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EFFICACY OF CCA AND TANALITH E TREATED PINE FENCE TO FUNGAL DECAY AFTER TEN YEARS IN SERVICE

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ABSTRACT

In response to environmental and health concerns over the use of CCA in wood preservation, there has been an increase in the development and use of arsenic and chromium free copper-based alternatives in recent years. One of the most promising alternatives to CCA is a copper-organic system, like Tanalith E, which is widely used out of the ground and is particularly suitable for wood used in outdoor applications. This is also accompanied by many attempts to understand the mechanism of copper tolerance by wood decay fungi. Samples made from CCA and Tanalith E impregnated fence (pine) palings that had been in service for ten years, were exposed to four different brown rot fungi (*Antrodia vaillantii, Leucogyrophana pinastri, Poria monticola* and *Gloeophyllum trabeum*) according to EN 113 procedure. After 16 weeks, the samples were isolated and their mass losses determined. They were also analysed using electron paramagnetic resonance (EPR). The CCA and Tanalith E treated wood were found to be resistant against the Cu-sensitive *G. trabeum*. *L. pinastri* and *P. monticola* decayed the Tanalith E but not the CCA treated samples. However, the Cu-tolerant *A. vaillantii* decayed both the Tanalith E and the CCA treated samples. The EPR spectra of the treated wood before and after exposure to the fungi, have shown the important role of oxalic acid in fungal tolerance of copper.

KEY WORDS: CCA, Tanalith E, wood preservation, copper tolerance, wood decay fungi, brown rot fungi

INTRODUCTION

Chromated copper arsenate (CCA) is currently the most extensively used wood preservative in the USA, Canada and in many European countries. However, environmental and health concerns with the use of CCA, including possible arsenic exposure to humans have resulted in its use being significantly restricted or limited (Pohleven 1998). In anticipation of the possible restriction or potential ban on chromium in wood preservatives, considerable research is being conducted in recent years to develop alternative, more environmentally friendly and effective wood preservatives. One of the most promising alternatives to CCA is a copper-organic system, like Tanalith E, which consists of copper, boron and azole (Nicholas and Schultz 2003). It is widely used in Europe and approved in more than 15 countries throughout the world and is suited for use above ground and is suitable for wood used in outdoor applications such as decking and other garden products.

A major problem concerning the use of copper based wood preservatives is copper tolerance by the target organisms especially the fungi. Though copper tolerant fungi are known for almost 50 years (Zabel 1954) and in spite of intense investigations carried out during the past decade, the mechanism of copper tolerance by these fungi is still not completely understood (Green et al. 1991, Stephan et al. 1996, Humar et al. 2001 and 2002a). This phenomenon is connected with oxalic acid excreted by the copper tolerant fungi, which reacts with copper in the preserved wood to form an insoluble and thus less toxic copper oxalate (Green et al. 1991, Humar et al. 2002a). Additionally, it has been reported that lowering of pH by oxalic acid also contributes to copper tolerance as well (Starkey 1973). During the initial stages of wood decay, significant and rapid acidification of the wood and oxalic acid production occur and consequently the toxic effect of copper is reduced (Humar et al. 2001, Clausen and Green 2003).

Impregnated timber at the end of its lifetime often contains high concentrations of the preservative. The main aim of this research was to determine the efficacy of CCA and a novel copper-based preservative, Tanalith E treated wood to brown rot wood decay fungi after ten years in service as fence palings. This is part of a larger research project to develop and optimise a large-scale novel process for bio-recycling of preservative treated wood wastes through bioremediation and bulk-reduction using wood decay fungi.

MATERIAL AND METHODS

The fence palings made of pine and vacuum impregnated with CCA (Type C) or Tanalith E that had been in service for 10 years were supplied by Arch Chemicals (Castleford, UK). Retention of preservative in the sapwood was determined using X-ray fluorescence. Experimental samples (1.5 x 2.5 x 5.0 cm) were cut from the impregnated part of these fences. For control, unimpregnated pine samples were used. After steam sterilization of the samples, they were exposed to four different brown rot fungi listed in Tab. 1. Exposure was performed according to the EN 113 procedure (ES 1989). Fungi cultures were grown and maintained on a 3.9% potato dextrose agar medium (PDA, Difco). Jars with PDA medium were inoculated with small pieces of fungal mycelium. One treated and one control wood sample were placed on a sterilized plastic grid in each inoculated jar and exposed to fungal decay for 16 weeks in the dark (25°C, RH 75%). Afterwards, the wood specimens were cleaned of mycelia and mass losses were determined gravimetrically. The experiment was performed in five parallels.

EPR spectra of the control and decayed samples were recorded at room temperature using Bruker ESP-300 X-band spectrometer (Microwave Frequency = 9.62 GHz, Microwave Power = 20 mW, Modulation Frequency = 100 kHz, Modulation Amplitude = 0.1 mT). Four matchsticks like samples (1 x 1 x 40 mm) were cut from each wood sample and inserted into the resonator separately. The various components of EPR parameters (tensor g, and hyperfine splitting tensor A) were determined directly from the spectra, where possible, for the respective paramagnetic species.

Tab. 1: Brown rot fungi used. Copper tolerance is described with marks according to the results of Pohleven et al. (2002). Mark 1 describes the highest copper tolerance; and 5 describes the highest copper sensitivity.

Fungi	Origin	Estimated Cu tolerance
Antrodia vaillantii	University of Ljubljana ZIM L037	Cu tolerant 1
Leucogyrophana pinastri	Buckinghamshire Chilterns University College UK	Cu tolerant 2
Poria monticola	BAM 102	Cu sensitive / Cu tolerant 3
Gloeophyllum trabeum	University of Ljubljana ZIM L017	Cu sensitive 5

RESULTS

The retention of the Tanalith E impregnated timber was between 1.8 and 1.9 kg/m^3 of Cu and that of the CCA impregnated timber it was 6.9 kg/m^3 of CCA. Both timbers investigated were without any visible signs of biological deterioration after 10 years in service. This could be due to the high preservative retention and the fact that they were used out of ground contact. From Tab. 2, it is evident that none of the copper sensitive strains, *Poria monticola* and *Gloeophyllum trabeum* as well as the copper tolerant strain, *Leucogyrophana pinastri* was able to decay the CCA impregnated samples. However, the copper tolerant strain, *Antrodia vaillantii* decayed the CCA impregnated wood with the average mass loss of 17.6%. Significantly higher mass losses were obtained for the Tanalith E treated samples. The highest mass loss was by the copper tolerant *A. vaillantii* (24.7%), followed by *P. monticola* (18.1%) and *L. pinastri* (17.7%). However, the copper sensitive *G. trabeum* was not able to decay the Tanalith E treated samples (Tab. 2).

Tab. 2: Mass losses of CCA or Tanalith E treated and untreated specimens after 16 weeks of exposure to brown rot fungi according to EN 113 procedure. Standard deviations are given in the parentheses.

Fungi	CCA	Tanalith E Mass loss [%]	Untreated
Antrodia vaillantii	17.6 (5.2)	24.7 (1.5)	34.1 (4.8)
Leucogyrophana pinastri	0.0 (0.1)	17.7 (0.6)	30.2 (6.2)
Poria monticola	0.0 (0.0)	18.1 (3.4)	39.8 (3.6)
Gloeophyllum trabeum	0.0 (0.0)	1.3 (0.3)	45.4 (7.2)

From the EPR spectra of the unexposed CCA treated wood samples, three different EPR signals can be distinguished: Cr(III) EPR signal (g = 1.982, linewidth = 50 mT), Cu(II) EPR signal (g_0 = 2.067) and the free radical signal (g0 = 2.003) (Fig. 1). Similar parameters (signals) for CCA treated wood are reported in literature (Hughes et al. 1994, Humar et al. 2002b). This result indicates that with the exception of the EPR signal of Cr(V), there has been no significant changes in the composition of CCA preservative in the wood due to weathering.

After exposure of the CCA treated samples to G. trabeum, there were no changes to the shape as well as the EPR parameters. On the other hand, exposure of the samples to the copper tolerant fungi *A. vaillantii* and *L. pinastri* resulted in significant changes of the EPR spectra. The Cr(III) and Cu(II) EPR signals completely disappeared and a copper oxalate EPR signal appeared (g = 2.18, linewidth = 51 mT). In addition, the intensity of the free radical EPR signal increased. EPR spectra of specimens exposed *L. pinastri* and *P. monticola* are not shown as they are comparable with the *A. vaillantii* ones (Fig. 1).

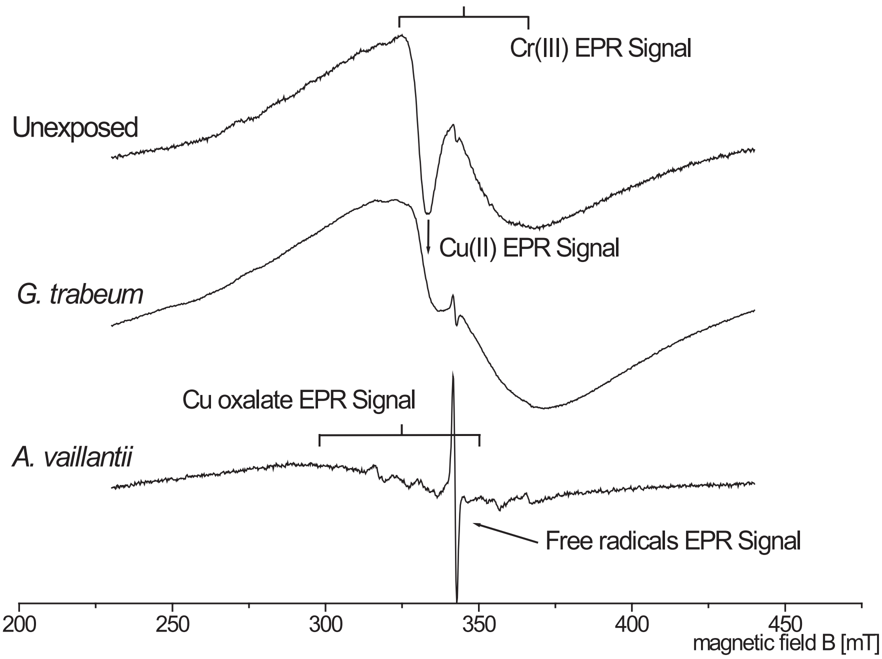

Fig. 1: EPR spectra of CCA treated samples unexposed and exposed to brown rot fungi Gloeophyllum trabeum and Antrodia vaillantii for 16 weeks.

The wood impregnated with Tanalith E exhibited an anisotropic Cu(II) EPR spectrum ($g_{\perp}$ = 2.076, g_{II} = 2.278, a_{II} = 15.5 mT) (Fig. 2). After 16 weeks of exposure to the fungi, changes of the EPR spectra were similar to those observed for the exposed CCA treated wood. From the EPR spectra of impregnated wood exposed to the fungi *A. vaillantii, P. monticola* and *L. pinastri* transformation of Cu(II) EPR signal to copper oxalate signal and an increase in intensity of the free radical EPR signal could be resolved. On the other hand, there were no significant changes on the EPR spectra of the Tanalith E impregnated wood exposed to the copper sensitive fungus G. trabeum (Fig. 2).

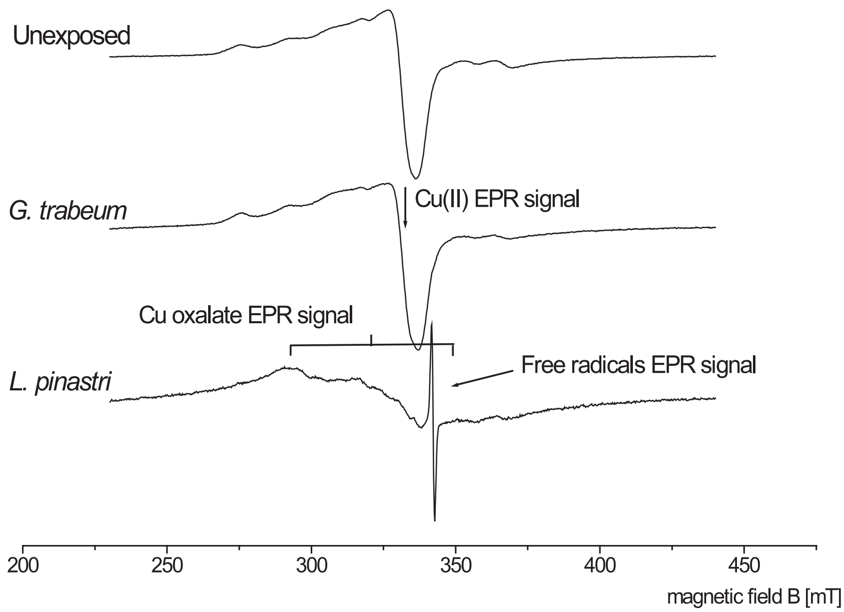

Fig. 2: EPR spectra of Tanalith E treated samples unexposed and exposed to brown rot fungi Gloeophyllum trabeum and Leucogyrophana pinastri for 16 weeks.

DISCUSSION

From the EPR spectra of unexposed CCA treated wood (Fig. 1), with the exception of Cr(V) signal, the copper and chromium signals have the same parameters as those reported in literature for CCA impregnated wood. During the fixation process, chromium was reduced from diamagnetic Cr(VI) to paramagnetic Cr(III) via Cr(V) as an intermediate. The EPR signal of Cr(V) was found to be present two years after impregnation (Hughes et al. 1994, Humar et al. 2002b). However, due to exposure of the sample to weathering when in service, this signal could not be resolved. It was suggested that water environment affects chromium reduction and this could explain the disappearance of the Cr(V) signal in the sample (Hughes et al. 1994, Humar et al. 2002b).

The mid point of the toxic values of CCA treated wood when tested without prior ageing against the most aggressive fungus according to the EN 113 guidelines (ECS 1994) is 2.5 kg of CCA/m^3. The preservative retention of the CCA treated sample was found to be three times higher (6.9 kg/m^3) than in guidelines even after ten years of natural weathering. This explains the resistance of the timber against *P. monticola*, *L. pinastri* and *G. trabeum*. However, in contrast to these fungi, exposure of the CCA treated wood to *A. vaillantii* resulted in about 17.6% mass loss (Tab. 2). *A. vaillantii* is reportedly a fungal strain that exhibits the highest copper tolerance on several screening and other laboratory tests (Humar et al. 2001, 2002a, Pohleven et al. 2002).

After exposure of the CCA treated wood to the copper tolerant strain *A. vaillantii*, instead of the Cu(II) EPR signal, another signal with a measured g_0 value of 2.18 and line width about

51 mT appeared. These parameters correlate well with those reported in the literature for copper oxalate (Srivastava et al. 1980, Humar et al. 2002a). Copper oxalate is not soluble, and therefore not fungitoxic (Richardson 1997). In addition, there was a significant decrease of the Cr(III) which could be due to the formation of chromium oxalate, as this compound cannot be resolved from the EPR spectra (Lahiry and Kakkar 1982). Similar, but less intense changes of the EPR spectra were observed for the samples exposed to *L. pinastri* and *P. monticola*. However, for the CCA treated samples exposed to these two fungal strains, no mass losses were obtained and we therefore believe that the formation of copper oxalate is not the only mechanism responsible for copper tolerance. On the other side, the EPR spectra of the CCA treated samples exposed to *G. trabeum* showed that the intensity and shape of Cr(III) and Cu(II) EPR signals were unaffected (Fig. 1). This could be due to the fact that *G. trabeum* excretes less oxalic and other organic acids. Furthermore, this spectrum also showed that copper remained in original toxic form and thus protected the CCA impregnated wood blocks from being decayed. From the EPR spectra of CCA treated wood exposed to *A. vaillantii*, a signal assigned to free radicals can be resolved as well (Fig. 1). Occurrence of free radicals during brown rot decay is well known and described, as they are part of the process of cellulose degradation (Green and Highley 1997, Qian et al. 2003).

Tanalith E impregnated wood gave a Cu(II) EPR signal consisting of a quadruplet signal at low field and an unresolved absorption at higher field. This is typical for Cu(II) ions in an anisotropic state. However, the shape and parameters of the EPR spectra (Fig. 2) are similar to those obtained for copper sulfate treated wood (Hughes et al. 1994, Humar et al. 2002a). Though the retention of copper in Tanalith E treated wood is comparable to that in the CCA impregnated wood, it has to be considered that besides copper there are other co-biocides present in the Tanalith E treated wood and these could have resulted in the different performance against the fungal strains. After ten years in service and weathering, the Tanalith E treated timber was found to be resistant against the copper sensitive strain *G. trabeum* but not against other less copper-sensitive strains. The highest average mass loss (24.7%) was obtained for the samples exposed to Cu-tolerant *A. vaillantii*. The fact that the Tanalith E treated timber was not resistant against the copper tolerant fungi, is not significant, as this preservative is predominantly used for the protection of wood in hazard class 3 where these copper tolerant fungi do not attack wood.

From the EPR spectra of Tanalith E treated timber exposed to the wood decay fungi (Fig. 2), there were no changes in the spectra of samples exposed to the copper sensitive *G. trabeum*. This further proves the efficacy of Tanalith E to this fungus. On the other hand, exposure of the Tanalith E treaded wood to the other wood decay fungi, showed a significant decrease of the Cu(II) EPR signal and an increase of copper oxalate and free radicals signals. The reason for these changes is the excretion of oxalic acid by these fungi and cellulose degradation.

CONCLUSIONS

CCA and Tanalith E treated wood were found to be resistant against a copper sensitive brown rot decay fungi strain *Gloeophyllum trabeum* after 10 years in service as fence palings.

The brown rot decay fungi *Leucogyrophana pinastri* and *Poria monticola* decayed the Tanalith E treated wood but not the CCA treated one. However, the copper tolerant *Antrodia vaillantii* decayed both the Tanalith E and CCA treated wood.

From the EPR spectra of the treated wood exposed to the wood decayed fungi, the important role that oxalic acid plays in fungal tolerance can be resolved.

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