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WOOD UNDER FRESH WATER: EFFECT ON THE CHEMICAL PROPERTIES AND ON DECAY RESISTANCE

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ABSTRACT

This study aimed to evaluate the effect of waterlogging on the chemical properties and on decay resistance of two fast-growing eucalypt species. Samples of spotted gum and rose gum wood were placed underwater and taken out at different times: after 4, 8 and 12 months. Chemical properties were performed via wet chemical quantification (Tappi standards), monomeric sugars by HPLC, and qualitative evaluation of extractives by Py-GC/MS and ATR-IR spectroscopy; biological performance was investigated using *Trametes versicolor* and *Gloeophyllum trabeum* rot fungi. The results showed slight changes to the chemical properties of both wood species, with an increase in lignin represented by the reduction of sugars due to waterlogging. Both species showed higher susceptibility to *Gloeophyllum trabeum* mainly in the sapwood, with no influence on decay resistance due the waterlogging.

Keywords: Chemical composition, decay fungi, waterlogged wood, wood extractives, wood underwater.

INTRODUCTION

Under certain conditions of exposure, wood may rapidly decompose due to organic decay or natural weathering (Hyvönen *et al.* 2005). The exposure to water in certain circumstances, as in constructions, in the building sector, can cause several damages to wood.

On the other hand, the conditions underwater, with moist and without air, reduces the decomposition of wood, which is evidenced by archaeological objects and ships, found after many centuries at the sea or lakes in relatively well conditions (Fojutowski *et al.* 2014). Besides, in some industries of forestry sector, logs are reserved underwater while wait the production process as a mean of avoiding cracks and fungi and insects deterioration (Lourençon *et al.* 2015).

In this context, many countries, mainly at 1950s and 1970s, had their forests flooded due the construction of dam projects, and since the mid-1990s this resource have been explored (Fojutowski *et al.* 2014). It is well know the relatively well preserved structures of these woods; however, the most part of the scientific studies are focused on culture heritage.

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Furthermore, studies of wood exposed to underwater conditions can be used to provide support to the search for new information about underwater archaeological objects (Björdal 1999, Jensen and Gregory 2006, Tamburini *et al.* 2014), flooded forests (Kooye 2011, Tenenbaum 2004), and wood underwater to preserve its structure before some industrial application.

A preliminary study showed an increase of homogeneity and specific gravity of these two eucalypt varieties after waterlogging (Lourençon *et al.* 2015). Nevertheless, studies related to chemical compounds and biological resistance in wood submerged to fresh water could supplemental the first investigation. To understand the role that water (as an isolated factor) plays in the submerged wood, it was chosen a non-real-world submerged wood situation, composed by a controlled environment where there was no influence of sand, mud, weathering and/or organisms.

The aim of our research was to evaluate the effect on the chemical properties and on decay resistance in spotted gum and rose gum wood caused by the submergence of samples in fresh water for twelve months.

MATERIAL AND METHODS

Raw materials and experimental submergence

Spotted gum (*Corymbia citriodora* Hill & Johnson) and rose gum (*Eucalyptus grandis* Hill ex Maiden) wood from a fast-growing forest plantation were harvested in Southern Brazil (31º45'48"S 52º29'02" W). The Köppen classification of this region is Cfa. For each species, three fifty-year old trees were randomly extracted according to ASTM (1999). The first log (1 m length) of each tree was cut. Then, four small logs (each 25 cm) were cut. One small log was utilized as control sample (T_0) (not submerged) and the three others were submerged in drinking water boxes (from a public network), at ~20°C, that were changed every two weeks (to avoid bacterial degradation), and taken out at different times: 4, 8 and 12 months (T_4, T_8 and T_{12}, respectively). After the immersion, a central plank was cut from each small log and the sapwood was separated from heartwood by macroscopic distinction. All these samples were placed in a climatic chamber (20ºC and 65% relative humidity) to stabilize the moisture content in ~12%. Then, samples were prepared for chemical analyses and decay resistance (Figure 1). Diagnostic checks (*T*-test of normality and Bartlett test of homogeneity of variances) were performed in order to evaluate whether the data is suitable for analysis of variance (ANOVA) tests. When the null hypothesis was rejected, the average values were compared with a Tukey Test at the level of significance of 1%.

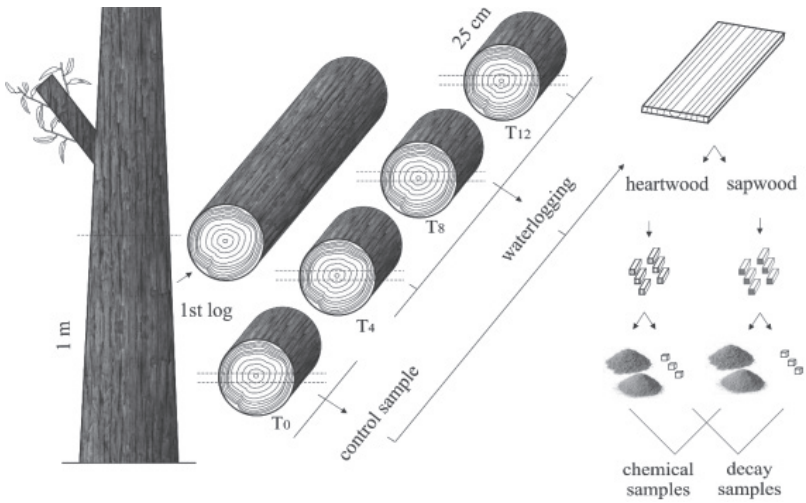

Figure 1. Scheme of sample performed.

Wet chemical quantification

The prepared heartwood and sapwood samples were milled in a knife-mill (Willey type) and classified in 40-60 mesh (fraction 40/60), according to the procedures of TAPPI (1996). The moisture content (wet basis) of wood samples, ethanol:toluene (1:2) extractives, Klason lignin, hot water soluble substances and NaOH solubility$_{(1\%),}$ were determined in triplicate, according to TAPPI Standards (TAPPI, 1993, 1997a, b, 1998a, b).

Monomeric sugars quantification

High performance liquid chromatography (HPLC) was used for the quantification of the main sugars in the wood. The first filtrate from the Klason lignin test was analyzed by a Jasco LC-Net II/ ADC equipment with a photodiode array detector MD-2018Plus, refractive index detector RI-2031Plus and Rezex ROA Organic Acid H^+ (8%) column. Dissolution of 0,005 N H_2SO_4 with 100% of deionised and degassed water was used as a mobile phase. The conditions of the samples' injection were 4ºC; 0,35 mL/min flow and volume of 20 µL.

Classification of extractives by infrared spectroscopy (ATR-IR)

The lipophilic extractives obtained through ethanol/toluene wood extraction (a mix of three independent replicates), at utmost times (T_0 and T_{12}), were analyzed by ATR-IR spectroscopies. The study was developed in a Nicolet Nexus 570 equipment by direct transmittance in a single-reflection ATR System, at a resolution of 4 cm^{-1} for 32 scans ranging from 700 cm^{-1} to 4000 cm^{-1}.

Identification of extractives by Py-GC/MS

The ethanol/toluene extracts, at utmost times (T_0 and T_{12}), were analyzed by Pyrolysis coupled with gas chromatography/mass spectrometry (Py- GC/MS). The pyrolysis was carried out using a CDS analytical Pyrobrobe 5150. The pyrolysis temperature was set at 650ºC for 15 sec with a heating rate of 2ºC $msec^{-1}$. The products were then analyzed by a GC-MS instrument. The GC (7890A)-MS (5975C inert MSD with Triple-Axis Detector) Agilent was equipped with a capillary column HP-5MS ((5%-Phenyl)-methylpolysiloxane, 60 m x 0,25 mm). Helium was used as the carrier gas. The oven program started at 50ºC and was held for 2 min at this temperature. Then, it was raised to 120ºC at 10ºC/min and held for 5 min; raised to 280ºC at 10ºC/min, held for 8 min and finally raised to 300ºC at 10ºC/min and held for 10 min. For the characterization of extractives, identified through NIST08 Mass Spectral Library, as a selection criteria, were discarded compounds area less than 1%, and after that, only compounds with 70% of minimum probability of certainty were considered, summarizing 32 main compounds.

Resistance of wood to *Trametes versicolor* and *Gloeophyllum trabeum*

Decay resistance of submerged woods was evaluated by *in vitro* tests, according to PN-EN113 Standard (PN-EN113 2000), adapted. Potato agar was used as substrate and *Gloeophyllum trabeum* (Persoon ex Fries) Murrill and *Trametes versicolor* (Linnaeus ex Fries) Pilat fungi were used to promote brown and white decay, respectively. Nine samples of each wood, specie and time were separately exposed to decay for 16 weeks.

Mass loss after fungal attack was calculated by the difference between the oven-dried mass of the samples before and after the test.

RESULTS AND DISCUSSION

Chemical evaluation of wood

There was no statistical difference in the content of lipophilic extractives (ethanol/toluene) as a function of waterlogging (Table 1). These were expected results due to extractives type - only extracted by nonpolar solvents; hence, not removable by water (polar). Differently, the results reported by Fojutowski *et al.* (2014), showed a decrease of extractives in oak wood (without specific analysis between heartwood and sapwood) after two years underwater, probably due to longer time underwater, salt and other environment influences.

Table 1. Chemical quantification of spotted gum and rose gum wood over a period of one year submerged.

Content (%)		Spotted gum				Rose gum			
		heartwood Mean (SD)	F-ratio	sapwood	F-ratio	heartwood	F-ratio	sapwood	F-ratio
Extractives	T_0	5,7 (0,9)	$1,3^{ns}$	1,9 (0,2)	$2,8^{ns}$	2,1 (0,9)	$2,0^{ns}$	2,3 (0,9)	$1,2^{ns}$
	T_4	6,8 (1,8)		1,4 (0,4)		2,1 (0,5)		1,7 (0,2)	
	T_8	7,0 (1,3)		1,5 (0,0)		3,1 (1,0)		1,7 (0,5)	
	T_{12}	6,9 (1,2)		1,3(0,5)		2,4 (0,1)		2,1 (0,7)	
Hot water solubility	T_0	5,4 (0,5) *a*	$8,0^{**}$	2,9 (1,7)	$0,7^{ns}$	4,0 (1,9)	$2,3^{ns}$	2,9 (0,8)	$3,5^{ns}$
	T_4	5,0 (0,4) *a*		3,5 (1,6)		2,0 (1,0)		4,1 (1,3)	
	T_8	6,0 (1,9) *a*		2,5 (1,0)		3,9 (1,7)		2,4 (1,6)	
	T_{12}	8,0 (0,1) *b*		2,5 (0,4)		3,6 (0,8)		4,4 (0,8)	
NaOH solubility$_{(1\%)}$	T_0	15,9 (2,0)	$0,5^{ns}$	9,6 (2,0)	$0,7^{ns}$	20,8 (2,2)	$0,5^{ns}$	10,6 (0,5)	$0,3^{ns}$
	T_4	14,7 (2,8)		11,9 (2,4)		20,6 (2,7)		10,4 (0,6)	
	T_8	15,3 (4,1)		11,4 (1,7)		17,7 (1,4)		9,8 (1,6)	
	T_{12}	18,5 (3,1)		10,1 (2,1)		21,6 (4,2)		9,8 (1,1)	
Insoluble lignin	T_0	26,7 (3,3) *ab*	$9,3^{**}$	22,1 (1,2) *a*	$28,0^{**}$	30,8 (0,4) *a*	$11,3^{**}$	25,9 *a*	$19,9^{**}$
	T_4	22,2 (1,1) *a*		20,0 (2,9) *a*		27,3 (5,4) *a*		25,3 *a*	
	T_8	35,4 (3,7) *c*		34,2 (3,1) *b*		41,3 (4,3) *b*		35,2 *b*	
	T_{12}	32,5 (4,7) *bc*		30,8 (3,1) *b*		40,2 (1,8) *b*		34,8 *b*	

**Significant at 99% of confidence level. ns: not significant

The only difference in hot water-soluble compounds results was an increase at T_{12} in the heartwood for spotted gum. This isolated result could be associate to the available situation of the heartwood samples in the hot water analysis (able to be extracted by water), different from the waterlogging. The $NaOH_{(1\%)}$ solubility results could not be used to evaluate differences by the waterlogging, as well.

Higher differences are found for the lignin content (Table 1) which, for both species and woods, presented an increase from T_8. Increased lignin due to wood waterlogging has been observed (Fojutowski *et al.* 2014). The highest lignin percentage presented in the last two times (T_8 and T_{12}) is related to the reduced values of other wood components that can be extracted or degraded with water, such as salts, simple carbohydrates, polysaccharides and some phenolic substances (Silvério 2008). In addition, Krogell *et al.* (2013) and Song *et al.* (2013) have previously shown that hemicellulose can be obtained using water as solvent.

Therefore, if the carbohydrate content (Figure 2) has lower values as a function of time, the lignin proportion - difficult to extract due to an amorphous and complex structure (Chakar and Ragauskas 2004) -presented higher values, compensating the reduction in proportion, since the balance should add up to 100%. An analogy can be made with wood heat treatment, wherein the proportional increase of lignin occurs due to thermal decomposition of the hemicelluloses (Esteves *et al.* 2011, Mohareb *et al*. 2012).

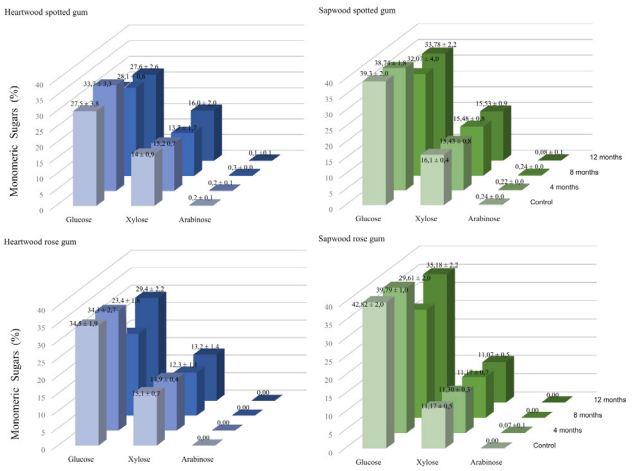

Figure 2. Monomeric sugars in heartwood and sapwood of spotted gum and rose gum.

Chemical evaluation of extractives

The ATR-IR spectra of extractives from waterlogged wood and control samples for both spotted gum and rose gum are shown in Figure 3. The band at 1030 cm^{-1} related to C-O stretching (ether bond), present in carbohydrates, aromatic compounds (Barbosa *et al.* 2005) and alcohols (Silverstein *et al.* 2005), decreased in the waterlogged (T_{12}) sapwood samples for spotted gum due to a possible removal of some carbohydrates with waterlogging. For the rose gum extractives, no specific behavior was observed.

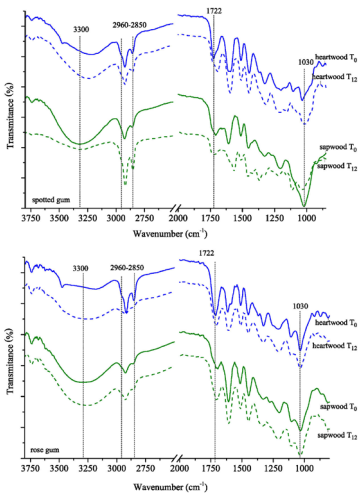

Figure 3. ATR-IR spectra of extractives of spotted gum and rose gum wood.

The band at 1722 cm^{-1} related to C=O stretching present in carboxylic acid, aldehydes, esters and ketone (Barbosa *et al.* 2005, Silverstein *et al.* 2005) had a higher intensity in the heartwood of spotted gum and sapwood of rose gum; such a difference is associated with the distinct chemical composition of each species.

The C-H (methyl and methylenes) bond vibration at 2960-2850 is common to several classes of aliphatic compounds such as fatty acids and esters, long-chain alcohols and steroids (Silvério 2008,

Silverstein *et al.* 2005, Sócrates 1979). The highest intensity of this region was observed in the sapwood at T_{12} for spotted gum, related to extractive compounds with these substances.

The band at 3300 related to OH stretching indicates the presence of carboxylic acids and alcohols (Barbosa *et al.* 2005, Silvério 2008). In the sapwood of spotted gum, a decrease of this band's intensity at T_{12} can be observed, which indicates a decrease in hydroxyl groups (Ajuong and Breese 1998), possibly removed by water in the waterlogging. For the rose gum, the higher intensity in the sapwood for both times (T_0 andT_{12}) is clear.

In addition, thirty-two major compounds with highest concentrations in the extractives of sapwood and heartwood for both species were identified by Py-GC/MS analysis (Figure 4). The active principles with their retention time (RT) and the percentage of the total area in the extractives for both species are presented in Table 2.

For spotted gum, an increase in the phenolics and polyphenolic compounds (peaks 5, 11, 16, 17 and 19), from T_0 to T_{12}, and a decrease for limonene (peak 6) were observed in the heartwood, while the phenolic compounds (peaks 16 and 19) decreased and the limonene increased (peak 6) in the sapwood.

The phenolic and terpene compounds are the main cause for the natural decay resistance of the wood (Costa *et al.* 1997, Mossi *et al.* 2010). Thus, on one hand, the resistance of sapwood decreases with a reduction in phenolics, while on the other, it increases with the terpene increase.

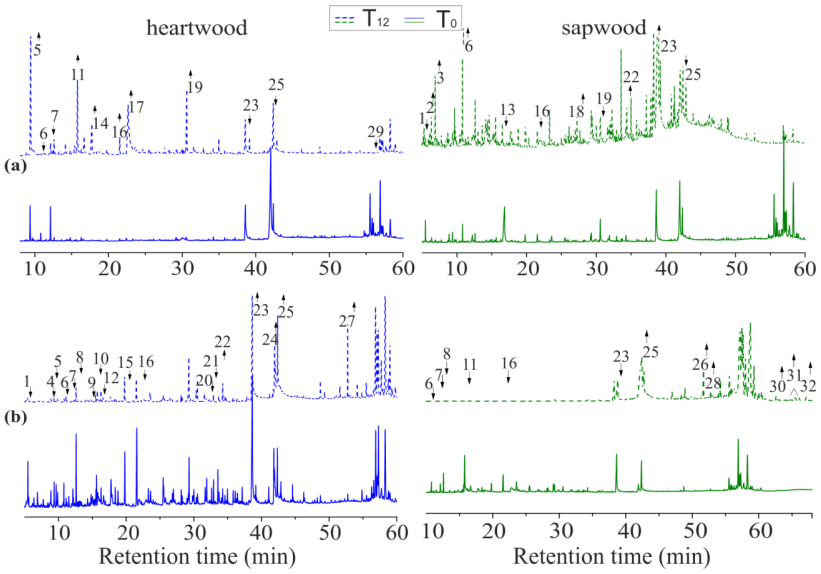

Figure 4. Chromatogram of lipophilic extractives from heartwood and sapwood of *Corymbia citriodora* (a) and *Eucalyptus grandis* (b) for control samples (T_0) and waterlogged samples (T_{12}).

The fatty acids (peaks 23 and 25) of heartwood showed a reduction from T_0 to T_{12}. Conversely, in the sapwood (peaks 22 and 23) fatty acids were higher in T_{12}. This behavior was confirmed by band 2960-2850 cm^{-1} in the ATR-IR analysis, where the highest intensity was observed for the sapwood at T_{12}.

Table 2. Composition of lipophilic extractives from heartwood and sapwood for both spotted gum and rose gum.

			Spotted gum				Rose gum			
			Heartwood		Sapwood		Heartwood		Sapwood	
Peak	RT	Compound	% Area				% Area			
			T_0	T_{12}	T_0	T_{12}	T_0	T_{12}	T_0	T_{12}
1	5,47	Furfural	-	-	1,14	0	1,13	0,18	-	-
2	6,32	p-xylene	-	-	0,28	0,35	-	-	-	-
3	6,86	Styrene	-	-	0,21	1,02	-	-	-	-
4	8,86	2-Furancarboxaldehyde, 5-methyl-	-	-	-	-	0,61	0,18	-	-
5	9,5	Phenol	2,56	8,75	-	-	0,82	0,16	-	-
6	10,8	Limonene	0,49	0,04	0,79	1,01	-	-	-	-
6	10,8	D-limonene	-	-	-	-	0,5	0,13	0,60	0
7	12,12	Phenol, 4-methyl	2,41	0,66	-	-	0,51	0,10	0,87	0,01
8	12,6	Phenol, 2-methoxy-	-	-	-	-	1,68	0,39	1,15	0,01
9	14,8	Phenol, 2-ethyl-	-	-	-	-	0,5	0,16	-	-
10	15,62	Phenol, 2-methoxy-4-methyl-	-	-	-	-	0,68	0,09	-	-
11	16	Benzenediol	0	5,76	-	-	-	-	-	-
11		1,2-Benzenediol	-	-	-	-	-	-	5,75	0,02
12	16,28	Benzofuran, 2,3-dihydro-	-	-	-	-	0,47	0,35	-	-
13	16,7	2 -furancarboxaldehyde, 5 - (hydroxymethyl)	-	-	6,88	0,16	-	-	-	-
14	17,75	2- propenoic acid, 2-methyl-, 2-methylpropyl ester methylpropyl ester	0	3,43	-	-	-	-	-	-
15	19,8	2-Methoxy-4-vinylphenol	-	-	-	-	1,90	1,03	-	-
16	21,52	Phenol, 2,6-dimethoxy	0,32	1,14	0,55	0,29	3,11	1,03	1,92	0,02
17	22-23	1,2,4-Benzenetriol	0,18	9,65	-	-	-	-	-	-
18	27,24	Pentadecane	-	-	0,12	0,35	-	-	-	-
19	30,56	Phenol, 3,4,5-trimethoxy-	0,39	5,13	1,72	0,54	-	-	-	-
20	31,9	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	-	-	-	-	0,89	0,19	-	-
21	32,9	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	-	-	-	-	0,58	0,21	-	-
22	34,35	Tetradecanoic acid	-	-	0,37	0,91	0,5	0,75	-	-
23	38,58	n-hexadecanoic acid	6,36	3,28	6,41	9,53	7,8	9,73	5,88	4,07
24	41,93	Oleic acid					2,25	4,44		
25	42,38	Octadecanoic acid	20,8	4,50	8,51	3,50	2,33	7,1	4,81	10,05
26	51,7	Succinic acid, decyl 5-methoxy-3-methylphenyl ester	-	-	-	-	-	-	0,07	1,89
27	52,75	Squalene	-	-	-	-	0,19	1,93		
28	52,76	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	-	-	-	-	-	-	0	0,55

RT - Retention time (min); T_0-control samples, T_{12} - time 12 months.

The phenolic compounds (heartwood: peaks 5, 7, 8, 9, 10 and 16; sapwood: peaks 7, 8, 11 and 16) and limonene (heartwood and sapwood: peak 6) decreased at T_{12}. Rose gum wood extractives presented higher phenolic compounds in the heartwood than spotted gum.

However, the fatty acids in the heartwood (peaks 22, 23, 24 and 25) and sapwood (peak 25) increased at T_{12}, which corroborates with the ATR-IR results; a higher intensity in the region 2960-2850 cm^{-1} that includes these compound classes. In this work, the fatty acids represent the higher areas in the rose gum extractives composition (both in the sapwood and in heartwood) and are the main constituents of this species (Barbosa *et al.* 2005, Kilulya *et al.* 2012, Silvestre *et al.* 2001).

The results showed that there was a partial migration of extractives compounds in both directions of the wood (from heartwood to sapwood and from sapwood to heartwood) and also to water, producing a phenomena of diffusion and counter-diffusion. However, there was no increase or decrease observation in the extractives content (Table 1) appearing due to waterlogging, at any time, only changes in their composition.

Wood decay resistance

The results of the wood mass loss (Table 3) showed greater susceptibility of both wood species to *Gloeophyllum trabeum* (Gt) fungus. This brown-rot fungus degrades preferentially polysaccharides of wood and partially oxidizes the lignin (Aguiar and Ferraz 2012, Hammel *et al.* 2002). Also, both wood species and fungi showed a higher mass loss in the sapwood (Motta *et al.* 2013) and consequently better decay resistance in the heartwood (Costa *et al.* 2003, Wiedenhoeft 2010) mainly due to the higher content of phenolic extractives in this wood.

Table 3. Mass loss of spotted gum and rose gum wood exposed to *Trametes versicolor* and *Gloepyllum trabeum* attack.

Specie	Exposure time	*Trametes versicolor* heartwood	*Trametes versicolor* sapwood	*Gloeopyllum trabeum* heartwood	*Gloeopyllum trabeum* sapwood
		Mass loss (%)			
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
spotted gum	T_0	2,04 ± 0,57	3,95 ± 1,10	5,67 ± 1,51	14,11 ± 3,83
	T_4	2,15 ± 0,71	3,1 ± 1,08	5,78 ± 2,68	16,26 ± 9,32
	T_8	1,90 ± 0,52	4,41 ± 2,83	3,02 ± 0,83	16,4 ± 4,03
	T_{12}	1,40 ± 0,28	3,88 ± 1,97	3,53 ± 1,08	13,84 ± 5,32
F-ratio		$1,90^{ns}$	$0,43^{ns}$	$3,30^{ns}$	$0,48^{ns}$
rose gum	T_0	0,57 ± 0,24	1,33 ± 0,50	1,38 ± 0,52	23,06 ± 4,17
	T_4	0,62 ± 0,31	0,95 ± 0,55	2,34 ± 1,35	23,32 ± 4,76
	T_8	0,71 ± 0,12	1,19 ± 0,77	1,99 ± 1,57	25,98 ± 4,32
	T_{12}	0,91 ± 0,18	1,18 ± 0,97	0,99 ± 0,85	18,03 ± 3,07
F-ratio		$2,62^{ns}$	$0,32^{ns}$	$1,54^{ns}$	$2,31^{ns}$

S.D.- standard deviation. ^{ns}not significant

As can be seen in Table 3, the wood decay resistance did not change as a function of waterlogging in fresh water. Otherwise, wood submerged in seawater showed a decrease after 6 months (Fojutowski *et al.* 2011), 1 year (Pomian *et al.* 2010) and 2 years (Fojutowski *et al.* 2014).

Differences between the studies are mainly due to means of submersion. Wood submerged in seawater has the possibility of suffering an initial weakening by marine organisms, which would consequently cause further degradation in the fungi tests, since the material structure was previously decayed, thus facilitating an enzyme attack during the rot samples.

CONCLUSIONS

Waterlogging of spotted gum and rose gum wood may cause an increase in lignin content due to the reduction of sugars, naturally extracted by water. Concerning to biological results, this work showed neither improvement nor decrease on decay resistance of submerged wood.

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