of a ground wood sample that had been treated with a 0.25% solution and extracted in methanol. These data were used for example calculations that are subsequently shown.

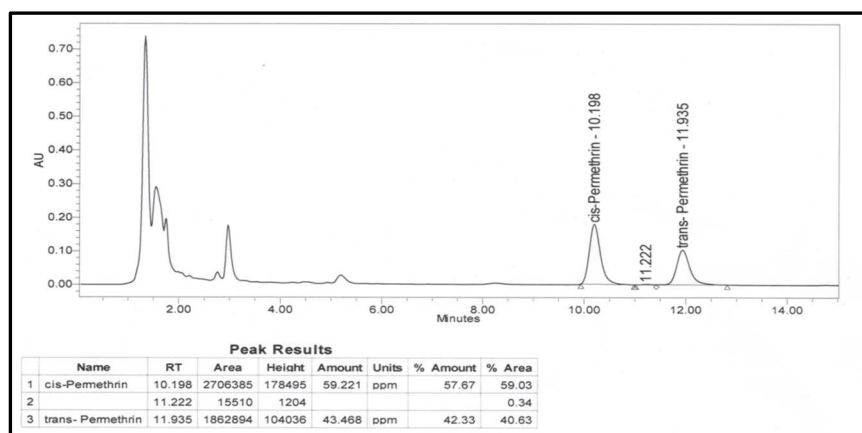


Figure 3. Chromatogram for methanol solution used to extract permethrin from ground wood treated with a 0.25% permethrin treatment solution.

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The ppm for each isomer obtained from the HPLC output was summed and used in each of the equations outlined previously. In this example, the sample dry weight was 0.2517 grams. The cap, vial and sample were 13.5254 grams, the cap and vial were 9.975 grams. The amount of methanol used was 5 ml and the density of the methanol was 0.792. This data was used in either calculation for all samples from either methanol or ethanol extraction. Using the data from Figure 3 and the weights mentioned above, we calculated 0.2008% m/m permethrin using the AS/NZS 1605.3 method and 0.2040% permethrin using the AWP A43-14 method. For the FPL and cooperator samples, mean percent permethrin was compared with one-way ANOVA within treatments and between the calculation methods for each treatment. For percent recovery of permethrin, we divided the average percent permethrin extracted from the wood by the original percent permethrin in the wood percent for a particular treatment.

RESULTS AND DISCUSSION

AWPA E1 Termite Testing

Cooperator Samples Test

Table 1 provides a summary of the means and statistical analysis for percent mass loss and percent mortality. The water control treatment incurred the highest mass loss that was significantly higher than all other treatments. Two other non-permethrin treatments also incurred high mass loss which did not differ statistically from one another, but did differ significantly from all other treatments. Although termites consumed less wood in the no permethrin treatments compared to the water treatment, the block ratings for these treatments were 0 indicating complete failure. No mass loss occurred in high, med and low IPBC/PER treatments.

Percent mortality was lowest in the non-permethrin and water treatments. Mortalities in these treatments were not significantly different from one another, but were significantly lower than low, med and high IPBC/PER treatments that all incurred 100% mortality.

FPL Termite Test

Table 2 provides a summary of the means and statistical analysis for percent mass loss and percent mortality. The water control treatment incurred the highest mass loss that was significantly higher than all other treatments. No mass loss occurred in any of the permethrin treatments. Percent mortality was lowest for the water treatment and was significantly lower than the permethrin treatments which all incurred 100% mortality.

Table 1. Results of an AWP A43-14 no-choice test showing mass loss and termite mortality with statistical results after exposure of *Reticulitermes flavipes* to IPBC/Permethrin treated wood.

Mass Loss				Mortality			
Treatment	Mean Mass Loss (%)	Standard Deviation	ANOVA	Treatment	Mean Mortality (%)	Standard Deviation	ANOVA
High IPBC/PER	0.00	0.00	A	High IPBC/PER	100.00	0.00	A
Mid IPBC/PER	0.00	0.00	A	Mid IPBC/PER	100.00	0.00	A
Low IPBC/PER	0.00	0.00	A	Low IPBC/PER	100.00	0.00	A
No IPBC/PER	55.10	3.42	B	No IPBC/PER	17.14	6.91	B
No IPBC/PER	51.97	3.84	B	No IPBC/PER	15.93	2.50	B
Water	70.41	5.11	C	Water	13.82	3.64	B

Values sharing a capital letter are not significantly different at $\alpha=0.05$.

Table 2. Results of a second AWP A43-14 no-choice test showing mass loss and termite mortality with statistical results after exposure of *Reticulitermes flavipes* to permethrin treated wood.

Mass Loss				Mortality			
Treatment	Mean Mass Loss (%)	Standard Deviation	ANOVA	Treatment	Mean Mortality (%)	Standard Deviation	ANOVA
Water	25.40	4.52	A	Water	33.40	6.47	A
0.04	0.00	0.00	B	0.04	100.00	0.00	B
0.07	0.00	0.00	B	0.07	100.00	0.00	B
0.25	0.00	0.00	B	0.25	100.00	0.00	B

Values sharing a capital letter are not significantly different at $\alpha=0.05$.

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HPLC Analysis of Permethrin Treated Wood – FPL

Figures 4A and 4B show the average percent of permethrin in the blocks determined by extraction using each solvent for wood blocks treated with the three solution concentrations (0.04, 0.07, 0.25% permethrin). Figure 4A used the permethrin calculation based on the AS/NZS 1605.3 method. Figure 4B used a variation of calculations in the AWP A43-14 standard. No significant differences were found between sets extracted with ethanol or methanol for each treatment level.

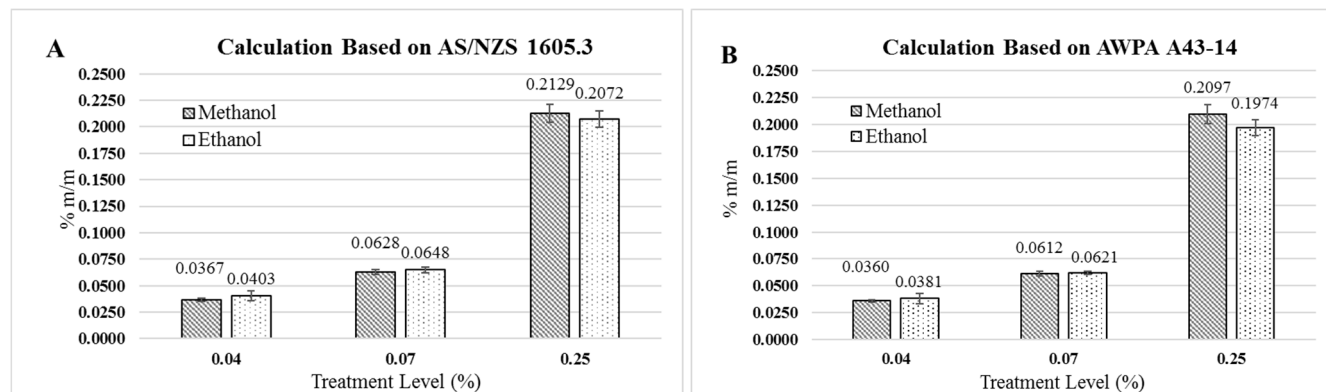


Figure 4. Average percent permethrin in wood recovered using methanol or ethanol and two calculation methods, 4A or 4B for wood blocks treated at the three retentions (0.04, 0.07, 0.25% permethrin).

Percent Recovery

Table 3 shows the percent recovery for permethrin in the treated samples for the different solvents and calculation methods. We divided the average percent permethrin extracted from the wood by the original percent permethrin in the wood or a particular treatment. For example, the average percent permethrin from blocks treated with a 0.0465% solution, extracted with methanol and calculated using the AS/NZS 1605.3 method was 0.0367% (Figure 4A). This was divided by 0.0482% and multiplied by 100 to give 76.5% permethrin recovered.

Table 3. Percent permethrin recovery using two different solvents for three concentrations of permethrin from SYP treated blocks using two calculation methods.

AS/NZS 1605.3				AWPA A43-14			
Treatment Solution	% PER Uptake in Wood	Recovery (%)		Treatment Solution	% PER Uptake in Wood	Recovery (%)	
		Methanol	Ethanol			Methanol	Ethanol
.0465 (.04)	0.0482	76.5	83.5	.0465 (.04)	0.0482	74.6	79.0
.0679 (.07)	0.0934	67.2	69.4	.0679 (.07)	0.0934	65.5	66.5
.2608 (.25)	0.3277	65.0	63.2	.2608 (.25)	0.3277	64.0	60.2

Percent recovery was higher with ethanol as the solvent compared to methanol for both the 0.04 and 0.07% treatments, but the difference was not significant. Recovery using methanol as the solvent was higher for the 0.25% treatment compared to ethanol. Better recovery might be facilitated by soxhlet extraction of wood shavings compared to sonication as shown by Šťávoř et al. 2011, but this approach was not performed in this study.

HPLC Analysis of Permethrin Treated Wood: Cooperator Samples

Figures 5A and 5B show the average percent of permethrin in samples treated by Troy Corporation from the first termite resistance test. Although these samples had been treated with a mix of IPBC/Permethrin, we analyzed for permethrin only. As in the FPL results previously described, we used a calculation for permethrin based on the AS/NZS 1605.3 (Figure 5A) or the AWP A43-14 method (Figure 5B). Although samples treated with the high concentration of permethrin resulted in higher levels of permethrin in the methanol extract, no significant differences were found between sets extracted with ethanol or methanol for this level or the low and medium level treatments.

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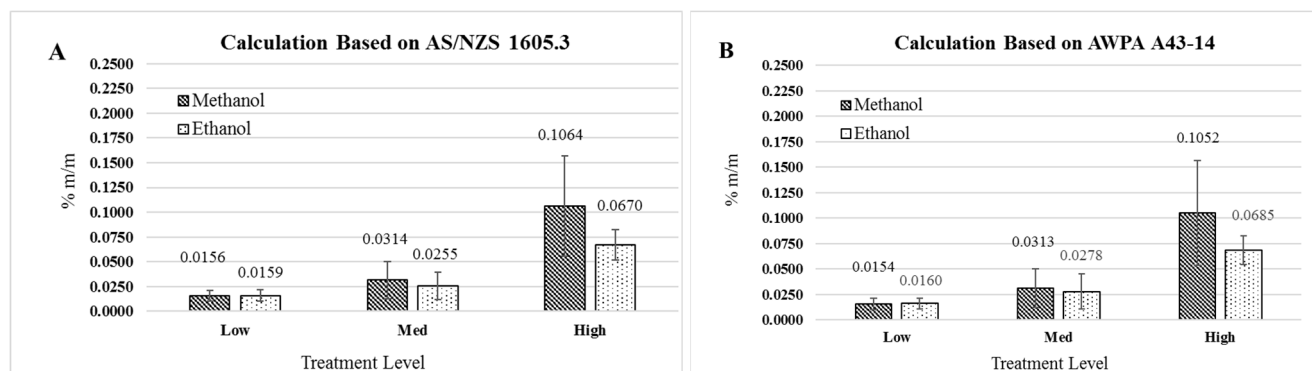


Figure 5. Average percent permethrin in wood recovered using methanol or ethanol and two calculation methods, 5A or 5B for wood blocks treated at the three retentions (low, medium, high % permethrin).

CONCLUSIONS

All levels of permethrin in wood were effective against subterranean termites in an AWP A1-15 termite test. All untreated or non-permethrin treated samples incurred high mass loss from termites and cause lower termite mortality.

Analysis of treated blocks via HPLC and using ethanol or methanol as extraction media indicated that there was no difference in recovery when either methanol or ethanol is used. The use of a calculation to account for solvent and sample density did not give better results than a simple calculation using total solvent volume. This data suggests that either methanol or ethanol could be used as effective solvents to remove permethrin from treated wood samples. Since IPBC is commonly used as a co-biocide with permethrin in wood preservation, methanol may be a more versatile solvent due to its wide polarity range. This study did not focus on the analysis of IPBC and permethrin together. Future work will examine this along with potential modifications to the extraction methods that will improve the percent recovery of active ingredient.

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