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Original article

Methodology to assess both the efficacy and ecotoxicology of preservative-treated and modified wood

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Abstract –
• Wood used in outdoor conditions out of ground contact is susceptible to weathering, inducing both fungal decay and leaching of components to the environment.
• This paper presents a methodology to determine these two parameters for untreated, preservative-treated and modified wood. Therefore, the wood was first leached and subsequently exposed to fungal decay of the most prominent wood-rotting fungi. The crustacean *Daphnia magna* was exposed to the leachates to provide information on their impact on the environment.
• Combining both parameters reveals that preservative-treated wood and modified wood are capable of protecting the wood adequately for application under use class 3 conditions without posing a threat to the environment.
• This proves the suitability of the concept of combining efficacy and ecotoxicology for the evaluation of new types of wood treatments.

basidiomycetes / Cu-based preservatives / *Daphnia magna* / furfurylation / thermal modification

Résumé – Méthodologie pour l'évaluation de l'efficacité et de l'écotoxicité du bois traité et modifié.
• Le bois utilisé à l'extérieur sans contact avec le sol est susceptible de s'effriter, induisant des dégradations fongiques et le lessivage de composés dans l'environnement.
• Cet article présente une méthodologie pour déterminer ces deux paramètres pour du bois non traité, traité avec des agents de protection et modifié. Ensuite le bois a été tout d'abord lessivé puis exposé à des attaques fongiques du plus important champignon lignivore. Le crustacé *Daphnia magna* a été exposé aux lixiviats de manière à évaluer leur impact sur l'environnement.
• La combinaison des deux paramètres fait apparaître que les bois traités et modifiés confèrent une protection suffisante pour les usages de classe 3 sans danger pour l'environnement.
• Cela prouve la pertinence du concept qui combine efficacité et écotoxicité pour l'évaluation de nouveaux types de traitement des bois.

basidiomycètes / traitement à base de cuivre / *Daphnia magna* / furfurylation / modification thermique

1. INTRODUCTION

Wood is a very valuable material which is rather cheap, easy to process, has good strength properties and an aesthetic appeal. Due to the numerous wood species lots of combinations of these properties are available. That is why wood is in demand as building material, both for interior applications as well as outdoor end-uses. For wood in exterior applications out of ground contact (use class 3) enhanced wood properties are needed to prevent fungal deterioration. To resolve this problem both wood preservation using biocides and non-biocidal strategies can be envisaged. Since these exterior applications are susceptible to weathering some components may leach from the wood, regardless of the strategy used to protect it. This way, these components become available in nature and may pose a threat to the environment. Therefore, when evaluating new products the efficacy against fungi, basidiomycetes in particular, as well as the ecotoxicological profile are important. This is also in line with the intention of the European Biocidal Product Directive (1998). Up to now no overall approach has existed to combine these two important evaluation criteria. The purpose of this paper is to elaborate on methodology to determine and combine these two parameters.

One of the most used heavy duty wood preservatives in a biocidal strategy is CCA (salts of copper, chromium and arsenic). However, in several European countries and the USA this product is subject to limitations concerning the production, trade and use of it (Donath et al., 2006; Hingston et al., 2001; Lande et al., 2004a). Meanwhile, optimised alternatives to CCA are on the market. Closest to CCA are other copper-based products which combine copper with organic molecules

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such as azoles, amines, quat (alkaline copper quaternary ammonium salts) and HDO (bis[N-cyclohexyldiazeniumdioxy] copper) (Cowan and Banerjee, 2005). Common to all preservatives is that their toxicity to fungi is dependent on their mode of action (Lande et al., 2004a). A major disadvantage of the broad-spectrum Cu-based wood preservatives is their susceptibility to leaching, inducing a subsequent potentially high ecotoxicity (Townsend et al., 2005).

In a non-biocidal protection strategy it is not the purpose to add a compound to the wood which is toxic to degrading organisms, but to change the chemical structure of the wood in such a way that it becomes unattractive/unrecognisable to micro-organisms. Another way is to lower the fibre saturation point of the wood below the minimal moisture content of the wood necessary for fungal degradation (Boonstra et al., 1998). Since the primary purpose of most wood modification techniques is to improve properties of the wood such as dimensional stability, hydrophobicity, fire retardance, mechanical properties and aesthetic appearance of the wood, but also its resistance to wood deterioration, they can be an alternative to the more traditional preservative treatments of wood (Lande et al., 2004a; Tjeerdsma and Militz, 2005). It is also the purpose to produce a material that can be disposed of at the end of its life without environmental hazard (Hill, 2006). In this paper so-called thermally and chemically/impregnation modified wood as defined by Hill (2006) are covered. More precisely, thermally modified spruce according to the Plato process is assessed (Plato International bv, the Netherlands). This two-step heat treatment consists of an initial hydrothermal treatment of the wood, followed by a drying step and finalised by curing, after which conditioning of the wood takes place (Boonstra et al., 1998; 2007; Hill, 2006; Lande et al., 2004a). LCA studies commissioned by Plato Wood have shown that the product has superior environmental performance compared with materials such as concrete and PVC, as well as preservative-treated wood products (creosote and CCA) (Hill, 2006). Kamdem et al. (2000) have already reported that both toxic and non-toxic compounds were formed during a one-step heat treatment. Due to the lack of quantification of these products they were not able to decide whether the final product was toxic or not. The durability of thermally modified wood and different hypotheses for the mechanisms are reported in several publications (Boonstra et al., 1998; 2007; Hakkou et al., 2006; Kamdem et al., 2002; Tjeerdsma et al., 1998). It was shown that neither the increase in the hydrophobic character of the wood, nor the generation of new extractives during heat treatment are responsible for this increase in durability. In contrast to the degradation and/or modification of hemicelluloses, which are generally considered as an important nutritive source for the development of wood-rotting fungi, the modification of the lignin network and changes in the external conditions affecting the microenvironment are thought to affect the decay mechanism of thermally modified wood, increasing its resistance against fungal attack. They also stipulate that it is difficult to distinguish which heat treatment effect contributes to the improved resistance against fungal attack.

Besides this thermal modification, chemical modification, and more specifically, furfurylation was also included. Both southern yellow pine (SYP) and maple were treated according to a process developed by Kebony asa (Norway), the so-called Kebony® treatment. SYP was also treated with the BioRez™ solution, following a process developed by TransFurans Chemicals bvba (Belgium). The furfuryl alcohol (FA) of both the Kebony and BioRez solutions are derived from furfural originating from hydrolysed agricultural wastes. The Kebony treatment of wood consists of an impregnation with FA including additives using a full-cell process, followed by a curing step at elevated temperatures to induce polymerisation and ended by kiln drying of the wood (Lande et al., 2004b). Besides grafting of the FA or polyfurfuryl alcohol to wood cell-wall polymers, homopolymerisation and copolymerisation with additives or wood extractive substances also take place during the process (Lande et al., 2004a).

Despite all the research efforts performed up to now it remains difficult to find a way to treat wood combining sufficient efficacy against fungal decay and providing an excellent ecotoxicological profile of the leachates at the same time. That is why the objective of this research was to find a methodology to evaluate wood for usage in outdoor applications without ground contact, taking both the efficacy of the wood against fungal degradation as well as the ecotoxicology of the wood leachates into account. In a first attempt it was the purpose to include on the one hand the most abundant wood-destroying fungi, and on the other hand, a fast, easy and low-cost evaluation of the ecotoxicity of the wood leachates. Therefore, the resistance of preservative-treated and modified wood to the basidiomycetes *Coniophora puteana*, *Postia placenta* and *Trametes versicolor* and the ecotoxicology of the wood leachates using *Daphnia magna* were evaluated. Since Waldron et al. (2003) already demonstrated the importance of the leaching procedure and in order to have a more general picture of the ecotoxicity in outdoor performance, two different leaching procedures were evaluated. In that respect it was preferred to include both a harsh as well as a mild leaching procedure. Also, different harvesting times were considered.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Reference wood species

Since treated wood is used to either benchmark or outperform other wood available, several reference wood species were included in the research. Both tropical and temperate wood species covering both softwood and hardwood species were used (Tab. I). As native wood species oak, beech and Scots pine were chosen. Furthermore, wood derived from the domesticated species Douglas fir and black locust were examined. Because of their high natural durability (EN 599, 1996; Lincoln, 1994), the tropical wood species padauk, azobé, merbau and bangkirai were also included. They exhibit an inherent toxicity due to the abundant presence of extractives in the wood. It is already proven that these extractives may leach out under laboratory conditions, using a variety of extraction methods (Hillis, 1987; Van Eetvelde et al., 1998). As such, these extractives may harm the

Table I. Overview of wood species used, their botanical name, origin and natural durability (Lincoln, 1994).

Wood species	Botanical name	Origin	Natural durability (EN 350–2)
Softwoods			
Scots pine sapwood	*Pinus sylvestris* L.	Europe, Asia	5
Scots pine heartwood	*Pinus sylvestris* L.	Europe, Asia	3–4
Douglas fir	*Pseudotsuga menziesii* (Mirb.) Franco	North America, Europe[1]	3
Temperate hardwoods			
Beech	*Fagus sylvatica* L.	Europe	5
Oak	*Quercus robur* L. / *Q. petraea* Lieblein	Europe, Asia	2–3
Black locust	*Robinia pseudoacacia* L.	North America, Europe[1]	1–2
Tropical hardwoods			
Abiurana	*Pouteria guianensis* Aubl.	South America	1–2
Azobé	*Lophira alata* Banks ex Gaertn.f.	West Africa	1(-2)
Bangkirai	*Shorea laevis* Ridl.	Southeast Asia	2
Merbau	*Intsia bijuga* (Colebr.) Kuntze.	Southeast Asia	1–2
Padauk	*Pterocarpus soyauxii* Taubert	West Africa	1
Piquia	*Caryocar villosum* (Aubl.) Pers.	Central America	2–3

[1] Domesticated in Europe (material used for this research).

environment. Two relatively new wood species on the European market originating from South America were added; namely, abiurana and piquia. All these tropical wood species are frequently used in water constructions such as canal lining and lock gates or in other outdoor applications such as exterior joinery, cladding, fencing and garden furniture, indicating they are suited for use in use class 3 or even higher use classes. In these outdoor applications they may pose a potential hazard to the (aquatic) environment and are therefore examined in this test setup.

2.1.2. Preservative-treated wood

Scots pine sapwood was treated with different wood preservatives that are on the market for heavy duty application, mainly copper-based wood preservatives. In this respect CCA was the reference wood preservative (Tanalith CO, Arch Timber Protection nv, Belgium; 30.6% CrO_3, 11.1% CuO, 17.3% As_2O_5), and three other Cu-based wood preservatives were included; namely, a Cu-amine (Impralit KDS, Rütgers Organic Gmbh, Germany; 20.5% $CuCO_3$-$Cu(OH)_2$, 8% H_3BO_3, 10% polymer betain), a Cu-azole (Tanalith E 3492, Arch Timber Protection nv, Belgium; 20.5% $CuCO_3$, 4.5% H_3BO_3, 0.23% tebuconazole, 0.23% propiconazole) and a Cu-quat (Kemwood ACQ 1900, CSI Kemwood AB, Sweden; 38–44% copper tetra-amine-dihydrogencarbonate, 4.8% N-alkyldimethylbenzylammoniumchloride QAC). It was decided to impregnate the wood with the preservatives at about 10 kg of product retention per m^3 of sapwood. This is close to the average retention of these products as prescribed for usage under class AB of the Nordic Wood Preservation Council (NTR No. 73, 2005).

Scots pine sapwood specimens (50 × 25 × 15 mm^3 (longitudinal × tangential × radial)) were placed in a vessel and a vacuum was induced for 20 min before adding the solution to the vessel. After reinstalling the vacuum for 5 min, the vacuum was released and the specimens stayed submerged for another 2 h. Afterwards the specimens were subjected to a fixation drying in ambient conditions for 48 h and subsequently at 60 °C until they reached a constant mass. The masses prior to and after treatment were determined and allowed the calculation of the obtained retention.

2.1.3. Modified wood

In this research modified wood can be divided into two groups; namely, thermally modified wood and furfurylated wood. The thermally modified spruce (*Picea abies*) used in this research was sampled during the development of the hydrothermal Plato process. Furfurylated wood according to two different scaling-up processes was also included. In the first process, SYP (30% WPG) and maple (20–25% WPG) were treated according to a furfurylation process using a monomeric furfuryl alcohol treating solution. For the second furfurylation process, SYP was treated according to a process applying an oligomeric furfuryl alcohol-based solution (20–30% WPG). The main end-uses for both thermally modified and furfurylated wood are garden furniture, fencing, cladding and joinery (Hakkou et al., 2006).

2.2. Leaching procedures

It was the purpose of this study to set up a methodology to evaluate both the fungal resistance of the wood (either treated or modified) as well as the ecotoxicity. Since the preservative-treated wood was aimed at use class 3 (EN 335-1, 2006) and following the European Standard EN 599-1, the specimens were first leached according to the European Standard EN 84 (1996) and then subjected to fungal decay according to the European Standard EN 113 (1996). For testing equivalent natural durability assessment reference is made to CEN/TS 15083-1 (2006) which is similar, but not using dose response as a basis.

Wegen et al. (1998) stated that for ecotoxicity estimation, testing of the 24-h leachate is suited as a worst-case consideration. Concerning the wood in question, they recommend using the 14-day EN 84 leachate of treated timber. It is, however, a basic problem to generate a leachate containing realistic concentrations of depleted components (Melcher and Wegen, 1999). Therefore, a second series of treated wood blocks was subjected to the milder OECD part 1 leaching procedure (CEN/TR 15119, 2005), developed for usage in use class 3. In view of equal treatment of all specimens, two series of the modified wood blocks were also subjected to leaching, each series according to one of both leaching methods.

The leaching procedure according to the European Standard EN 84 was used as a worst-case scenario. The 12 replicates for each treatment were divided into two groups. The water volume to wood ratio used was 5, i.e. 562.5 mL water for every 6 specimens. The leaching procedure consisted of an initial impregnation with distilled water. The water was subsequently replaced 2 h after the impregnation and at 24 h and 48 h, and another seven times in the next 12 days at intervals of not less than one day and not more than three days. The first (24 h) and last leachates were retained for further use in the ecotoxicity tests. This leaching procedure differs from the OECD part 2 leaching procedure (OECD, 1984), which is developed for wood in use classes 4 or 5 (EN 350-2, 1994): (1) the cross-sections of the specimens are not sealed in EN 84, (2) the ratio wood surface to water is 40 m^2/m^3, whereas this is 50 in OECD part 2, and (3) the exposure time is only 14 days according to EN 84 and 30 days according to OECD part 2.

A more realistic and much 'milder' OECD part 1 leaching procedure was also performed. Using this method, both cross-sections were sealed with 2 layers of a 2-component polyurethane finish. This time the ratio wood surface area/water volume was kept constant at 40, being 600 mL of water for 6 specimens. No impregnation of the specimens was performed, but immersions in water were used to simulate rain events. One rain day consisted of 3 separate rain events. This means that the specimens were in groups of six specimens submerged three times a day in 600 mL distilled water for 1 min. Between the submersions the specimens were allowed to dry under ambient conditions. In the next 14 days every third day was a rain day. The leachates were collected after the first and fifth rain days and used for ecotoxicity testing. Regardless of the leaching procedure used, both pH and total hardness were determined on all leachates. To lower the impact of pH on the ecotoxicity testing, pH values lower than 6.0 were adjusted with NaOH (0.5 mol/L) to a value between 6.0 and 7.0.

2.3. Ecotoxicity testing

The ecotoxicity of the leachates was evaluated with the freshwater crustacean *Daphnia magna* using the Daphtoxkit procedure (Daphtoxkit F^{TM} magna, 2001), which is based on the OECD guideline 202 (1984). Five concentration series of the pooled duplicate leachates (1:2 dilution series) using four replicates and a control series were used for each treatment. Five neonates were transferred into each well and the micro-well test plates were subsequently incubated for 48 h in the dark at 20 °C. After 24 h and 48 h of exposure, the inhibition of mobility of the daphnids was recorded. The toxicity data obtained as 50% effect endpoint values (EC_{50}s) in % of dilution were calculated according to the Trimmed Spearman-Karber method (Hamilton et al., 1977; United States Environmental Protection Agency, 2006). The EC_{50}values were subsequently transformed into toxic units (TUs) with the formula of Sprague and Ramsay (1965) as cited in Manusadžianas et al. (2003) (Eq. (1)).

$$TU = \frac{1}{EC_{50}(\%)} \times 100 \qquad (1)$$

Since each leaching procedure yielded two leachates (after 1 and 14 days) a multi-stage evaluation of the four leachates was considered. First of all, the leachates originating from the harshest leaching procedure, being the EN 84 leachates obtained after 1 day, were evaluated, since it was considered that these leachates had potentially the highest environmental impact. If no significant toxicity for *D. magna* was observed, the evaluation stopped here, since real-life leaching, which is milder, should not cause any toxic effect. If, in contrast, a considerable ecotoxicity was detected then a second step was performed. In this step both the EN 84 leachates after 14 days and the OECD part 1 leachates of the first rain day were examined. When these latter leachates still exhibited a toxic response, then the leachates of the fifth rain day (OECD part 1) were also evaluated. An arbitrary ecotoxicity evaluation scale was used based on the 1:2 dilutions of the leachates. In that respect, leachates with less than 2 TUs were considered not toxic, especially since all leachates of untreated wood belong to that class (see results). They have already been used for a long time in use class 3 conditions and are considered socially acceptable. In line with this, in this paper the consecutive classes are called hardly toxic (2–4 TUs), slightly toxic (4–8 TUs), toxic (8–16 TUs) and quite toxic (> 16 TUs).

2.4. Decay resistance

The evaluation of the protective effectiveness against basidiomycetes of the Scots pine sapwood specimens treated with wood preservatives was done based on the European Standard EN 113 (1996). However, no concentration range was considered, but merely one treating level was assessed. In contrast, modified wood cannot be evaluated using the wood preservatives approach, but could be evaluated based on the natural durability approach (CEN/TS 15083-1, 2006). Although modified wood is not a new wood species, the characteristics of the original wood species are changed in such a radical way that it could be considered as a new wood species/product (Van Acker, 2003).

Both the EN 113 and the CEN/TS 15083-1 tests are very similar and hence the test setup used allows several approaches. After the leaching procedures, the wood blocks were given the time to dry in ambient conditions and they were subsequently γ-sterilised. Kolle-flasks with a malt-agar culture medium were inoculated with *Coniophora puteana* or *Postia placenta* for softwood (Scots pine sapwood and spruce) and with *Coniophora puteana* or *Trametes versicolor* for hardwood (maple). In each EN 113 test flask, one untreated control wood block was put beside one preservative-treated wood block. However, in each CEN/TS 15083-1 test flask, two modified wood blocks were put next to each other. For both test setups untreated control specimens were used to test the virulence of the fungi. To take factors that have an influence on the mass other than fungal attack into account, additional preservative-treated/modified wood blocks were aseptically put into uninoculated culture vessels to determine a correction factor. After 16 weeks of exposure, adhering mycelium was taken away and the specimens were weighed after oven drying at 103 °C, which allowed the calculation of the mass loss.

3. RESULTS

3.1. Ecotoxicological evaluation

The ecotoxicity results obtained for the various treatments are summarised in Table II. As the table indicates, no considerable toxicity (< 2 TUs) was observed for untreated wood, and this is valid for all wood species and leaching methods evaluated. As can be expected, a pronounced ecotoxicity was determined for wood treated with Cu-based preservatives. No

Table II. Product retention values and ecotoxicity (expressed as toxic units at 48 h) to *Daphnia magna* of the leachates of the treated wood.

Wood species/Treatment	Toxic units for different leaching procedures			
	EN 84		OECD part 1	
	1 day	14 days	1 day	14 days
Untreated softwoods				
Scots pine sapwood	< 2	< 2	< 2	–
Scots pine heartwood	< 2	–	–	–
Douglas fir	< 2	–	–	–
Untreated temperate hardwoods				
Beech	< 2	–	–	–
Oak	< 2	–	–	–
Black locust	< 2	–	–	–
Untreated tropical hardwoods				
Abiurana	< 2	–	–	–
Azobé	< 2	< 2	–	–
Bangkirai	< 2	–	–	–
Merbau	< 2	< 2	–	–
Padauk	< 2	–	–	–
Piquia	< 2	–	–	–
Wood[1] treated with preservatives				
CCA at 10.4 kg/m^3	> 16	4.9	< 2	–
Cu-azole at 11.3 kg/m^3	> 16	2.6	< 2	–
Cu-amine at 10.9 kg/m^3	> 16	< 2	2.6	< 2
Cu-quat at 10.8 kg/m^3	> 16	4.5	3.7	< 2
Modified wood				
Furfurylated SYP[2] (process 1)	5.8	7.5	< 2	–
Furfurylated maple[3](process 1)	> 16	> 16	< 2	–
Furfurylated SYP[2] (process 2)	< 2	< 2	< 2	–
Thermally modified Spruce[4]	3.6	< 2	< 2	–

- : EC_{50} not determined; [1] Scots pine sapwood, *Pinus sylvestris* L.; [2] *Pinus* spp.; [3] *Acer pseudoplatanus* L.; [4] *Picea abies* (L.) Karst.

difference in ecotoxicity could be observed for the Cu-based preservatives based on the leachates after 24 h EN 84 leaching, since they all show TU values over 16. After 14 days' leaching the leachates of Scots pine sapwood treated with CCA (4.9 TUs) or Cu-quat (4.5 TUs) are classified as slightly toxic, whereas leachates of treatments with Cu-azole (2.6 TUs) are rated hardly toxic and leachates of treatments with Cu-amine (< 2 TUs) are considered not toxic. The ecotoxicity of leachates obtained according to the OECD part 1 procedure for nearly all treatments is lower than those after 14 days' EN 84 leaching. Treatments of Scots pine with CCA or a Cu-azole are rated as being not toxic (< 2 TUs), while treatments with a Cu-amine (2.6 TUs) or a Cu-quat (3.7 TUs) are still considered hardly toxic. Therefore, these last two leachates were analysed after 14 days' OECD part 1 leaching. This led to the conclusion that the leachates were no longer toxic after 14 days' OECD part 1 leaching (< 2 TUs).

Table II also indicates that both the modification process and the wood species influence the ecotoxicological response to *D. magna* of modified wood. Leachates of thermally-treated spruce were hardly toxic (3.6 TUs) after 24 h EN 84 leaching and the ecotoxicity even diminished after 14 days or when leached according to the OECD part 1 leaching (1 day). These last two leachates were classified as not toxic (< 2 TUs). Wood furfurylated with a monomeric FA solution displayed certain ecotoxicity and this toxicity seems to depend both on the wood species as well as on the leaching procedure. SYP leachates seem to have an inherently lower toxicity than leachates of maple, although they have a slightly higher WPG (30% WPG for SYP compared with 20–25% WPG for maple). The EN 84 leachates of the furfurylated SYP were classified as slightly toxic (5.8 TUs), whereas those of furfurylated maple were quite toxic (> 16 TUs). A striking point of agreement between the two wood species furfurylated with the monomeric solution is the fact that the toxicity does not seem to diminish over time. The ecotoxicity towards *D. magna* of leachates of oligomeric furfurylated SYP has astonishing low values. The ecotoxicity was, regardless of the leaching procedure and harvesting time, always considered not toxic (< 2 TUs).

3.2. Fungal resistance

Since the natural durability of the untreated wood species is already known (Tab. I), it was not determined again. Figure 1 gives an overview of the mass losses obtained for the preservative-treated and modified wood after exposure to various fungi for 16 weeks. The figure shows that both the CCA- and Cu-azole-treated Scots pine specimens were fully protected against fungal attack by both *C. puteana* and *P. placenta*. The Cu-amine-treated wood, in contrast, was treated at

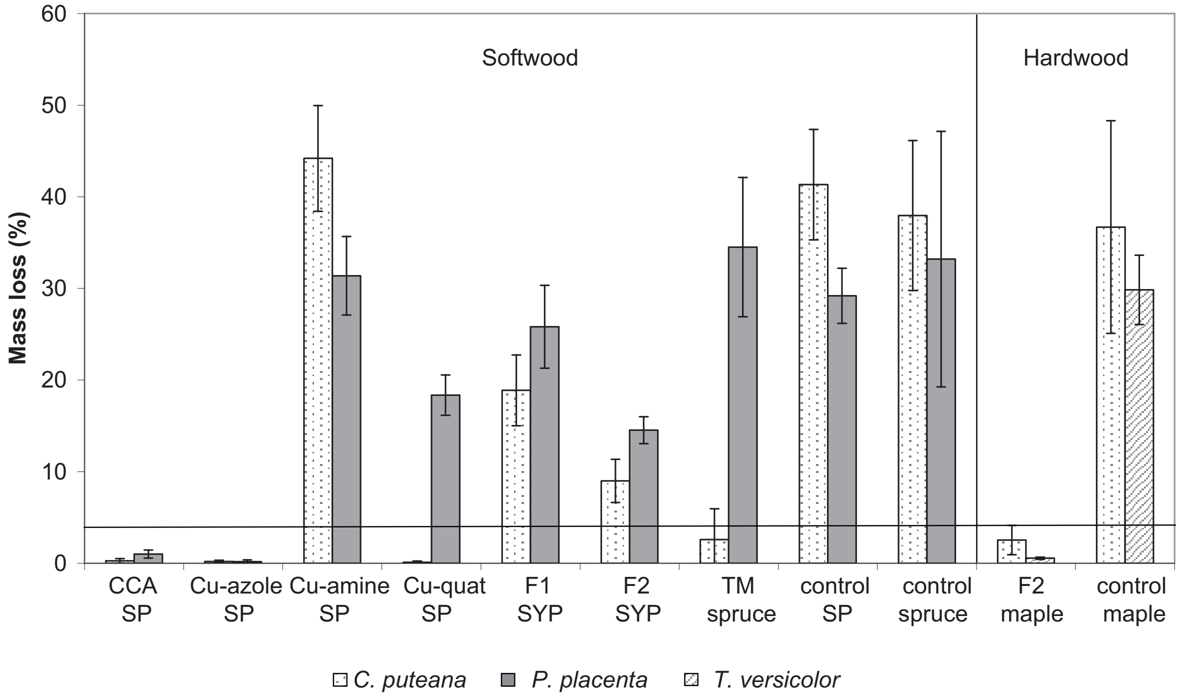

Figure 1. Mass losses of Scots pine sapwood (SP) specimens impregnated with four different Cu-based wood preservatives, furfurylated (F) southern yellow pine (SYP) and maple, thermally modified (TM) spruce and control specimens. The horizontal line is situated at 3% mass loss (CEN/TS 15083-1, 2006; EN 113, 1996).

a retention level below the toxic value and could not be differentiated from untreated Scots pine sapwood. This indicates that the wood was not sufficiently protected at a retention level of circa 10 kg/m^3. The Cu-quat-treated specimens show a sufficient protection against attack by *C. puteana* since the mass loss is below the 3% limit; however, the mass losses caused by *P. placenta* were considerably higher.

For the thermally modified spruce the mass loss caused by *P. placenta* did not differ from that of untreated spruce, whereas the mass loss of the wood induced by *C. puteana* was below the 3% threshold (Fig. 1). Both furfurylation processes could reduce the decay of SYP by *C. puteana* and *P. placenta*, although none of them could be identified as a fully effective preservative. The furfurylated maple is protected effectively both against attack by the brown rotter *C. puteana* and the white rotter *T. versicolor*. When considering modified wood as a new wood species, the natural durability can be determined based on the mass losses obtained after 16 weeks' exposure to the fungi (CEN/TS 15083-1, 2006). The durability class should be determined using the highest mass loss, and so the thermally modified spruce ends up in durability class 5, despite the good protection against *C. puteana*. The monomeric furfurylation of SYP induced an improvement in durability by 2 classes, whereas the oligomeric furfurylation process can enhance the durability by only 1 class. Due to the furfurylation maple is rated in durability class 1, whereas untreated maple is rated 5. This again shows that not only the ecotoxicology, but also the fungal resistance is influenced both by the wood species as well as by the modification process parameters.

4. DISCUSSION

4.1. Ecotoxicological evaluation

Table III gives an overview of ecotoxicity values for leachates from different wood species and treatments as presented in the literature. The table confirms for nearly all evaluated untreated wood species that their leachates are not toxic (< 2 TUs) towards *D. magna* (Van Eetvelde et al., 1997; 1998). This is also valid for the tropical wood species, which have an inherent toxicity due to the presence of extractives. This means that, despite the pronounced colour of the leachates due to the presence of extractives, they are not rated toxic to *D. magna* under the circumstances as described in this paper. The data show the importance of the leaching regime since azobé, padauk and merbau give different toxicity values depending on the leaching procedure used. The highest values are recorded for leachates obtained according to the ENV 1250.2 leaching procedure (ENV 1250.2, 1994; Van Eetvelde et al., 1998), followed by those acquired according to the OECD part 2 leaching procedure (Van Eetvelde et al., 1997). The values obtained with the leaching regimes in this research are even lower (Tab. II). It is known that the ENV 1250.2 procedure is harsh, mainly due to stirring of the water during leaching. The OECD part 2 procedure is designed to test wood exposed under use classes 4 and 5, which are the highest possible use classes (EN 335-1, 2006). In fact, it is more or less a modification of the biological efficacy testing protocol EN 84, which was developed to test wood used under use class 3

Table III. Literature values of the toxicity of leachates of different wood products (expressed as toxic units) to *Daphnia magna*.

Wood species	Exposure time		
	24 h[1]	24 h[2]	48 h[1]
Untreated wood			
Scots pine sapwood	–	< 2	< 2
Oak	–	–	< 2
Black locust	–	–	< 2
Azobé	3.3	< 2	4.4
Bangkirai	< 2	< 2	< 2
Merbau	> 32	16.7	> 32
Padauk	–	–	2.5
Wood treated with preservatives			
CCA-C Scots pine	–	–	> 32
CCB Scots pine	–	–	> 32
Cu-organic at 10 kg/m^3	–	–	> 32
Cu-organic at 8 kg/m^3	–	–	> 32
Modified wood			
Scots pine Plato	–	–	2.3
Beech Plato	–	–	< 2
Scots pine VisorWood (furfurylated)	–	–	1.5[3]
Scots pine (acetylated)	–	–	3.7

[1] Leached according to ENV 1250.2 (ENV 1250.2, 1994; Van Eetvelde et al., 1998); [2] Leached according to OECD 202 part 2 (OECD, 1984; Van Eetvelde et al., 1997); [3] Leached according to EN 84 (EN 84, 1996; Lande et al., 2004a).

conditions (Willeitner and Peek, 1998). This EN 84 leaching is still considered to be severe due to the water impregnation stage (Hingston et al., 2001). The OECD part 1 leaching procedure is designed to assess leachates in use class 3. Despite the important influence of the leaching procedure it is still surprising that merbau gives rise to such a high variation in ecotoxicity.

The thermally modified spruce used in this study showed low toxicity of the leachates towards the crustacean *D. magna*. Although even lower toxicities obtained with the ENV 1250.2 leaching method are reported by Van Eetvelde et al. (1998) for Plato-treated Scots pine and beech, the TUs found in this research for EN 84 leachates after 1 day of thermally modified spruce are still classified as hardly toxic (3.6 TUs). This research shows that whatever compounds are formed during the heat treatment of spruce as used in this research, the leachable part is only slightly to not toxic to the crustacean *D. magna*.

The ecotoxicology of furfurylated wood was earlier reported by Lande et al. (2004a). They reported no toxicity (< 2 TUs) towards *D. magna* for VisorWood leachates. This VisorWood is Scots pine furfurylated according to the same process as Kebony, but with a different treating solution (Hakkou et al., 2006). Lande et al. (2004a) put forward two reasons for the low leachate toxicity of VisorWood. The first reason could be that the furfurylated pine wood consisted mainly of sapwood with low extractive content. It is believed that the differences in natural extractive quantities are the reason for the differences among the pine samples tested and not the furfurylation process. The second reason could be the reaction between FA and the extractives. The reaction may immobilise some of the extractives, thereby reducing their leachability. These reasons seem only partly valid for the furfurylated wood in this research. This research proved that the extractives of both untreated Scots pine sapwood and heartwood are not toxic to *D. magna*. This means that differences in natural extractive quantities of Scots pine, being present between sapwood and heartwood, cannot lead to a toxic response in one case and a non-toxic response in another case of the leachates towards *D. magna*, although they might be responsible for small differences in ecotoxicity among samples. SYP furfurylated according to the first process using monomers has a slightly toxic leachate (5.8 TUs), whereas that treated according to the second process using oligomers was not toxic (< 2 TUs). The difference in ecotoxicity between the two furfurylation processes is, however, very significant. It is likely that the treating solution and process parameters (including the curing and drying steps) during furfurylation play an important role in this. They may therefore also influence the possible reaction between FA and extractives. Also, the wood species as such seems important. Monomeric furfurylated maple (20–25% WPG) gives higher leachate toxicity than monomeric furfurylated SYP (30% WPG). The authors therefore do believe that the differences in ecotoxicity are caused by a combination of factors: wood species, treating solution, WPG and furfurylation process used. Because of the complexity of parameters with an influence on the leachate toxicity and the limited knowledge of the influence of each of them on the global ecotoxicity, it remains difficult to make an overall statement about the ecotoxicology of leachates of furfurylated wood against *D. magna*. The overall conclusion on furfurylated wood is that impact on ecotoxicity of leachates can be steered by means of optimised treatment parameters.

Van Eetvelde et al. (1998) have reported toxicities of Scots pine treated with different Cu-based formulations. Regardless of the type of Cu-based formulation, all EN 1250 leachates had a toxicity of over 32 (Tab. III). This is in correspondence with the high toxicities found in this research with the EN 84 leaching method; however, no exact TUs were determined in the range over 16. Table II also shows that there is a rapid decline in toxicity after 14 days. This indicates the decrease in leachable components over time. The low TUs found after 24 h OECD part 1 leaching prove a positive assessment for long-term usage of preservative-treated wood.

The multi-stage approach has an added value over evaluating the ecotoxicology of a single leachate. To determine the protective effectiveness of wood preservatives, the EN 84 leaching can be used as an ageing method. If only the first leachate after 1 day was evaluated, no distinction between the Cu-based treatments would have been possible. The distinction in ecotoxicology of their leachates is possible due to the subsequent evaluation of the EN 84 leachates after 14 days. Comparing the TUs of both harvesting periods with each other shows that the ecotoxicology towards *D. magna* of leachates of preservative-treated wood diminishes fast over time, while this is not the case for modified wood. Since thermally modified spruce and oligomeric furfurylated SYP already had low TUs after 1 day, this is not surprising, but as stipulated before, this constant toxicity over both harvesting periods is marked for monomeric furfurylated SYP and maple. Considering the OECD part 1 leachates, no ecotoxicity was detected. As a comprehensive conclusion of this multi-stage evaluation, it can be said that preservative-treated wood has an impact on the environment when exposed under heavy duty conditions, but this ecotoxicity diminishes fast as time goes by. When, in contrast, the preserved wood is used in less severe circumstances, the leachates seem to have a limited impact on the environment. For modified wood, the conclusion depends on the modification process used. Leachates of thermally modified spruce and oligomeric furfurylated SYP are considered only slightly to not toxic, regardless of the exposure conditions. When SYP or maple is furfurylated with a monomeric solution, the ecotoxicology clearly depends on the exposure conditions; the harsher they are, the more the leachates pose a threat to the environment. Ongoing research showed that not the treating solution as such, but more specifically, the treatment process parameters influence the leachability of compounds.

In view of the most realistic leaching procedure and ecotox test for wood applied in use class 3 conditions, the OECD part 1 leachates after 1 day's exposure and consecutive evaluation of immobility of *D. magna* after 48 h seem the most suited. Evaluated in this way, the untreated wood species are all classified as non-toxic. For the preservative-treated wood, only those impregnated with about 10 kg/m^3 Cu-amine or Cu-quat showed low toxic values and none of the modified wood specimens gave toxic leachates. Nevertheless, it must be stressed that the ecotoxicity of the leachates was evaluated only with the crustacean *D. magna*. For a more comprehensive ecotox profile, organisms of different trophic levels should be included. While (sub)chronic/long-term ecotoxicity is also important, it is outside the scope of this paper and therefore not examined.

4.2. Fungal resistance

Increased durability of thermally modified wood has been reported by several authors (Boonstra et al., 1998; Kamdem et al., 2002). Research has indicated that the change in wood properties depends upon the species treated and the exact conditions employed in the process. Tjeerdsma et al. (1998) stated that heat treatment according to the Plato process revealed the highest improvement for resistance against brown rot fungi, and more specifically against *C. puteana*. This is confirmed in this research where the resistance of the thermally modified spruce against the brown rot *C. puteana* was increased a lot (mass loss below 3%), but the efficacy against *P. placenta* did not differ significantly from that of untreated spruce. Several authors state that the exact conditions of heat treatment have a significant effect on improved properties. This means that by changing the process parameters emphasis can be put on strength, dimensional stability or durability (Tjeerdsma et al., 1998).

Although Kebony 30 is classed as durable and Kebony 100 as highly durable (Hill, 2006), not many publications provide detailed information of biodegradation of furfurylated wood. Lande et al. (2004b) reported that furfurylation of wood gave high protection against biodegradation (fungi, marine borers and termites) at moderate to high levels of modification. They found reduced mass losses after 16 weeks for VisorWood-treated Scots pine specimens treated at 20–75% WPG, whereas full protection (ML < 3%) against *P. placenta* was achieved at a WPG level of 120%. All mass losses were lower than 10% and thus all furfurylated material can be allocated to durability classes 1 and 2 (CEN/TS 15083–1, 2006). In this research only furfurylated maple (20–25% WPG) achieved durability class 1, to which padauk, azobé and, to a lesser extent, abiurana and black locust also belong. This is a considerable improvement in durability compared with untreated maple, which is rated not durable just like untreated beech or Scots pine sapwood (durability class 5). For SYP moderate improvements from durability class 5 to 4 or 3 were recorded, depending on the furfurylation process used. This is quite good, since the furfurylated wood is mostly used in use class 3 conditions. Douglas fir, oak, piquia and Scots pine heartwood are also used under this biological use class without additional treatment.

Although CCA has already served for a long time as a wood preservative and has shown its efficacy in practice (Hingston et al., 2001; Mazela et al., 2005), not many data are provided on the toxic values for basidiomycete attack. Cockroft (1974) collected the data available in 1974 and reported that the toxicity value for CCA-treated Scots pine amounted to 2.3 ± 1.4 kg/m^3 and 2.7 ± 0 kg/m^3 against *C. puteana* and *P. placenta*, respectively. In this research it was not the purpose to determine this toxic value, but to evaluate the performance of impregnated Scots pine, as currently in use. The sapwood retention of 10.4 kg/m^3 CCA, which is higher than that approved by the Nordic Wood Preservation Council (NTR No. 73, 2005), was anyhow able to protect the wood completely. It can therefore be expected that lower loadings would also suffice to protect the wood. Humar et al. (2004) were able to show that after 10 years' outdoor exposure CCA-treated Scots pine (6.9 kg/m^3 of CCA) was still able to protect the wood sufficiently based on brown rot decay resistance in an EN 113 test. This was valid for both copper-sensitive strains and a copper-tolerant strain. In contrast, Tanalith E-impregnated specimens (1.8–1.9 kg/m^3 of Cu) could only protect the wood sufficiently against attack by *Gloeophyllum trabeum* and not against *P. placenta*. In this research the loadings of Cu-azole were lower, but slightly higher than the approved ones (NTR No. 73, 2005) and full protection was achieved against both *P. placenta* and *C. puteana*. Of course, it is not possible to fully compare 10-year natural weathering with artificial weathering/leaching according to EN 84. Both the Cu-amine and the Cu-quat were applied at a lower loading than the approved ones (NTR No. 73, 2005), so it could be expected that higher protection can be achieved with higher concentrations.

To evaluate the performance of treated wood under use class 3 conditions against basidiomycetes, both the natural durability approach and the preservative approach can be used. This research has shown that equal performance can be achieved using the biocidal strategy applying Cu-based solutions or when modifying the wood.

To determine the durability, evaluation according to CEN/TS 15083-1 seems the best option. The thus determined durability should at least be equal to 3 to be suited to using the wood in outdoor conditions out of ground contact. Table I shows that all untreated wood species, except Scots pine sapwood and beech, are classified into durability class 3 or higher. This classification was made based on durability according to EN 350–2 (for in ground contact) and practice. The preservative-treated wood considered in this paper was not tested using a dose response as in EN 113, but as induced durability evaluated similarly to natural durability (Fig. 1). The Cu-amine- and Cu-quat-treated wood were not fully durable; nevertheless, the Cu-quat-treated wood was at the limit for durability class 3. Concerning modified wood, furfurylated maple was classified as very durable. Monomeric furfurylated SYP (30% WPG) reached durability class 3 and was therefore, in the light of this research, sufficiently treated. When SYP is furfurylated using the oligomeric solution (20–30% WPG) a durability close to that of class 3 is obtained. The wood might therefore require a slightly higher treatment level. Since the tested thermally modified spruce is not durable against *P. placenta* but very durable against *C. puteana* there seems to be space left for improvement of the treatment conditions. Performance in practice can, however, differ significantly from the lab test results since the protection mechanism induced is moisture control-driven and lab tests enforce higher moisture content. Similar data could be observed for Scots pine heartwood (Van Acker et al., 1999). Therefore, field tests are needed to get a full picture of the performance of preservative-treated and modified wood in practice.

4.3. Combining ecotoxicology and efficacy

The monomeric furfurylated maple in this research could resist fungal attack very well and is classified as very durable. In contrast, its leachates after 1 day's and 14 days' EN 84 leaching are rated as quite toxic (> 16 TUs). The same tendency, although less marked, is observed for the monomeric furfurylated SYP using the same process. The improvement in protective efficacy against basidiomycetes is limited to durability class 3, but the corresponding ecotoxicology of the EN 84 leachates is also limited to slightly toxic (4–8 TUs). For the oligomeric furfurylated SYP the efficacy against basidiomycetes is only slightly improved to durability class 4, but the corresponding ecotoxicology is very low and in the same range as that of untreated wood (< 2 TUs). The thermally modified spruce leads to some confusing results, in that the efficacy against *P. placenta* is not improved at all compared with untreated spruce, whereas the durability against *C. puteana* is very high. The ecotoxicology of the leachates against *D. magna* is hardly toxic (2–4 TUs) to not toxic (< 2 TUs), depending on the leaching procedure used.

For the preservative-treated Scots pine, irrespective of the type of Cu-based formula used, the EN 84 leachates of the treated wood after 1 day are quite toxic (> 16 TUs). However, as stipulated before, this toxicity diminishes fast over time (14 days' EN 84 leaching). Although no significant differences in ecotoxicity towards *D. magna* are observed, major differences in efficacy can be observed. Both CCA and Cu-azole treatments are very effective in protecting the wood against fungal attack at the retention used. For the Cu-quat treatment a discrepancy in protective effectiveness between different fungi is found. Since the Cu-amine treatment of Scots pine sapwood resulted in a lower concentration than approved by the Nordic Wood Preservation Council, higher retention rates are desirable to achieve good protection of the wood against fungal attack.

5. CONCLUSION

Most ecotox TUs determined in this research are in line with what has been reported in the literature. Those values that differ from the literature could be attributed to differences in leaching procedures. Again, it is shown that the type of leaching procedure is crucial in the assessment and should therefore always be mentioned when reporting leaching or ecotoxicity values (Waldron et al., 2003). Combining the results of the ecotoxicological study and the wood protection efficacy test of each product reveals that certain products can guarantee a good protection, but have a high ecotoxicological value, and vice versa. The methodology proposed in this research could help to develop a product/process aiming for treatment of wood to be used under use class 3 conditions, since it may help to find a good balance between the efficacy against decay by basidiomycetes and the ecotoxicology of the leachates of the treated wood.

This research used a range of treated wood materials, each time at a chosen treatment level or even using process conditions still being optimised. The indications of commercial products are not intended to evaluate them in general but the specific test material was mainly to stimulate extra discussion on the importance of using material fit for purpose for use class 3 applications. High retention values or harsher treatment conditions might induce higher ecotoxicity of the leachates. Some close-to-optimal conditions resulted in some results which do not necessarily correspond to practice. This seems to be the case for the furfurylated maple and SYP used in this research, which were not yet treated under optimal conditions. Scots pine sapwood impregnated with Cu-quat, and even more with Cu-amine, would require a somewhat higher retention level to perform adequately. The retention levels used here, however, reveal the difficulty with increased ecotoxicity. Tropical wood species were included in this work as they are considered functional and fit for purpose for use class 3 applications and might be considered for benchmarking both for durability and ecotoxicity assessment. The methodology as applied in this paper proves to be suited to evaluating both parameters adequately and may therefore be used during the development phase of a new product as well as at the final certification of it.

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